TAXONOMIC REVISION OF THE GENUS *CHILOSCHISTA* LINDL. (ORCHIDACEAE) IN THAILAND

THOTSAPORN CHANOKKHUN

A Thesis Submitted in Partial Fulfillment of the Requirement for the
Degree of Master of Science in Environmental Biology
Suranaree University of Technology
Academic Year 2022

การทบทวนอนุกรมวิธานกล้วยไม้สกุลเอื้องพญาไร้ใบในประเทศไทย

นายทศพร ชนกคุณ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาชีววิทยาสิ่งแวดล้อม
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2565

TAXONOMIC REVISION OF THE GENUS *CHILOSCHITA* LINDL. (ORCHIDACEAE) IN THAILAND

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

Thesis Examining Committee

(Assoc. Prof. Dr. Surapon Saensouk)
Chairperson

(Assoc. Prof. Dr. Santi Watthana)
Member (Thesis Advisor)

(Assoc. Prof. Dr. Nooduan Muangsan)
Member (Thesis Co-Advisor)

(Asst. Prof. Dr. Pongthep Suwanwaree)
Member

(Assoc. Prof. Dr. Pimwadee Pornpongrungrueng)
Member

(Assoc. Prof. Dr. Chatchai Jothityangkoon)
Vice Rector for Academic Affairs
and Quality Assurance

(Prof. Dr. Santi Maensiri)
Dean of Institute of Science

ทศพร ชนกคุณ : การทบทวนอนุกรมวิธานกล้วยไม้สกุลเอื้องพญาไร้ใบในประเทศไทย (TAXONOMIC REVISION OF THE GENUS *CHILOSCHISTA* LINDL. (ORCHIDACEAE) IN THAILAND) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.สันติ วัฒฐานะ, 84 หน้า.

คำสำคัญ: ไฟโลเจนี อนุกรมวิธาน สถานภาพการอนุรักษ์

กล้วยไม้สกุลเอื้องพญาไร้ใบ เป็นกล้วยไม้ที่มีความสวยงามแปลกตา นิยมนำมาปลูกเลี้ยงเป็นไม้ประดับ มีความผันแปรของลักษณะที่หลากหลาย โดยก่อนหน้านี้มีการรายงานทางอนุกรมวิธานของกล้วยไม้สกุลนี้ที่พบในประเทศไทย รวม 11 ชนิด แต่บางชนิดมีลักษณะที่คล้ายคลึงกัน จึงควรทำการศึกษาตัวอย่างเพิ่มเติมในธรรมชาติและจากพิพิธภัณฑ์พืช เพื่อตรวจสอบการจำแนกระดับชนิดจากลักษณะทางสัณฐานวิทยาแล้วนำไปวิเคราะห์ความสัมพันธ์ทางพันธุกรรม (Phylogenetic analysis) เพื่อยืนยันสถานะของแต่ละชนิดในแผนภาพความสัมพันธ์ทางวิวัฒนาการ ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาทบทวนทางอนุกรมวิธานจากข้อมูลทางสัณฐานวิทยาและอณูชีววิทยาเพื่อยืนยันการจำแนกของแต่ละชนิด ซึ่งการทบทวนทางอนุกรมวิธานได้ทำการศึกษาเปรียบเทียบจากตัวอย่างที่เก็บใหม่จากธรรมชาติและตัวอย่างอ้างอิงในพิพิธภัณฑ์พืชทั้งในและต่างประเทศ และใช้หลักการกำหนดชนิดด้วยลักษณะฐานวิทยาเป็นแนวทางในการกำหนดระดับชนิด ผลการศึกษาครั้งนี้พบกล้วยไม้สกุลเอื้องพญาไร้ใบจำนวน 7 ชนิด ในประเทศไทย ได้แก่ เอื้องพญาไร้ใบสิงขร (*Chiloschista extinctoriformis* Seidenf.) เอื้องพญาไร้ใบปากเป็ด (*C. exuperei* (Guill.) Garay) เอื้องพญาไร้ใบดอกแดง (*C. lunifera* (Rchb.f.) J.J.Sm.) เอื้องพญาไร้ใบจุดประ (*C. parishii* Seidenf.) เอื้องพญาไร้ใบคางยาว (*C. rodriguezii* Cavestro & Omerod) เอื้องพญาไร้ใบ (*C.usneoides* (D.Don) Lindl.) และเอื้องพญาไร้ใบดอกเขียว (*C. viridiflava* Seidenf.) โดยมีการเพิ่มชื่อพ้อง 2 ชนิด ได้แก่ *Chiloschista trudelii* Seidenf. (ชื่อพ้องของ *C. viridiflava* Seidenf.), *C. ramifera* Seidenf. (ชื่อพ้องของ *C. lunifera* (Rchb.f.) J.J.Sm.) ส่วน *C. lindstroemii* Dalstrom & Kolan. น่าจะเป็นชื่อพ้องของ *C. parishii* Seidenf. ลักษณะทางอนุกรมวิธานที่สามารถใช้แยกชนิด คือ ลักษณะของกลีบปาก อย่างไรก็ตามกลุ่มของ *C. usenoides* ที่ประกอบด้วย *C. lunifera*, *C. parishii*, *C. usneoides* และ *C. viridiflava* ใช้ได้เพียงลักษณะของสีดอกในการแยกชนิด สำหรับการศึกษาความสัมพันธ์ทางพันธุกรรมระดับโมเลกุล (Molecular phylogeny) ด้วยยีน *mat*K และ ITS ด้วย Maximum Likelihood (ML) และ Posterior Propability (PP) ด้วยโปรแกรม RaxM BlackBox และ MrBayes ตามลำดับ ในเว็บไซต์ CIPRESS Science Gateway v. 3.3 ยืนยันว่าสกุล

เอื้องพญาไร้ใบอยู่ในกลุ่มโมโนไฟเลติก อย่างไรก็ตาม ยังต้องการตัวอย่างและยีนที่จะนำมาวิเคราะห์ความสัมพันธ์ทางพันธุกรรมเพิ่มเติม ผลของการประเมินสถานภาพทางการอนุรักษ์ด้วยข้อมูลการกระจายพันธุ์และจำนวนประชากรของแต่ละชนิดพบว่า *C. extinctoriformis, C. rodriguezii* และ *C. exuperei* เป็นชนิดที่เสียงต่อการสูญพันธ์ (VU) ซึ่งต้องทำการศึกษาชีววิทยาเพื่อการอนุรักษ์ รวมถึงจัดทำแผนอนุรักษ์ชนิดพันธุ์อย่างเร่งด่วน

สาขาวิชาชีววิทยา
ปีการศึกษา 2565

ลายมือชื่อนักศึกษา_____
ลายมือชื่ออาจารย์ที่ปรึกษา_____
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

THOTSAPORN CHANOKKHUN : TAXONOMIC REVISION OF THE GENUS *CHILOSCHISTA* LINDL. (ORCHIDACEAE) IN THAILAND. THESIS ADVISOR : ASST. PROF. SANTI WATTHANA, Ph.D. 84 PP.

Keyword: Taxonomy, Phylogeny, Conservation status.

The orchid genus *Chiloschista* Lindl. is one of the striking and beautiful orchids which are popular for ornamental purpose. They have variable morphological characteristics. Previous taxonomic studies reported 11 species in Thailand, but some species are closely similar. So, field observation and herbarium study should be performed to clarify the species based on morphology including the phylogeny position of each species derived from morphological species delimitation. This study aimed to study taxonomic revision based on morphological variation and molecular phylogeny to confirm the species identification. For the taxonomic revision, the additional specimens collected in Thailand as well as from Herbaria, in Thailand and foreign countries, were compared to study the variation. The species delimitation was employed the species concept based on morphology. It was revealed that, 7 species found in Thailand which are *Chiloschista extinctoriformis* Seidenf., *C. exuperei* (Guill.) Garay, *C. lunifera* (Rchb.f.) J.J.Sm., *C. parishii* Seidenf., *C. rodriguezii* Cavestro & Omerod *C. usneoides* (D.Don) Lindl. and *C. viridiflava* Seidenf. Two new synonyms were added which are *C. trudelii* Seidenf. (synonym of *C. viridiflava* Seidenf.) and *C. ramifera* Seidenf. (synonym of *C. lunifera* (Rchb.f.) J.J.Sm.). While *C. lindstroemii* Dalstrom & Kolan. is doubtful to be a synonym of *C. parishii* Seidenf. The taxonomic characters are the labellum morphology. However, *C. usenoides* complex comprised *C. lunifera, C. parishii, C. usneoides* and *C. viridiflava*, in which the color pattern can be used to distinguish among those similar species. The molecular phylogeny study based on *mat*K and ITS with Maximum Likelihood (ML) and Posterior Propability (PP) analysis using RaxM BlackBox and MrBayes on XSEDE in CIPRESS Science Gateway v.3.3 confirmed that seven species are monophyletic group. However, it needs more

samples of each species and more genes to include in the further phylogeny analysis. The result on conservation status evaluation based on distribution and number of the population of each species showed that *C. extinctoriformis, C. rodriguezii* and *C. exuperei* are vulnerable (VU) species. They need urgently to study the conservation biology as well as proper species conservation management, especially the disturbed population by human activities to protect species extinction from the wild.

School of Biology
Academic Year 2022

Student's Signature _____
Advisor's Signature _____
Co-advisor's Signature _____

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my advisor and co-advisor, Assistant Professor Dr.Santi Watthana and Associate Professor Dr.Nooduan Muangsan for their encouragement, valuable advice and guidance in all of this research.

In addition, I am grateful for the teachers of Biology for suggestions and all their help.

I would like to thank Mr.Tammanoon Jitpromma for your help in the work and all of the graduate students and staff at the School of Biology for their kind help and support.

This research work was support by OROG grant and Research Unit under the Institute of Research and Development, Suranaree University of Technology, including the financial supporting from Fundamental Fun, Taxonomy of the Genus *Chiloschista* Lindl. (Orchidaceae) and Conservation Status in Thailand (FF1-104-65-12-66(15)) and Evolution relation of the genus *Chiloschista* Lindl. (Orchidaceae) in Thailand and publishing biological information for leaning (FF1-104-66-12-110(F)) under the Thailand Science, Research, and Innovation (TSRI).

Finally, I most gratefully acknowledge my parents for all their support throughout the period of study.

Thotsaporn Chanokkhun

CONTENTS

CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF ABBREVIATION

bp	base pair
DMSO	Dimethyl sulfoxide
DNA	Deoxy-ribonucleic acid
mM	millimolar
PCR	Polymerase chain reaction
pmol/µL	picomole per microliter
µL	microliter
°C	degree Celsius

CHAPTER I

INTRODUCTION

The taxonomy is one part of the systematic including four components: Description, Identification, Nomenclature, and Classification. Taxonomic work can help to define the organisms, which supplement the information of biology for communication (Simpson, M. G., 2010). It is the biological subject which helps us to know what they look like, which group they are and what their names are.

The orchid is high diversity in the world belonging to family Orchidaceae. They are brilliantly colored flowering plants with massive diversity, with over 880 genera and 27,000 species—in fact more than reptiles, birds and mammals combined (Givnish, T. J., Spalink, D., Amess, M., Lyon, S., Hunter, S., Zuluaga, A., Iles, W., Clements, M., Kalin, M., Leebens-Mack, J., Endara, L., Kriebel, R., Neubig, K., Whitten, W., Williams, N., and Cameron, K., 2015). Christenhusz and Byng (2016) counted 284,281 flowering plant species of angiosperms (monocots: 74,273 species; eudicots: 210,008 species) of which orchids makeup 8.4%. They are the largest flowering plant (angiosperm) family as a result of their highly variable life history strategies. The classification of orchids is different from the past to recent. Since the early 1800s, the orchidologist has classified the orchids based on the morphology of their column (Brown, S., 1810; Lindley, J., 1840; Pfitzer, E., 1887; Schllechter, R., 1926; Swartz, O., 1800).

Recent plant classification is based on several biological evidence apart from the morphology, DNA, anatomy, palynology, etc. (Judd, W. S., Campbell, C. S., Kellogg E. A., Stevens, P. F., and Donoghue, M. J., 2016; Simpson, M. G., 2010). The molecular study is now more important and widely accepted information for the plant including the orchid classification (Reece, J. B., Urty, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., and Jackson, R. B., 2014).

Morphological traits are conveniently applied for species delimitation and generic classification. However, for the generic unit, especially the orchid, there are

sometimes no evolutionary relationships because of convergent evolution. While the molecular evidence naturally gives more information which can be used to separate into the distinguished species (Xu, Q., Zhang, G., Liu, Z., and Luo, Y., 2014), including the generic circumscription (Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y., 2015). Indeed, taxonomy of the species should include molecular evidence as well as morphological study (Chase, M. W., Cameron, K. M., Barrett, R. L., and Freudenstein, J. V., 2015). At the present, taxonomists use the DNA data for supported orchid classification (Reece, J. B., Urty, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., and Jackson, R. B., 2014). In the other word, the species identification by molecular evidence is more reliable. In this study, I am interested in using the molecular evidence for species delimitation for the genus *Chiloschista* because there are many species having similarity characters resulting in difficulty to identification.

The genus *Chiloschista* Lindl. has been reported as 29 species in the world, distributed from India to South China and South Asia. (https://powo.science.kew.org). They are only epiphytic orchids belong to the subtribe Aeridinae under the subfamily Epidendroideae. It is one of the striking orchids showing epiphytic roots and long pendulous inflorescence deserving potential to economic value. Seidenfaden (1988) listed the distribution of this genus as Myanmar, Thailand, Nepal, and Bhutan as well as a large area of the Indian Himalayan. Recently reports showed the distribution in Bhutan as well; Wang Chu River in the Chhukha district (Gyeltshen, N., Gyeltshen, C., Tobgay, K., Dalstrom, S., Gurung, D. B., Gyeltshen, N., and Ghalley, B. B., 2020). For Thai species, Seidenfaden (1988) reported that there are 9 species. Later in 2005 and 2020, there were 2 new species which were described from Thailand (Cavestro, W., and Ormerod, P., 2005; Dalstrom, S., and Kolanowska, M., 2020).

This genus is one of the orchid genera with having some closed related species by means of morphological traits, complex group (Seidenfaden, G., 1988). The classification in the present is still not clear because the morphological character of *Chiloschista* has similarity in nature, especially species group of *C. parishii* Seidenf., *C. viridiflava* Seidenf., *C. useneoides* (D.Don) Lindl., *C. lunifera* (Rchb.f.) J.J. Sm., *C. ramifera* Seidenf. and *C. yunnanensis* Schltr. etc. Moreover, Seidenfaden (1995) stated that he felt uncertain about how many species should be recognized in the

genus. Thus, it needs to observe the variation in the natural population to delimit the species boundary. The identification by using DNA sequences should be employed to confirm whether they are good species or not. Finally, I shall revise the taxonomy of the genus *Chiloschista* in Thailand to reveal the accepted species based on morphological and molecular evidence, the accepted name following the International Code of Nomenclature for Algae, Fungi and Plants (ICN). Then I will provide the description of each accepted species, illustration, key to species and ecological note as well as distribution map.

From a conservation point of view, natural populations of *Chiloschista* are declining due to deforestation and over collection, same as other strikingly orchid species (Pedersen, H.Æ., 2010). This study will fulfill the Flora of Thailand Project which attempts to reveal species diversity in Thailand. Such taxonomic study is fundamental basic information which can be applied for proper conservation management on species survival and sustainable use (Chase, M. W., Cameron, K. M., Barrett, R. L., and Freudenstein, J. V., 2003).

1.1 Research objectives

1. To do taxonomic revision of the genus *Chiloschista* Lindl. in Thailand.

2. To confirm the species identification of Thai species by using molecular phylogeny information based on ITS and *mat*K.

1.2 Scope and limitations

All specimens of the genus *Chiloschista* Lindl. collected in Thailand and deposited in various herbarium such BK, BKF, C, CMU, K, PSU and QBG (the abbreviations follow Thiers, 2010) including my collection from the field trip during this study shall be compared the morphology for survey the variation of various populations to delimit the species based on morphological species concept. I shall indicate the taxonomic characters to separate the taxonomic unit considered by discrete characters. The morphological species concept shall be applied.

I shall consult previous taxonomic work such as Seidenfaden (1988) to identify the species as well. Then, each tentatively accepted species shall be analyzed the

phylogenetic tree based on one nuclear and one chloroplast gene. Then, I shall follow the phylogenetic species concept applying the monophyletic clade for the taxonomic unit (i.e. Simpson, M. G., 2010). Finally, nomenclature shall be involved according to The International Code of Nomenclature (ICN) to reveal the accepted name.

After having solutions on taxonomic classification and nomenclature, I shall prepare key to species or subspecies and varieties if any as well as species citation, description, distribution, ecology, conservation status and taxonomic note. The result of this study will be a part of the taxonomic revision for the Flora of Thailand.

CHAPTER II

LITERATURE REVIEW

2.1 Classification of family Orchidaceae

The orchidaceae is one of the largest family of flowering plants, having over 750 genera, with about 27,000 species (Chase, M. W., Cameron, K. M., Barrett, R. L., and Freudenstein, J. V., 2015; Givnish, T. J., Spalink, D., Amess, M., Lyon, S., Hunter, S., Zuluaga, A., Iles, W., Clements, M., Kalin, M., Leebens-Mack, J., Endara, L., Kriebel, R., Neubig, K., Whitten, W., Williams, N., and Cameron, K., 2015). They occur in almost every habitat in the world (Chase, M. W., Cameron, K. M., Barrett, R. L., and Freudenstein, J. V., 2003). Stevens (2020) reported on his well-known Angiosperm Phylogeny Website that the Orchidaceae classified into order Asparagales with closed related to Liliales, and Monocotyledons clade. Boryaceae, Blandfordiaceae, Lanariaceae, Asteliaceae and Hypoxidaceae clade is the sister group of the Orchidaceae. Recently, the Orchidaceae is classified as follow:

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Clades: Angiosperms

Clades: Monocots

Order: Asparagales

Family: Orchidaceae

The orchidaceae is the mycorrhizal herb with protocoms which has no radicle. More than 75% of their habits are epiphyte, the less are terrestrial orchids, often tuberous, and lithophyte. Roots consist of velamen, a persistent outer layer of dead cells. Leaves are entire margin, spirally arranged, usually distichous and often with a basal sheath, rarely reduced to sales. Inflorescences are usually paniculate or racemose. Flowers are usually resupinate, the phenomenon of twisted through 180 degrees of pedicel. The floral shape is lateral symmetry. The flowers consist of 3 colored sepals and 3 colored petals. One petal is strongly differentiated into a highly modified lip or labellum. Male and female organs completely adnate, forming a column. The anther usually has 1, some has 2 or 3. Pollens are usually in pollinia which are large waxy or granular masses, usually having 2, 4 or 8. There is one inferior ovary. Fruit is usually a capsule with 1-6 longitudinal splitted ridges. Seeds are microseeds without phytomelanin (adapted from Utteridge and Bramley, 2015).

The revised and updated classification of the flowering plant is refered by Angiosperm Phylogeny Group (APG). The family Orchidaceae is placed within the order Asparagales recognised into 5 subfamilies, including Apostasioideae, Cypripedioideae, Orchidoideae, Epidendroideae, and Vanilloideae (Stevens, P. F., 2020; Pridgeon, A. M., Cribb, P. J., Chase, M. W., and Rasmussen, F. N., 1999) (Figure 2.1).

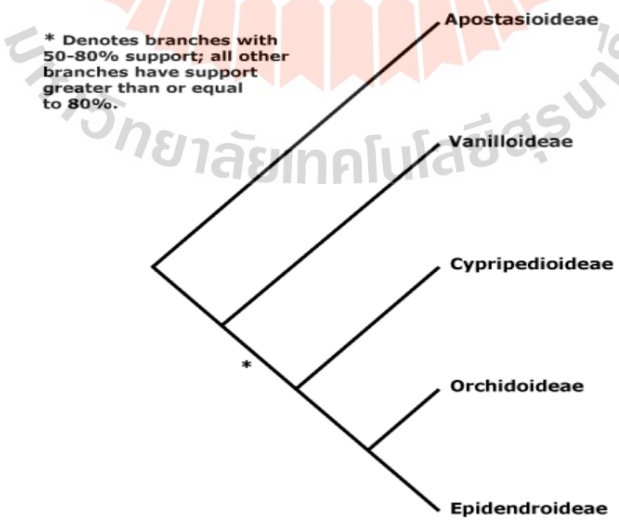

Figure 2.1 Phylogenetic tree of Orchidaceae (Stevens, P. F., 2020; Pridgeon, A. M., Cribb, P. J., Chase, M. W., and Rasmussen, F. N., 1999)

Apostasioideae is a small to medium-sized terrestrial perennial herbs that are woody bases. Roots are aerial, visible above the ground. Rhizome is often absent. Shoots are branched or unbranched, erect, leafy. Leaves are spiral, leathery, plicate. Inflorescences are terminal, racemose, few to many flowered. Bracts are persistent. Flowers are more or less regular, concolorous. Pedicels are usually short, twisted or not. Sepals are spreading, entire, cuspidate, with thickened midribs. Petals are free, cuspidate. Labellum is similar to petals or slightly broader. Column is short, straight to curved. Style fuses to the base of filaments only. Others are 2 or 3, each 2-locular. Pollen is powdery, not forming pollinia. Staminode is present or absent. Style is free in the upper part, cylindric, fleshy. Stigma is terminal, rounded to 2- or 3-lobed. Fruit is capsular or berrylike. Seeds are ovoid to ellipsoidal with a terminal appendage (Figure 2.2).

Figure 2.2 Apostasioideae: *Apostasia wallichii* R. Br.

Cypripedioideae is a small to large terrestrial, lithophytic, or rarely epiphytic herb. Roots are rather thick. Rhizome is rather short. Stems are unbranched, short to long and erect. Leaves are distichous arrangement having 1 to many. Young leaves are, plicate or conduplicate. Mature leaves green spread or suberect Leave texture is thin, fleshy, or leathery. The leave surface is glabrous or variously pubescent. The leave margin is entire. Some species have markings on the upper side of leaves. Inflorescence comprises 1 to several flowers, produced on the terminal part. Rachis is hairy, glandular, or glabrous. Floral bracts are distinct and conduplicate. Flowers are usually colorful. Pedicel is obscure to short. The Dorsal sepal is often hooded over the lip. Its shape is ovate, obovate, or elliptic. Its apex is obtuse, acute, or acuminate. Its surface is glabrous or pubescent. The Lateral sepals are usually merged to form a concave synsepal, like the dorsal sepal. Petals are free, spreading or pendulous and spiraling or not. Its shape is elliptic, ovate, linear, or oblanceolate. Its surface is glabrous or rarely pubescent on outer surface, usually ciliate on margins, sometimes warty on margins. Labellum is a deep pouch, slipper or urn shape. The front margin of the labellum incurves or erect. Side lobes are sometimes warty. Column is short. There are 2 anthers, each one having 2 locules. Pollen is powdery or viscid. Staminode is present, sessile or shortly stalked, often shield-shaped or transversely reniform. Stigma is subsessile or with stalk, dependent, convex, 3-partite. Ovary is 1-locular or 3-locular, glabrous or hairy. Fruit is an erect to pendulous capsule with 3-ribbed. Its shape is cylindric to almost ellipsoidal. Seeds are ellipsoidal with a thin testa. Only the genus *Paphiopedilum* has been recorded in Thailand (Pedersen, H.Æ., Kurzweil, H., Suddee, S., and Cribb, P. J., 2011) (Figure 2.3).

Figure 2.3 Cypripedioideae: *Paphiopedilum hirsutissimum* (Lindl. ex Hook.) Stein.

Orchidoideae is a terrestrial or very rarely epiphytic plant, producing tubers or fleshy, short to long rhizomes. Some species are holomycotrophic orchids having colorless of all part. The leaves of those holomycortrophic orchids reduced to colorless sheaths. Tubers vary from ovoid, spherical, ellipsoidal, digitate, ro cylindric-fusiform, solitary or in clusters. Its surface is hairy or glabrous. Rhizome is elongated and fleshy. 1- many leaves are spirally arranged at the basal part or along the stem. Some species are deciduous or rarely persistent for more than a year. The leaves are often sheathing at base, rarely spotted marking. Inflorescence arises from the terminal part producing 1 to many flowered. The peduncle is usually terete. Its surface is glabrous, hairy, or glandular. Floral bracts are linear, lanceolate, ovate, or elliptic, usually glabrous and green. Flowers vary from small to large, usually resupinate and often showy. The pedicel is often invisible. Dorsal sepal is often adnate to petals to form a hood over column. The lateral sepals are usually free, sometimes connate and oblique at base to form a spur-like mentum. Petals are entire or 2-lobed, often adnate to the dorsal sepal. The Labellum is entire or divided to 3-5-lobes. It is often saccate at base or with spurlike producing the nectar inside. Nectary spur is fusiform, clavate,

or cylindrical, rarely fused to ovary or often lacking. Column is basified or not. Anthers have one which is 2-locular, longer than or as long as rostellum. Pollinia has 2 or 4, sectile, attached by short to elongate caudicles to 1 or 2 viscidia. Two staminodes are lateral, usually present. Stigma is entire or with 2 lobes, concave to convex. Rostellum is usually 2- or 3-lobed, shorter than or as long as anther. Ovary is subcylindrical, glabrous or hairy. Fruit is a capsule with 3 ribs, longitudinally dehiscent. Seeds are fusiform to almost cylindrical, with a thin testa (Wu, Z. Y., Raven, P. H., and Hong, D. Y., eds. 2009) (Figure 2.4).

Figure 2.4 Orchidoideae: *Habenaria rhodocheila* Hance.

Vanilloideae is small to large terrestrial autotrophic or holomycotrophic, monopodial or sympodial herbs or scrambling or climbing vines. Roots are elongate, fleshy. Rhizome is short to elongate. Stems are erect or scrambling, unbranched or branched. Leaves have 1 to many, varied from short to long and sometimes reduced to scales. Leaves arrangement is alternate. Its texture is fleshy or leathery. Young

leaves are conduplicate and spread or suberect when mature. Leaves shapes vary from ovate, elliptic, lanceolate, ligulate to oblong. Its surface is glabrous. Inflorescences arise from the terminal or lateral parts, producing 1- to many flowers on raceme or panicle. Rachis is sometimes pubescent. Floral bracts are conduplicate. Flowers are often colorful. Pedicel is short. Sepals are free and subsimilar to each other. Its surface is glabrous to pubescent. Petals are free. Its surface is glabrous. Labellum is free or fused to the column base and divided into 3-lobs or entire lobe. It usually has a callus of hairs, papillae, or backward-facing scales, lacking a nectary spur-like. The column is elongated, slender and hooded at the tip. The anther has 1, located on the terminal part of the column. The pollinia is composed of loosen pollen grains. Stigma is 3-lobed, concave. The ovary is 1-locular or 3-locular. Its surface is glabrous or pubescent. Fruit is an erect to pendulous capsule with 3 ribs. Its shape varies from cylindric to almost ellipsoid. Its surface varies glabrous to pubescent. Seeds are spherical with a hard testa (Wu, Z. Y., Raven, P. H., and Hong, D. Y., eds. 2009) (Figure 2.5).

Figure 2.5 Vanilloideae: *Vanilla siamensis* Rolfe ex Downie

Epidendroideae is the perennial epiphytic, terrestrial, or lithophytic herbs or rarely scrambling climbers. It can be either sympodial or monopodial growth habit and varies from small to large size. The rhizomes are short or long and elongate and creeping. Some species are achlorophyllous or holomycotrophic orchid. Roots have a 1- to several layer of velamen, smooth to warty. The aerial root shape is terete to dorsiventrally flattened. Stems form a pseudobulb and are usually leafy. Sometime the leaves are often reduced in number. The internode has 1 or more. Leaves are entire margin. Its arrangement is alternate or occasionally opposite, often distichous. Its texture is frequently fleshy or leathery. Its shape varies from dorsi- ventral compressed to rarely terete or canaliculate, with a basal sheath, sometimes articulated at base. Inflorescences arise from the basal, lateral or terminal part of the stem. It can be erect to pendulous in spicate, raceme, or panicle. Flowers are small to large 1 - to many, often quite colorful, usually pedicellate, mostly resupinate, glabrous to hairy. Sepals are free. The dorsal is often dissimilar to laterals. The laterals sometimes adnate to column foot forming a conical, saccate or spurlike mentum. Petals are free or rarely adnate to sepals. It is similar or not to the sepals, often showy. Labellum is dominant, simple to strongly trilobed spur present or lacking. It is ornamented or not with various calli shapes and with or without a basal spur or nectary. It margins are entire to laciniate. Column is short to long, with or without a basal foot. The anther has 1 located on the terminal part of the column, caplike, attached to column apex by a short filament. The pollinia is mealy or waxy, 2- 8, sessile or on the strip and viscidium. The stigma is 3- lobed, concave. The rostellum is usually transverse, less commonly elongate and 1- 3- lobed. The ovary is inferior and has 1 locule. Fruit is a capsule, opening laterally by 3 or 6 slits. Seeds are fusiform to discoid, numerous, dustlike, lacking endosperm, sometimes markedly winged (Wu, Z. Y., Raven, P. H., and Hong, D. Y., eds. 2009) (Figure 2.6).

Figure 2.6 Epidendroideae: *Dendrobium friedericksianum* Rchb. f.

2.2 Classification of *Chiloschista* Lind.

The generic name of *Chiloschista*, derived from the Greek words *cheilos* meaning lip or rim and *schistos* (cleft), describes the cleft lip found on the flowers (Dassanayake, M. D., and Fosberg, F. R., 1981). John Lindley named the *Chiloschista* genus in 1832 based upon information from *Epidendrum usneoides* D. Don (Seidenfaden, G., 1988). Numerous authors did not accept the genus as valid until 1905 when J.J Smith reintroduced *Chiloschista* (Seidenfaden, G., 1988). Later Seidenfaden (1988) popularized acceptance of the genus in the late 1980's. The species of *Chiloschista* collectively range from China and the Indian subcontinent through Southeast Asia to Australia, the Palau Islands, and Fiji (Figure 2.7).

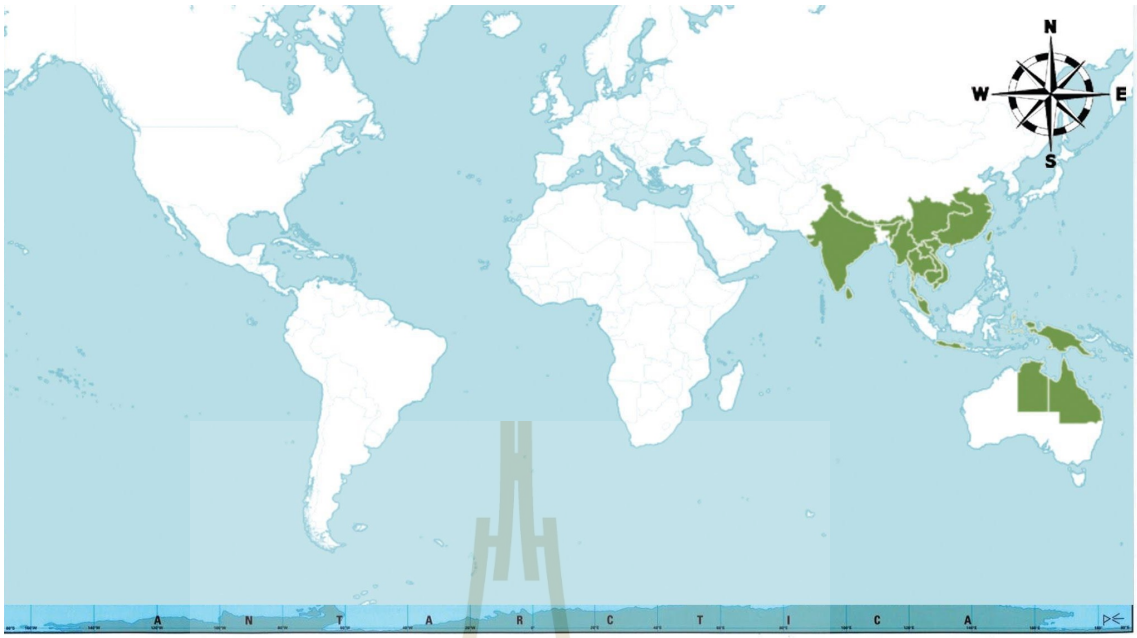

Figure 2.7 Map of Distribution of *Chiloschista* genus (Ref: Kew Science).

Phylogeny shows patterns and historical evolutionary relationships between species and sharing characteristics due to common ancestry. Phylogenies typically take the form of a tree diagram, or a phylogenetic tree. We can use a tree diagram that represents the history of evolution including learning great ideas about a species, but first we will treat the organism using taxonomy (named, classified and scientific rule) (Reece, J. B., Urty, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., and Jackson, R. B., 2014). We can use characters to reconstruct phylogenetic trees with two conceptual class divisions: (1) morphological (anatomy, organ morphology, and embryology); and (2) molecular from DNA sequences (nuclear DNA and chloroplast DNA). The selection of taxa for analysis includes both the group is ingroup (ingroup is a closely related group of taxa that has been examined for evolution relationships) and addition outgroup (outgroup is the group of taxa that is closely related but not a member of the ingroup). Typically, the outgroup is for comparison.

How can sequencing elucidate phylogenetic relationships? Topik, H., Peter, W., Tomohisa, Y., and Motomi, I. (2005) constructed phylogenetic analyses using two DNA sequences are *mat*K (Maturase K) and ITS (internal transcribed spacers). The phylogenetic analysis indicates *Chiloschista* is monophyletic in the *Arachnis* subclade, and repeatedly emphasizes characters (pollinia number, spur length, and column foot).

Produce phylogenetic trees for 2005 and 2012 studies for comparison. However, Topik, H., Peter, W., Tomohisa, Y., Motomi, I., and Rod, R. (2012) reexamined the Aeridinae subtribe with more DNA sequences (*mat*K, ITS, nrDNA; nuclear ribosomal DNA and cpDNA; Chloroplast DNA). The phylogenetic analysis determined relationships of the *Chiloschista* genus using the example species *C. viridiflava*. The result is an odd pairing of *C. viridiflava* with *Orthochilus difformis* as a sister group.

More recent results using Bayesian statistical methods generated posterior probabilities isolating the *Chiloschista* genus with weak parsimonious support grouping to the *Dimorphorchis* and *Thrixspermum* clade (Wood, J., 2014). Later, Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y. (2015) produced a molecular phylogeny analysis using mostly new DNA sequences (ITS, atpI-H, *mat*K, psbA-trnH, and trnL-F) from 211 taxa 74 Aeridinae subtribe genera. Their phylogenetic analysis employed the maximum parsimony (MP) and Bayesian inference (BI). Then, the BI consensus tree inferred from the combined dataset was chosen for the discussion of phylogenetic relationships showing the Bayesian posterior probabilities and the bootstrap percentages on each branch of the phylogenetic tree. The result indicates that Aeridinae is monophyletic group (Figure 2.8).

Chiloschista is monophyletic with strong support from both Bayesian Posterior probabilities (PP) and bootstrap percentages (BP). The *Chiloschista* clade includes *C. yunnanensis*, *C. lunifera*, *C. parishii*, *C. pusilla* and one unidentified *Chiloschista* species with strong support (PP 100). From this study, they used only 5 *Chiloschista* species to analyze phylogeny. The *Chiloschista* clade is a sister group of clades of *Phalaenopsis*, *Thrixspermum*, *Vanda*, *Aerides*, *Trichoglottis*, *Abdominea*, *Gastrochillus* and *Cheisostoma* clades (Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y., 2015).

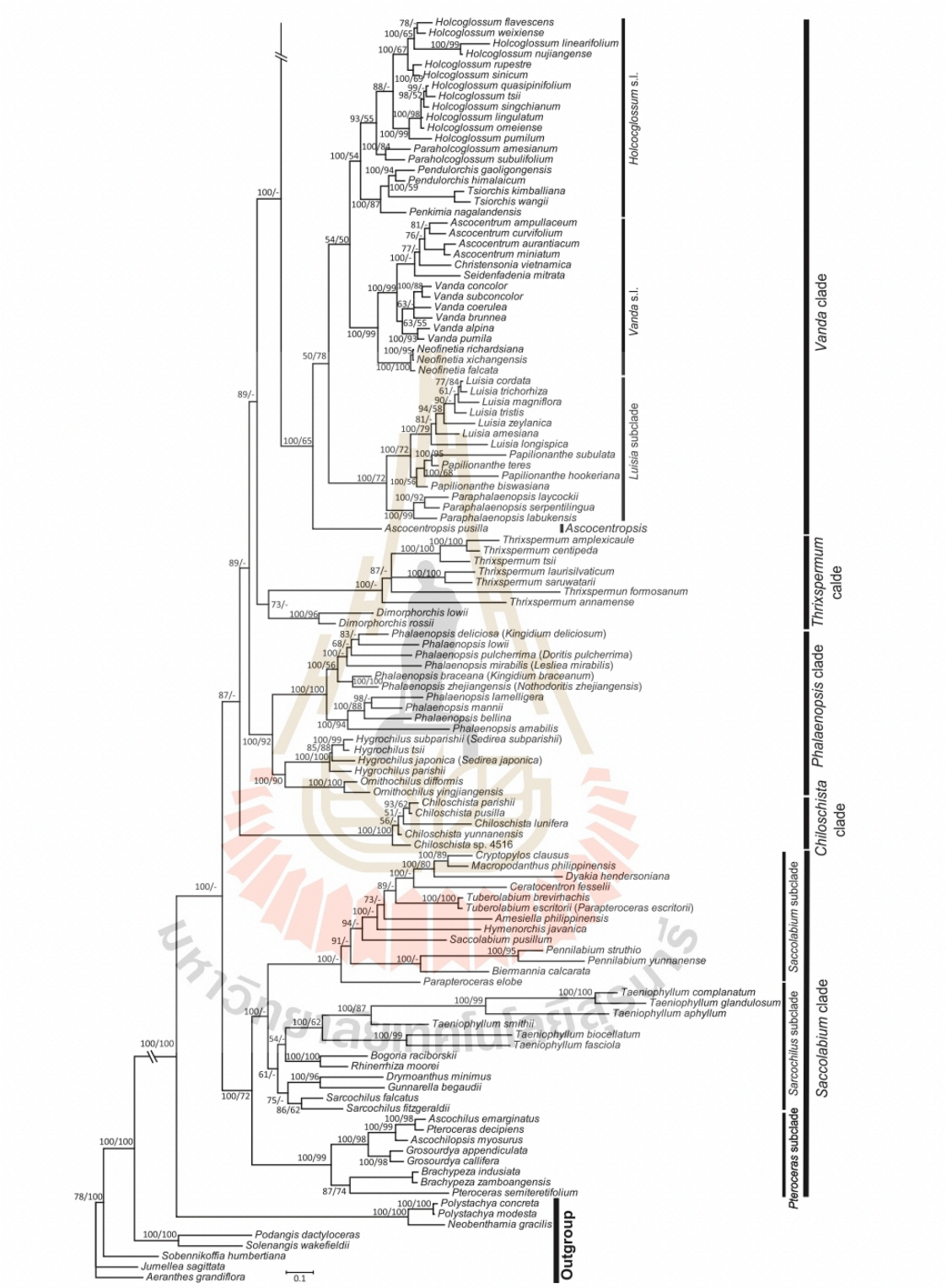

Figure 2.8 Part of phylogenetic tree of subtribe Aeridinae based on nuclear and chloroplast genes, showing a monophyletic *Chiloschista* (Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y., 2015).

Chiloschista can be recognized by being leafless epiphytic baring thick green roots but sometimes the scale leaves are produced. Inflorescences are racemose, sometimes branching at the basal part, often hairy, pendulous. Flowers are medium size up to 1.5 cm in diameter. Labellum is sac-like, without appendage inside, producing oblong side-lobes. The midlobes of the labellum are small. Operculum with or without a long thread appendage at both sides (Figure 2.9). It can be classified as follow:

Family: Orchidaceae

Subfamily: Epidendroideae

Tribe: Vandinae

Subtribe: Aeridinae

Genus: *Chiloschista* Lindl.

Figure 2.9 *Chiloschista* genus in Thailand. A. Plant (Epiphytic species) and B. Flower yellow color (in sepal and petal has faintly spotted brown).

2.3 Taxonomic history of *Chiloschista* in Thailand

Seidenfaden and Smitinand (1963) firstly reported the name of *C. lunifera* (Rchb.f.) in Thailand. Later in 1988, he re-identified this specimen as *C. parishii* Seidenf. as a new species and reported 9 species were found. Smitinand (2014) also reported 9 species of *Chiloschista* in Thailand. However, some species have few location reports, especially in Thailand. In 2005, Cavestro and Ormerod (2005) described *C. rodriguezii* Cavestro & Ormerod, an endemic species found in Mae Hong Son Province. Later, Dalstrom (2020) described *C. lindstroemii* Dalström & Kolan, a closed species with *C. parishii* Seidenf. found in Kanchanaburi Province. Accounting from available taxonomic literature, there are totally accepted 11 species of *Chiloschista* found in Thailand (Table 2.1).

Table 2.1 *Chiloschista* species and distribution in Thailand.

No.	Species	Locations in Thailand	Conservation status*	References
1	*C. extinctoriformis* Seidenf.	Prachuap Khiri Khan (Khao Luang)	EN (Endangered)	Seidenfaden, G. (1988), Smitinand, T. (2014)
2	*C. exuperei* (Guill.) Garey.	Chiang Mai (Mae Ai), Phetchabun (Nam Nao)	-	Seidenfaden, G. (1988)
3	*C. lindstroemii* Dalström & Kolan.	Kanchanaburi	-	Dalstrom (2020)
4	*C. lunifera* (Rchb. f.) J. J. Sm.	Mae Hong Son (Pang Ma Pha), Chiang Mai	-	Seidenfaden, G. (1988), Smitinand, T. (2014)
5	*C. parishii* Seidenf.	Tak (Fang), Lampang (Huay Mae Tam), Prae, Chieng Mai (Doi Suthep, Doi Saket), Chayaphum (Nong Bua Deng), Loei (Wang Saphung), Korat (Khao Yai, Pak Chong), Nakorn Nayok, Phetchaburi (Ban	-	Seidenfaden, T. (1988)

Table 2.1 *Chiloschista* species and distribution in Thailand (Continued).

No.	Species	Locations in Thailand	Conservation status*	References
		Nagorn), Prachuap (Upper Pran River), Kanchanaburi (Sisawat), Krabi (Ban Keng), Satul (Tung Nui)		
6	*C. pusilla* (*Koen.*) Schltr.	Betong; Pattani	-	Seidenfaden, G. (1988)
7	*C. ramifera* Seidenf.	Nakhon Ratchasima (Pak Chong), Saraburi (Sam lan)	EN (Endangered)	Seidenfaden, G. (1988)
8	*C. rodriguezii* Cavestro & Ormerod	Mae Hong Son	-	Cavestro and Ormerod (2005)
9	*C. trudelii* Seidenf.	-	-	-
10	*C. usneoides* (D. Don) Lindl.	Chiang Mai (Doi Chiang Dao, Fang)	-	Seidenfaden, G. (1988)
11	*C. viridiflava* Seidenf.	Chiang Mai (Doi Saket)	EN (Endangered)	Seidenfaden, G. (1988)

* Refer to Chamchumroon, V., Suphuntee, N., Tetsana, N., Poopath, M. and Tanikkool, S., 2017.

CHAPTER III

MATERIALS AND METHODS

3.1 Taxonomic revision

3.1.1 Examined specimens and morphological data collection

The morphological examination of *Chiloschista* herbarium and spirit specimens from the following herbaria are investigated: Herbarium, Royal Botanic Gardens, Kew, England (K), Botanical Garden, Natural History Museum, University of Copenhagen, Denmark (C), The Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Thailand (BKF), Queen Sirikit Botanic Garden Herbarium, Thailand (QBG), and Department of Biology, Faculty of Science, Chiang Mai University, Thailand (CMUB). The herbarium pictures available online at JSTOR Global Plants (http://plants.jstor.org) are assessed. Type specimens will be paid more attention. The relevant taxonomic literature will be consulted. Moreover, I intend to observe the variation of the orchid in the natural population during my field trip in 2021 and 2022. The phenology and ecology will be noted during the field trip. Representative individuals in each population shall be made as voucher specimens by means of dried or spirit (alcohol 70% with a drop of glycerin) preparations. The pictures of morphological characters will be done with careful notes.

All vegetative characters will be measured from dried herbarium and living plants, while reproductive characters will be measured under stereomicroscope with using scale Leica slide 0.1 mm bars. Dried specimens will be soaked with hot water with a few drops of detergent to soften before measurement. The botanical glossary of Stern (1989) will be followed for writing the description of each taxon (Figure 3.1).

3.1.2 Taxonomic problem solution

I grouped the specimens based on morphology into different groups judging by dissimilarity of the character set representative as the distinctive species. Indeed, the traditional morphology species concept were applied to circumscribe the species

boundary (Van Steenis, C. G. G. J., 1957; Davis, P. H., and Heywood, V. H., 1963) which accepted species were characterized by discontinuous variation in several characters. Whenever there was much similarity among taxa, the taxonomic unit of subspecies or varieties was categorized judging by non-overlapping and overlapping geographic ranges, respectively. The discrete characters were used as taxonomic characters to construct the identification key.

3.1.3 Nomenclature problem solution

After grouping the available specimens as distinct species, I identified by comparing with the type specimens of nomenclature carefully. The accepted name of each species will be justified according to similarity with the type specimens. The first description and protologues of the scientific name shall be consulted. If there is more than one type specimen matching with the species group, the International Code of Nomenclature (ICN) will be followed to judge the accepted name of the species. All synonyms shall be noted.

3.1.4 Documentation

Literature, description, illustration, ecology, distribution and vernacular names, as well as some notes for each species were documented. Distribution maps of each species in Thailand also was provided. The IUCN Standards and Petitions Committee's 2019 version of the Guidelines for Applying the IUCN Red List Categories and Criteria, Version 14, was utilized for conservation status.

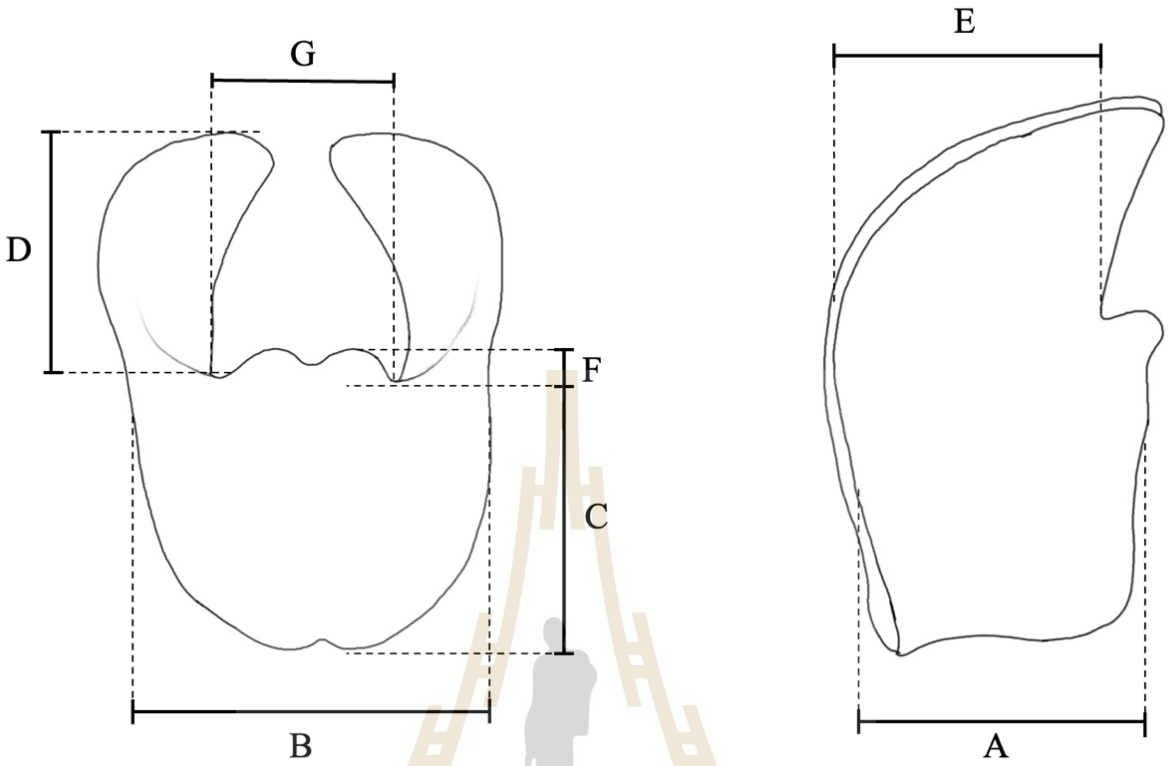

Figure 3.1 Floral diagram of *Chiloschista* for measuring: A. labellum length, B. labellum and hypochile width, C. hypochile height, D. sidelobe length, E. sidelobe width, F. midlobe length, G. midlobe width.

3.2 Phylogeny study

3.2.1 Studied specimens

According to molecular phylogeny, all *Chiloschista* species based on Seidenfaden (1988) circumscription found in Thailand are required to be ingroup species. At least 2 specimens for each species from different populations are used for constructing the phylogenetic tree. I collected the samples to extract DNA when I had seen the flowers. So, I could identify the specimen correctly. Part of young inflorescence and green roots were taken for DNA sequencing, due to leafless species. The samples will be dried by silica gel suddenly. The voucher specimens will be prepared and deposited at Queen Sirikit Botanic Garden (QBG). To compare with new

sample from this study, the *mat*K and ITS sequences from GenBank that are currently available were downloaded (Table 3.1) and used as ingroup. According to Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y. (2015); Topik, H., Peter, W., Tomohisa, Y., and Motomi, I. (2005) and Topik, H., Peter, W., Tomohisa, Y., Motomi, I., and Rod, R. (2012), I selected *Hygrochilus parishii* Pfitzer (syn. of *Phalaenopsis hygrochila* J.M.H.Shaw*, Ornithochilus yingjiangensis* Z.H.Tsi (syn. of *Phalaenopsis yingjiangensis* (Z.H.Tsi) Kocyan & Schuit. *, O. Difformis* (Wall. ex Lindl.) Schltr. (syn. of *Phalaenopsos difformis* (Wall. ex Lindl.) Kocyan & Schuit.*, Taeniophyllum glandulosum* Blume*, Thrixspermum annamense* (Guillaumin) Garay*, T. centipeda* Lour. and *Vanda alpina* (Lindl.) Lindl., gained from GenBank, as the outgroup due to being a member of a sister clade and closely related with *Chiloschista* clade.

Table 3.1 The available maturase K (*mat*K) and internal transcribed spacer (ITS) gene of *Chiloschista* genus and outgroup species from GenBank accession numbers. A hyphen (-) indicate missing data, and the remaining sequences are from GenBank.

No.	Species	Vouchers	nrITS	*mat*K
1	*Chiloschista cf.extinctoriformis*	K022	-	-
2	*C. extinctoriformis*	T.Chanokkhun 483	-	-
3	*C. exuperei*	T.Chanokkhun 295	-	-
4	*C. exuperei*	T.Chanokkhun 514	-	-
5	*C. lunifera*	T.Chanokkhun 476	-	-
6	*C. lunifera*	T.Chanokkhun 490	-	-
7	*C. parishii*	T.Chanokkhun 486	-	-
8	*C. parishii*	T.Chanokkhun 489	-	-
9	*C. parishii*	T.Chanokkhun 511	-	-
10	*C. rodriguezii*	T.Chanokkhun 313	-	-
11	*C. usneoides*	T.Chanokkhun 297	-	-
12	*C. usneoides*	T.Chanokkhun 325	-	-
13	*C. viridiflava*	T.Chanokkhun 469	-	-
14	*C. viridiflava*	T.Chanokkhun 565	-	-
15	*Chiloschista* sp.	T.Chanokkhun 468	-	-
16	*C. yunnanensis*	Z.J.Liu 6094	KJ021015	KJ021019
17	*Hygrochilus parishii*	Z.J.Liu 3336	KF545876	KF545887
18	*Ornithochilus difformis*	Z.J.Liu 4111	KF545878	KF545889
19	*Ornithochilus yingjiangensis*	Z.J.Liu 7160	KF545879	KF545894
20	*Taeniophyllum glandulosum*	Z.J.Liu 5458	KJ733455	KJ733612
21	*Thrixspermum annamense*	Z.J.Liu 4972	KF545883	KF545893
22	*Thrixspermum centipeda*	Z.J.Liu 5389	KJ733456	KJ733621
23	*Vanda alpina*	Z.J.Liu 6145	KC244656	KC244660

3.2.2 DNA extraction

DNA was extracted from plants in accordance with the manufacturer's instructions using the Genomic DNA Isolation Kit (Plant), (Bio-Helix, Taiwan). The protocol was done as follow:

1) Sample Preparation: the samples were made with dried plant tissue weighing 25 mg or 50 mg. The material was ground with a mortar and pestle to a fine powder under liquid nitrogen.

2) Lysis: Before the sample was ground, 500 µL of Buffer PL and 0.5 µL of RNase A (50 mg/mL) were added. The sample was then transferred to a 1.5 mL microcentrifuge tube after fully dissolving. After that, it was incubated for 30 minutes at 75 °C while being turned upside down every ten minutes. It was then transferred to a new 1.5 mL microcentrifuge tube containing the supernatant after being centrifuged at 14,000 x g for 5 minutes.

3) DNA Binding: The sample was mixed with the equal amount of 350 µL of isopropanol and vortex for 5 seconds to clear the supernatant. The mixture was then transferred to a column PC, which was then centrifuged for 30 seconds at 14,000 x g. The column PC was then put back into the collection tube after the flow-through had been removed.

4) Wash: The column PC was then filled with 400 µL of the buffer W1, and the mixture was centrifuged at 14,000 x g for 30 seconds. The column PC was centrifuged for 30 seconds at 14,000 x g after 600 µL of Buffer W2 (with additional ethanol) was added. The flow-through was then removed, and the column PC was put back into the same collection tube. Centrifuging once more for two minutes at 14,000 x g removes the residual buffer W2.

5) DNA Elution: The supernatant was transferred from the dried Column PC to a new 1.5 mL microcentrifuge tube. 50 µL of the Pre–Heated buffer BE or TE was added to the supernatant, which was then placed in the middle of the column matrix as left for 3 minutes at 75 °C. DNA that has been purified is eluted by centrifugation at 2 minutes for 14,000 x g. The extracted DNA was kept at -20 °C.

3.2.3 PCR Amplification and Sequencing

The *mat*K region was amplified by using a primer pairs, 390F and 1326R (Cuénoud, P., Savolainen, V., Chatrou, L. W., Powell, M., Grayer, R. J., and Chase, M. W., 2002). The total volume 50 µL amplification reaction contained 25 µL OnePCR Ultra, 2.5 µL each primer, see Table 3.2 (5 pmol/µL), 1 µL of template DNAs and 19 µL of free water. The polymerase chain reaction (PCR) profile included of an initial at 95 °C 5 minutes for premelting stage, followed by 30 cycles of denaturation at 95 °C 30 seconds , annealing at 55 °C 1 minutes, extension at 72 °C 40 seconds and a final extension at 72 °C 7 minutes (Kocyan, A., Vogel, E. F., Conti, E., and Gravendeel, B., 2008).

Amplification of ITS sequences was carried out using the primer pairs, 17SE and 26SE (Sun, Y., Skinner, D. Z., and Liang, G. H., 1994). The 50 µL amplification reaction comprised 25 µL OnePCR Ultra, 2.5 µL each primer, see Table 3.2 (5 pmol/µL), 1 µL of template DNAs, 1 µL of DMSO and 18 µL of free water. The polymerase chain reaction (PCR) profile consisted of an initial at 95 °C 5 minutes for premelting stage, followed by 30 cycles of denaturation at 95 °C 30 seconds, annealing at 55 °C 1 minutes, extension at 72 °C 40 seconds and a final extension at 72 °C 7 minutes (Kocyan, A., Vogel, E. F., Conti, E., and Gravendeel, B., 2008).

Table 3.2 The amplification reaction of gene sequences.

Loci	Primers	Sequences	References
*mat*K	390F	CGATCTATTCATTCAATATTTC	Cuénoud, P., Savolainen, V., Chatrou, L. W., Powell, M., Grayer, R. J., and Chase, M. W., 2002
	1326R	TCTAGCACACGAAAGTCGAAGT	
ITS	17SE (Forward)	ACGAATTCATGGTCCGGTGAAGTGTTCG	Sun, Y., Skinner, D. Z., and Liang, G. H., 1994
	26SE (Reverse)	GAATTCCCCGGTTCGCTCGCCGTTAC	

3.2.4 Phylogenetic analysis

Using Bioedit ver. 7.2.5 (Hall, 1999) and manual adjustments, the 23 nucleotide sequences of *mat*K, ITS and combined *mat*K and ITS from the ingroup and outgroup were aligned in this work. I tested the evolution model using jModelTest2 on XSEDE in the CIPRES Gateway version 3.3 (https://www.phylo.org/) before phylogenetic analysis. The software displayed the AIC of the concatenated sequences with TVM+G, ITS with GTT+G and *mat*K with TPM1uf+G. For the phylogenetic tree, RAxML BlackBox with 1,000 bootstrap repetitions (Felsenstein, J., 1985) was used for the maximum likelihood (ML) tree. MrBayes on XSEDE with a Markov chain Monte Carlo (MCMC) chain length of 1,000,000 for Bayesian analysis (BA) was run. Both of phylogenetic programs were employed in the CIPES Science Gateway version 3.3 (https://www.phylo.org/). The obtained trees from the online analysis were manipulated and labeled by using FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) to present in this study.

CHAPTER IV

RESULTS and DISCUSSIONS

4.1 Taxonomic revision

4.1.1 Morphology of *Chiloschista*

All species of *Chiloschita* in Thailand is an epiphytic orchid. Few species have habit type both epiphyte and lithophyte such as *C. usneoides* (Pearce and Cribb, 2002). Their stem obscure or very short and produce many roots. Their roots are flattened, ribbon-loke or subcylindrical, scabrid. It is usually grey-green when dry and can fast absorb water or vapor. Then the photosynthesis takes place at their root. They hardly produce leave, but sometime with small leaves during rainy season. Their leaves are elliptic or lanceolate with subacute apex and slightly bilobed. During flowering, there is no leave, showing only roots and inflorescence. The large and old individuals usually produce many inflorescences (Figure 4.1).

Inflorescences were lateral raceme in general. Healthy and old individuals seem to have few branches as being a panicle which were found in *C. lunifera* and *C. parishii.* The inflorescence is usually pendent or sometime erect with many-flowered in all Thai species. Sometime there are few flowers in a short inflorescence which may be related in its age. The rachis and peduncle are pilose in all thai species. The floral bracts are small with ovate-lanceolate shape, subtended the pedicel.

The flora part morphology is shown in Figure 4.2. Flowers are resupinate, ephemeral and fragrant. The pedicel including ovary is terete, terete and pilose in all species found in Thailand. Their color of sepals and petals are varied from white in *C. usneoides* to yellow in *C. rodriguezii*. In some species exhibits both white to pale yellow such as *C. rodriguezii.* In *C. lunifera*, there is a large reddish-brown patch on sepals and petals. While, *C. parishii*, *C. extinctoriformis* have reddish or purple sports. In contrast, *C. rodiguezii*, *C. usneoides*, *C. exuperei* have no any marking in the sepals and petals. The sepals and petals of *Chiloschista* vary from surrounded, ovate, elliptic

oblong to oblong. Some species such as *C. lunifera* has petals varied from oblong to rounded. While, *C. rodiguezii* has oblong and slight falcate petals. The color pattern of sepals and petals is a taxonomic character showing without any marking, spots and a blotch state. Seidenfaden (1988) used the characteristic of petal margin with sparsely cilliate as a taxonomic character. In this study showed that in *C. viridiflava* has cilliate or without, while of *C. parishii* is always cilliate. The labellum of the *Chiloschista* is concave to saccate to conical shape.

The labellum is upright and articulate with column foot. Their shapes are usually saccate with round tip hypochile. Exceptionally, in *C. extinctoriformis* has a conical and narrow tip hypochile and *C. rodiguezii* has a short cylindrical and obtuse tip. The epichile is 3 lobes consisting of 2 side lobes and a mid-lobe. Their side lobes are usually oblong. The mid-lobes are usually very small, except *C. exuperei* with having elongate oblong mid-lobe with retuse apex.

The column or androgynostemium of *Chiloschista* is a typical type of Epidendroid orchid, consisting of one incumbent anther and a stigma cavity below the anther. The pollinarium compose of 2 globular pollinia, which one is divided into 2 halves, one a litter smaller than another one, transparency strip and visidium.

Fruit of *Chiloschista* is an elongate capsule and 6-ridged. Inside of its capsule contains several thousand dust like seeds. However, the fruit specimens are difficult to identify. Thus, it is hard to indicate the morphological detail of each species.

Chiloschista can be recognized by being a leafless epiphytic orchid and the labellum erect and saccate with distinctly side lobes. The vegetative morphology of *Chiloschista* like the genus *Taeniophyllum* Blume in form of leafless epiphytic orchid. However, its flower quite different from the later genus.

Figure 4.1 *Chiloschista* habit: *Chiloschista viridiflava* Seidenf., showing its habit.

Figure 4.2 Morphology of *Chiloschista* flower.

4.1.2 Taxonomic treatment

***Chiloschista* Lindl.** Edwardes' s Bot. Reg. 18, sub. T. 1522. 1832; M. D. Dassanayake., Fl. Ceylon.: 188. 1981; Seidenf., Opera Bot. 95: 168. 1988; J. B. Comber., Orchids Java: 301. 1990; Seidenf., Opera Bot. 114: 409. 1992; Seidenf. & J.J. Wood, Orchids Penins. Malaysia Singapore: 655. 1992; Pridgeon et al., Gen. Orchid. 6: 152. 2014; N. Pearce & P. J. Cribb., Fl. Bhutan 3(3): 503. 2002; S. C. Chen et al. In Z. Y. Wu et al. (eds), Fl. China 25: 470. 2009. Type specimens: *Chiloschista usneoides* (D.Don) Lindl.—basionym: *Epidendrum usneoides* D.Don.

Epiphytic or lithophytic herbs, stem very short or indistinct, erect with commonly leafless, rarely with small leaves during vegetation period; roots flattened, ribbon- like or subcylindrical, grey- green, photosynthetic. *Leaves* (when present) oblong-elliptic to ligulate, obliquely subacute or unequally bilobed, sometime slightly falcate, articulate. *Inflorescence* lateral, racemose, rarely paniculate, erect or pendent, (few-)many-flowered, usually tomentose or rarely glabrous; floral bracts triangular or ovate-lanceolate. *Flowers* resupinate, mild to rather strong fragrant, white, greenish yellow to yellow, with or without reddish or purple spots or a large patch. *Sepals* and *petals* free, subequal, oblong-ovate, obovate, oblanceolate or oblong-elliptic, lateral sepals and petals usually laterally adnate to column foot by a broad base. *Labellum* clawed, articulate to apex of column foot, motile trilobed, basal portion concave or saccate, with an erect or ascending, entire or weakly bilobed, pilose with short and thick hairs callus on inner surface of posterior wall; side lobe erect; mid-lobe usually common abbreviated, transverse, entire or rarely bilobed, disc often pilose. *Column* semi-terete, foot perpendicular to column, broad, at least twice as long as column; clinandrium shallowly excavate, with a membranous posterior margin. *Anther cap* provided on either side with a long- filiform, glandular- tipped appendage, or appendages reduced to a small tooth pollinia four, waxy, stipe sublinear, visidium small; stigma transverse, deeply set; rostellum deflexed, shortly bifid after removal of pollinarium. *Capsule* cylindric with narrow at both ends, ridged; seeds dust-like.

Key to species

1 Labellum with a rounded pouch

2 Labellum midlobe distinct, oblong with emarginate apex.....2. *C. exuperei*

2 Labellum midlobe indistinct

3 Sepals and petals without any marking or with only faint marking at base of sepals and petals

4 Sepals and petal white or whitish yellow.....6. *C. usneoides*

4 Sepals and petals greenish yellow.....7. *C. viridiflava*

3 Sepals and petals with many reddish brown or purple spots or reddish a brown blotch

5 Sepals and petals with reddish brown or purple spots.....4. *C. parishii*

5 Sepals and petals with a reddish brown blotch.....3. *C. lunifera*

1 Labellum with a conical and short tubular pouch

6 Labellum with a conical pouch. Petals rounded.....1. *C. extinctoriformis*

6 Labellum with a short tubular-conical pouch. Petals oblong.....5. *C. rodriguezii*

1. *Chiloschista extinctoriformis* Seidenf., Opera Bot. 95: 178. 1988. Type specimen: *Kerr* 330 (holotype-K!) (Figure 4.3-4.4).

Epiphytic herb; stem very short, erect, simple. *Roots* silvery-green flattened, 1.5–2 mm wide, appressed to the host tree bark. *Inflorescence* axillary, pendulous raceme 10-12 cm long, arising near the stem apex; scape and rachis dull pale green, densely tomentose; scape 3.3-3.5 cm long, terete, with 3–4 distant triangular whitish scarious sterile bracts 2–3 mm long, 0.5–1 mm wide; rachis slightly zigzag, with 5–8 lax, spirally arranged flowers. *Flowers* widely opening, 1.1–1.2 cm across; sepals and petals whitish cream, with many brown spots; lip white to cream; base of side lobes with yellow base (inside); column whitish, column foot with dark purple variegated base; anther cap pale yellow; pollinia dull yellowish; pedicel and ovary terete, dull olive-green, straight or slightly curved, 2.5-3 mm long, densely tomentose with short hairs; floral bracts whitish, scarious, triangular, acute, 1.2–1.5 mm long, *ca.* 1.2 mm wide. *Dorsal sepal* broadly obovate to almost round, 5-6 mm long, 4.3-4.5 mm,

recurved, rounded apex; entire not ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Lateral sepals* broad elliptic, elliptic oblong, slightly oblique, 5–5.5 mm long, 3.5–4 mm wide, spreading, rounded at apex, entire not ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Petals* broadly ovate or ovate-oblong, 4.8–5 mm long, 3.5-4 mm wide, rounded at apex, entire with sparsely ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Labellum* erect, 2.2-2 mm long, 1.8-2 mm wide, 3.8-4 mm high, movably attached to the column foot apex, obscurely divided into a saccate hypochile and 3-lobbed epichile; hypochile concave to conical with narrow tip, 1.7-1.8 mm long, 1.8-2 mm wide, with a longitudinal median keel covered by thick and short hairs and dense longer thin hairs at apex of the keel; side lobes oblong, 3-3.2 mm long, 1.2-1.5 mm, erect, obtuse with entire margin; mid-lobe very small, *ca.* 0.5 mm long, *ca.* 1.5 mm wide. *Column ca.* 5 mm long including column foot; stigma concave, transversely lunate. *Anther cap* simple, helmet-shaped, *ca.* 1 mm long and wide, at front with broadly triangular, obtuse, 0.2–0.3 mm long, with thread like appendages *ca.* 1.3 mm long on each side; pollinia 2, globular, *ca.* 0.5 mm in diameter, each almost completely split into 2 unequal hemispheric halves; stipe (tegula) in the form of simple, oblong-lanceolate, *ca.* 1 mm long; viscidium attached distally, simple thin plate, wide oblong, 0.3 mm long, 0.2 mm wide. *Capsule* unknown.

Vernacular name: Ueang Phaya Raibai Singkhon (เอื้องพญาไร้ใบสิงขร)

Ecology: On tree trunk or branch in limestone forest, alt. 600-800 m. Flowering: April to May.

Distribution: Endemic to Thailand.

Specimens examined: SOUTH-WESRERN: Prachuap Khiri khan, Khao Luang, *Kerr* 330 (K!); Sinkhorn border market, 7.04.2017, *T. Pingyot, W. La-Ongsri, P. Tatiya & S. Satatha* 95 (QBG!); PENINSULAR: Phangnga, Sra Nang Mano Rah, *S. Watthana* and *S. Pumicong* 2031 (QBG!).

Conservation status in Thailand: Vulnerable (D2) In Thailand, it was found only two population in Prachuap Khiri Khan and Phang Nga Province. The local people who live near the population in Phang Nga inform that there are less than 500 mature individuals. While there is not any information about the population in Prachuap Khiri

Khan. So far, this species is an endemic species to Thailand. This species has been collected to sell in a wild plant market (Figure 4.5).

Notes: This species can be distinguished by having a narrow conical spur.

2. *Chiloschista exuperei* (Guillaumin) Garey, Bot. Mus. Leafl. 23,2: 166. 1972; Seidenf., Contr. Rev. Orchid Fl. Cambodia Laos Vietnam 25: 215. 1975; Opera Bot. 95: 171. 1988; Opera Bot. 114: 410. 1992.—*Taeniophyllum exuperei* Guillaumin, Bull. Mus. Natl. Hist. Nat. sér. 2, 29: 346. 1957. Type specimen: *Exupere* 70 (holotype-P! picture www.plant .jstore.org) (Figures 4.6-4.7).

Epiphytic herb; stem very short, erect, simple. *Roots* many, silvery- green flattened, 1.5 – 2 mm wide, addressed to the host tree bark. *Inflorescence* axillary, pendulous raceme 4.5-5 cm long, arising near the stem apex; scape and rachis dull pale green, densely tomentose; scape *ca.* 3 cm long, terete, with 3–4 distant triangular whitish scarious sterile bracts 2–3 mm long, 0.5–1 mm wide; rachis rather straight, with 7–8 lax, spirally arranged flowers. *Flowers* widely opening, *ca.* 1 cm across; sepals and petals whitish cream, without any marking; lip white to cream; side lobes cream, its base with yellow base (inside) ; column whitish, column foot with dark purple variegated base; anther cap pale yellow; pollinia dull yellowish; pedicel and ovary terete, dull olive-green, straight or slightly curved, 2.5-3 mm long, densely tomentose with short hairs; floral bracts whitish, scarious, triangular, acute, *ca.* 2.5 mm long, *ca.* 1 mm wide. *Dorsal sepal* broadly obovate to almost round, *ca.* 4.7 mm long, *ca.* 4.5 mm, recurved, rounded apex; entire not ciliate margin, abaxially densely hairy, adaxially sparsely hairy. *Lateral sepals* oblong, slightly oblique, 5–5.5 mm long, 3–3.5 mm wide, spreading, rounded at apex, entire not ciliate margin, abaxially and adaxially sparsely hairy. *Petals* oblong, 4.3-4.5 mm long, 2.3-2.5 mm wide, rounded at apex, entire with sparsely ciliate margin, abaxially and adaxially sparsely hairy. *Labellum* erect, 4-4.5 mm long, 3.3-3.5 mm wide, 3.8-4 mm high, movably attached to the column foot apex, obscurely divided into a saccate hypochile and 3-lobbed epichile; hypochile concave to short saccate with rounded tip, *ca.* 3 mm long, *ca* 2.3 mm wide, with a longitudinal median keel covered by thick and short hairs and dense longer thin

hairs at apex of the keel; side lobes oblong, 2.8-3 mm long, 1.8-2 mm, erect, obtuse with finely incised at margin; mid-lobe distinctly extended, oblong, 2.0-3.0 mm long, 1-1.5 mm wide, apex retuse. *Column* 3-4 mm long including column foot; stigma concave, transversely lunate. *Anther cap* simple, helmet-shaped, *ca.* 0.7 mm long, *ca.* 1 mm wide, at front with broadly triangular, obtuse, *ca.* 0.2 mm long, without thread like appendage; pollinia 2, globular, *ca.* 0.5 mm in diameter, each almost completely split into 2 unequal hemispheric halves; stipe (tegula) in the form of simple, oblong-lanceolate, *ca.* 1 mm long; viscidium attached distally, simple thin plate, quadrate, 0.2 mm long and wide. *Capsule* unknown.

Vernacular name: Ueang Phaya Raibai Pakpet (เอื้องพญาไร้ใบปากเป็ด)

Ecology: In hill evergreen forest, alt. 750-900 m. Flowering: March-April.

Distribution: South China, Laos, Vietnam, Thailand, Cambodia and Malaysia.

Specimens examined: NORTHERN: Chiang Mai, Pa Pae, Mae Taeng District, elevation 829 m., 23.05.2015, *S. Watthana & P. Momkaew* 4301 (QBG!). *Ibid.,* 18.02.64, *T.Chanokkhun* 295 (QBG!); Doi Lang, Mae Aye, *T.Chanokkhun* 479 (QBG!); Ibid., NC *G. Seidenfaden & T. Smitinand* 9610-14 (C); NORTH-EASTERN: Phetchabun, Nam Nao National Park, 750 m., *G. Seidenfaden & T. Smitinand* 8772 (C!); *Ibid*., *G. Seidenfaden & T. Smitinand* 8779 (C!).

Conservation status in Thailand: Vulnerable (D2). In Thailand, it was found in three localities in Chiang Mai and Petchabun. Available mapping showed Extent of occurrence (EOO) as 24,985 Km^2. From my field observation in 2022, in Doi Lang, Mae Aye, Chiang Mai Province, I visually estimated that there were less than 500 mature individuals. However, this orchid usually thrived on branch of Tea branches which may be cut in the future by famer. Thus, there is a risk to reduce the population. Like the population in Pa Pae, they live on Tea's branches. I estimated that there are mature individuals less than 250 mature individuals (Figure 4.8).

Notes: This species can be distinguished by having oblong with bi-lobes apex of labellum.

3. *Chiloschista lunifera* (Rchb.f.) J.J.Sm., Orch. Java 553. 1905; A.D.Kerr, Nat. Hist. Bull. Siam Soc. 23, 1-2: 204. 1969; Seidenf., Bull. Mus. Hist. Nat. (Paris) 3. s. 71, Bot. 5: 113. 1973; Contr. Rev. Orchid Fl. Cambodia Laos Vietnam 1: 25. 1975; Opera Bot. 114: 411. 1992. — *Thrixspermum luniferum* Rchb. f., Gard. Chron. 1868: 786. 1868. — *Sarcochilus lunifer* (Rchb. f.) Benth. ex Hook. f., Bot. Mag. 115: t. 7044. 1889. Type specimen: *Reichenbach* 41550b (lectotype-WU, selected by Seidenfaden, 1988)

Chiloschista indica J.J.Sm., Bull. Jard. Bot. Buitenzorg, ser. 2, 10: 101. 1913, nom. superfl. Type specimen: not indicated.

Chiloschista ramifera Seidenf., 95: 179. 1988. Type specimen: Cumberlege s.n. (holotype-C!) (Figure 4.9-4.10).

Epiphytic herb; stem very short, erect, simple. *Roots* many, silvery-green flattened, 1.5–2 mm wide, addressed to the host tree bark. *Inflorescence* axillary, pendulous raceme 7-20 cm long, arising near the stem apex; scape and rachis dull pale green to dull greenish purple, densely tomentose; scape 2.3-8 cm long, terete, with 3–4 distant triangular whitish scarious sterile bracts 2–3 mm long, 0.5–1 mm wide; rachis rather straight to zigzag, with 7–8 lax, spirally arranged flowers. *Flowers* widely opening, 1-1.2 cm across; sepals and petals whitish cream, with a large dark reddish brown blotch; lip white to cream tinct with orange; side lobes white to cream with orange patch at base, its base with yellow base (inside); column whitish, column foot with dark purple variegated base; anther cap pale yellow; pollinia dull yellowish; pedicel and ovary terete, dull olive-green, straight or slightly curved, 4-4.5 mm long, densely tomentose with short hairs; floral bracts whitish, scarious, triangular, acute, *ca.* 1.5 mm long, *ca.* 1.2 mm wide. *Dorsal sepal* broadly obovate to almost round, 5-6 mm long, 3.5-4 mm, recurved, rounded apex; entire or sparsely ciliate margin, abaxially densely hairy, adaxially glabrous. *Lateral sepals* ovate, rounded or elliptic-oblong, slightly oblique, 5-5.2 mm long, 3.2–3.5 mm wide, spreading, rounded at apex, entire not ciliate margin, abaxially densely hairy, adaxially glabrous. *Petals* broad ovate to oblong, 5-5.5 mm long, 3.3-3.5 mm wide, rounded to truncate at apex, entire with sparsely ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Labellum* erect, 2-2.2 mm long, 1.8-2.0 mm wide, 4-5 mm high, movably attached to the column foot

apex, obscurely divided into a saccate hypochile and 3-lobbed epichile; hypochile concave to saccacte with rounded tip, 1-1.2 mm long, 1.8-2 mm wide, with a longitudinal median keel covered by thick and short hairs thoroughly or upper half of the keel; side lobes oblong, 3.5-4 mm long, 2-2.5 mm, erect, obtuse with entire margin; mid-lobe very short, *ca.* 0.5 mm long, *ca.* 1 mm wide. *Column* 4.5-5 mm long including column foot; stigma concave, transversely lunate. *Anther cap* simple, helmet-shaped, *ca.* 1 mm long and wide, at front with broadly triangular, obtuse, *ca.* 0.2 mm long, with thread like appendages 1.5-1.8 mm long on each side; pollinia 2, globular, *ca.* 0.5 mm in diameter, each almost completely split into 2 unequal hemispheric halves; stipe (tegula) in the form of simple, oblong-lanceolate, *ca.* 0.7 mm long; viscidium attached distally, simple thin plate, quadrate, 0.3 mm long and wide. *Capsule* unknown.

Vernacular name: Ueang Phaya Raibai Dokdaeng (เอื้องพญาไร้ใบดอกแดง)

Ecology: On tree trunk or branches in dry dipterocarp forest or mixed deciduous forest, at alt. 600-1,000 m. Flowering: March-April.

Distribution: Assam, East Himalaya, Nepal, Laos, Myanmar, Thailand, Vietnam

Specimens examined: NORTHERN: Chiang Mai, Doi Chiang Dao Animal Sanctuary, 29.03.1995, *J. F. Maxwell* 95-272 (CMU!), Tak, Ban Tha Nuea, Tha Nuea Sub-district, Mae On District, *T.Chanokkhun* 476 (QBG!), Mae Hong Son, NW of Pang Mapha, elevation 600 m., *G. Seidenfaden & T. Smitinand* 7101 (C!); *Ibid.*, *G. Seidenfaden & T. Smitinand* 7171 (C), *G. Seidenfaden & T. Smitinand* 7194 (C), *G. Seidenfaden & T. Smitinand* 7195 (C); EASTERN: Nakhon Ratchasima, Pak Chong, elevation 350 m., 03.1964, *Cumberlege* 522 (K!), *Ibid.*, elevation 350 m., 13.05.1966, *G. Seidenfaden & T. Smitinand* 1362 (C!), Pak Chong, *Cumberlege s.n.* (C!); CENTRAL: Saraburi, Sahm Lahn, 125 m., 20.03.1974, *Maxwell* 74-216 (BK!).

Conservation status in Thailand: Near Threatened. In Thailand, the EOO is 35,321 km^2 based on available information which is not matched with any criteria of threatened categories (CR, EN and VU) However, this species is popular for ornamental purpose, so it may risk of disappear from natural population (Figure 4.11).

Notes: When Reichenbach (1868) named this plant as *Thrixspermum luniferum* Rchb.f., he did not cite type specimen, just said that the specimen was in British Museum. Seidenfaden (1988) cited the specimen from Reichenbach herbarium 41550b and 41549 left hand sketches. Seidenfaden (1988) named *Chiloschista ramifera* Seidenf. and indicated that it closed to *C. lunifera* but having branching inflorescences. I found that the branching inflorescence is a variable character depended on their age. Thus, in this treatment I lumped *C. ramifera* Seidenf. as a new synonym of *C. lunifera*.

4. *Chiloschista parishii* Seidenf., Opera Bot. 95: 176. 1988; Opera Bot. 114: 411. 1992; Type specimen: *S.B.Parish* 55 (holotype-WU!).—*Thrixspermum luniferum* auct. non Rchb. f.: 1868; Rchb. f., Tr. Linn. Soc. 30: 136, 1874; Parish., Mason, f. (ed.), Burma, its people and production. 2: 199. 1883.—*Sarcochilus luniferus* auct. non (Rchb. f.) Benth. ex Hook.f., Bot. Mag. 115: t 7044. 1889 (quoad descr. and plant); King & Pantling., Ann. Roy. Bot. Gard. 8: 207, P1, 276, 1898; Biswas., Ind. Forest. Rec. Bot. 3, 1: 52, 1941; Banerji., Candollea. 19: 218, 1964; Mehra & Vij., Taxon. 19: 110, 1970.—*Chiloschista lunifera* auct. non (Rchb. f.) J.J.Sm., Die Orchideen von Ambon.: 1905; Diels & Mansfeld., Dahl. no. 106. 11: 497. 1932; Holttum., Kew. Bull. 14: 273. 1960; Senghas., Die Orchidee. 13, 3: 99. 1962, Figure 1-2; Seidenf. & Smitinand., Orchids Thailand: 539. 1963, Figure 401; 821. 1965; Tuyama., Univ. Mus. Univ. 2: 181, 1971; Banerji & Thapa., J. Bomb. Nat. Hist. Soc. 70, 1: 28. 1973; Pradhan., Guide to identification and culture 2: 505, 1979; Rao & Deori., J. Ind. For. 3, 3-4: 258, 1980; Barthlott & Ziegler., Ber. Deutsch. Bot. Ges. 93: 391. 1980; Bechtel et al., The manual of cultivated orchid species.: 87. 1981; Banerji & Pradhan., The orchids of Nepal Himalaya.: 484. 1984; Misra., Biology, Conservation, and Culture of Orchids.: 315. 1986 (Figure 4.12-4.13).

Epiphytic herb; stem very short, erect, simple. *Roots* many, silvery-green flattened, 1.5–2 mm wide, addressed to the host tree bark. *Inflorescence* axillary, pendulous raceme 7-15 cm long, arising near the stem apex; scape and rachis dull pale green, densely tomentose; scape 1-2 cm long, terete, with 3–4 distant triangular whitish scarious sterile bracts 1.5–2.5 mm long, 1-2.5 mm wide; rachis rather straight, with 7–8 lax, spirally arranged flowers. *Flowers* widely opening, 1-1.3 cm across; sepals

and petals greenish yellow or yellow, with many reddish brown spots; lip white to cream; side lobes yellowish white, its base with reddish brown base (inside); column whitish, column foot with dark purple variegated base; anther cap pale yellow; pollinia dull yellowish; pedicel and ovary terete, dull olive-green, straight or slightly curved, 4-4.5 mm long, densely tomentose with short hairs; floral bracts whitish, scarious, triangular, acute, 2-2.5 mm long and wide. *Dorsal sepal* obovate to oblanceolate, 5-6 mm long, 3-4 mm, recurved, rounded apex; entire not ciliate margin, abaxially densely hairy, adaxially glabrous. *Lateral sepals* lanceolate-oblong, (not)-slightly oblique, 5–5.5 mm long, 3–3.5 mm wide, spreading, rounded at apex, entire not ciliate margin, abaxially and adaxially glabrous. *Petals* oblong, 5-5.5 mm long, 3.5-4 mm wide, rounded at apex or subtruncate, entire with sparsely ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Labellum* erect, 2.3-2.5 mm long, 1.8-2.0 mm wide, 5-5.5 mm high, movably attached to the column foot apex, obscurely divided into a saccate hypochile and 3-lobbed epichile; hypochile concave to saccate with rounded tip, 1-1.2 mm long, 1.8-2 mm wide, apex slightly grooved and obtuse, with a longitudinal median keel covered by thick and short hairs through or half of the keel; side lobes oblong, 2.5-3 mm long, 1.8-2 mm, erect, obtuse with entire margin; mid-lobe very short, *ca.* 0.5 mm long, *ca.* 1.5 mm wide. *Column* 4.5-5 mm long including column foot; stigma concave, transversely lunate. *Anther cap* simple, helmet-shaped, *ca.* 1.5 mm long, *ca.* 2 mm wide, at front with broadly triangular, obtuse, *ca.* 0.2 mm long, with thread like appendages 1.5-2 mm long on each side; pollinia 2, globular, *ca.* 0.5 mm in diameter, each almost completely split into 2 unequal hemispheric halves; stipe (tegula) in the form of simple, oblong-lanceolate, 1-1.3 mm long; viscidium attached distally, simple thin plate, wide quadrate, 0.5 mm long and wide. *Capsule* unknown.

Vernacular name: Ueang Phaya Raibai Judpra (เอื้องพญาไร้ใบจุดประ)

Ecology: On tree or branches in mixed deciduous forest or dry evergreen forest, alt. 500-1,500 m. Flowering: March-April.

Distribution: Andaman Is., Assam, Bangladesh, East Himalaya, India, Laos, Myanmar, Nepal, Thailand, Vietnam.

Specimens examined: NORTHERN: Chiang Mai, Fang District, *G. Seidenfaden & T. Smitinand* 2096 (QBG!), Chiang Dao District, 24.04.2022, *T.Chanokkhun* 480 (QBG!), Tha Ton Sub-district, Mae Ai District, 24.04.2022, *T.Chanokkhun* 489 (QBG!), Mae Chaem, 600 m., *Kerr s.n.* (K), Doi Suthep, 1,100 ft., *Kerr* 130 (K), Doi Saket, *G. Seidenfaden & T. Smitinand* 7403 (C), Chiang Rai province, Chiang Saen District, *T.Chanokkhun* 461 (QBG!), Mae Sai District, 29.04.2022, *T.Chanokkhun* 513 (QBG!), Pa Teung Sub-district, Mae Chan District, 24.04.2022, *T.Chanokkhun* 488 (QBG!), Lampang, Huai Mae Tam, 1,400 ft., *Kerr* 130b (K), Tak, Ban Tham Suea, Phra That Pha Daeng Subdistrict, Mae Sot, elevation500 m., 10.03.2011, *S. Watthana* 3708 (QBG!), Mae Ramat, *G. Seidenfaden & T. Smitinand* 7885 (K), Phitsanulok, trial to Nam Dam, Ban Rom Klao, Chattrakaan, elevation 100 m., 05. 30. 2006, *P. Suksathan* 3854 (QBG!); NORTH- EASTERN: Loie, Wang Saphung, *T. Smitinand* 441 (BKF), Phethabun, Heaw Sai waterfall, Nam Nao, 12.05.2012, *S. Watthana* 3426 (QBG!); EASTERN: Chayaphum, Nong Bua Daeng, 300 m., *Kerr* 0931 (K); Ibid., *Kerr s.n.* (K), Nakhon Ratchasima, Khao Yai, *Cumberlege s.n.* (BKF); Pak Chong, *Cumberlege.* 1362 (C); WESTERN: Kanchanaburi, Sri Sawat, 01. 04. 1975, *Sornsuay, Sakarin & Aditep* 153 (BKF); SOUTHERN: Satun, Thung Nui, elevation 50 m., 29.01.1961, *T. Smitinand* 7096 (BKF!).

Conservation status in Thailand: Least Concern. This species is so common in Thailand. They have been found more than 20 localities. Each population were usually many individuals (Figure 4.14).

Notes: Dalström & Kolan (2020) named a specimen collected from Kanchanaburi Province as *C. lindstroemii* Dalström & Kolan indicated that its feature closed to *C. parishii* but having large size of the flower. From my field observation, I found that it is a variable. However, I have not seen type specimens, *A.Lindstrom* 90-1571 (holotype-BK not seen). It needs further study.

5. *Chiloschista rodiguezii* Casvestro & Ormerod, Orchidophile (Asnieres) 166: 180, Figure 179-183, 2005. Type specimen: Thailand, Northern, Mae Hong Son, 500 m alt. September 2003. *W. Cavestro* s.n. (holotype-LY) (Figure 4.15-4.16).

Epiphytic herb; stem very short, erect, simple. *Roots* many, silvery- green flattened, 1.5–2 mm wide, addressed to the host tree bark. *Inflorescence* axillary,

pendulous raceme 8.5-10 cm long, arising near the stem apex; scape and rachis dull pale green, densely tomentose; scape 2.5-3 cm long, terete, with 3–4 distant triangular whitish scarious sterile bracts 2-2.2 mm long, 1-1.5 mm wide; rachis rather straight, with 7–8 lax, spirally arranged flowers. *Flowers* widely opening, 0.8-1 cm across; sepals and petals white to cream, without any marking; lip cream to yellow; side lobes yellow, its base with reddish brown tinct at base (inside); column whitish yellow, column foot with dark purple variegated base; anther cap (pale)-yellow; pollinia dull yellowish; pedicel and ovary terete, dull olive-green, straight or slightly curved, 2.8-3 mm long, densely tomentose with short hairs; floral bracts whitish, scarious, triangular, acute, *ca.* 1.5 mm long, *ca.* 1.2 mm. *Dorsal sepal* oblanceolate, 3.5-4 mm long, 2-2.5 mm, recurved, rounded apex; entire with sparsely ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Lateral sepals* oblanceolate or obovate, slightly oblique, 3.5–4 mm long, 3.2–3.5 mm wide, spreading, rounded at apex, entire with sparsely ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Petals* oblong slightly falcate, 3.5-4.0 mm long, 2.0-3.5 mm wide, subtrancate at apex, entire with sparsely ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Labellum* erect, *ca.* 2 mm long, *ca.* 2.5 mm wide, 3 mm high, movably attached to the column foot apex, obscurely divided into a saccate hypochile and 3-lobbed epichile; hypochile concave, cylindrical conical with obtuse and tip, *ca.* 2 mm long, *ca* 1.5 mm wide, with a longitudinal median keel covered by thick and short hairs through the keel; side lobes oblique triangular-oblong, 1-1.5 mm long, 1 mm, erect, subacute; mid-lobe very small, *ca.* 0.5 mm long, *ca.* 1 mm wide. *Column* 3-3.5 mm long including column foot; stigma concave, transversely lunate. *Anther cap* simple, helmet-shaped, *ca.* 1 mm long, *ca.* 1.3 mm wide, at front with broadly triangular, obtuse, *ca.* 0.1 mm long, with thread like appendages *ca.* 0.5 mm long on each side; pollinia 2, globular, *ca.* 0.3 mm in diameter, each almost completely split into 2 unequal hemispheric halves; stipe (tegula) in the form of simple, oblong-lanceolate, *ca.* 0.4 mm long; viscidium attached distally, simple thin plate, oblong, 0.4 mm long and 0.2 mm wide. *Capsule* unknown.

Vernacular name: Ueang Phaya Raibai Khangyound (เอื้องพญาไร้ใบคางยาว)

Ecology: On tree or branches in hill evergreen forest, alt. 800- 1,000 m. Flowering: April-May.

Distribution: Endemic to Thailand.

Specimens examined: NORTHERN: Mae Hong Son, Ban Den, Wat Chan, elevation 1,298 m., 29.04.2014, *M. Norseangsri* 10950 (QBG!), Ban Huai Phu Loei, elevation 1,063 m., 29.04.2014, *M. Norseangsri* 10989 (QBG!), Doi Pui, Huai Hi, elevation 1,544 m., 16.12.2016, *S. Watthana* 2605 (QBG!), Pang Uung, 19.02.2021, *T.Chanokkhun* 326 (QBG!), Pang Tong, 19. 02. 2021, *T. Chanokkhun* 313 (QBG!), Pang Mapha, *T.Chanokkhun* 477 (QBG!), Chiang Mai, Doi Maonlan, Phrao District, *T.Chanokkhun* 496 (QBG!).

Conservation status in Thailand: Vulnerable B1ac(iii,iv). This species has a narrow distribution, found only Northern Thailand with 8,698 Km^2 and found only 7 populations. Each population usually has a low number of mature plants (Figure 4.17).

Notes: This species is easily recognized by having tubular- conical pouch labellum.

6. *Chiloschista usenoides* (D.Don) Lindl., Edwards's Bot. Reg. 18: t. 1522. 1832; Wall. Cat. no. 7330. 1832, nom**.** nud**.**; Bot. Reg. 18: sub T.1522, 1832; 219. 1833; Sert. Orch. Frontesp.: 1840, Figure 4. – Gard. Chron.: 135. 1846; Lindley., J. Linn. Soc. 3: 43, 1859; Diels & Mansfeld., Dahl. no. 106. 11: 496. 1932; Holttum., Kew. Bull. 14: 273. 1960; Ghose., Orch. Rev. 78: 296. 1970; Hara et al., An enumeration of the flowering plants of Nepal.: 35. 1978; Banerji & Thapa., J. Bomb. Nat. Hist. Soc. 70, 1: 28. 1973; Pradhan., Guide to identification and culture 2: 505, 1979; Seidenf. & Arora., Nord. J. Bot. 2: 11, 1982; Banerji & Pradhan., The orchids of Nepal Himalaya.: 484. 1984; Deva & Naithani., The orchid flora of North West Himalaya.: 365. 1986, Figure 208. — *Epidendrum suneoides* D.Fon in Prodr. Fl. Nepal.: 37. 1825.—*Sarcochilus usneoides* (D.Don) Rchb.f. in W.G.Walpers, Ann. Bot. Syst. 6: 497. 1863.—*Thixspermum usneoides* (D.Don) Rchb.f. in Xenia Orchid. 2; 120. 1867. Type specimen: *Wallich* 7330 (neotype-K! selected by Seidenfaden 1988) (Figure 4.18-4.19).

Epiphytic herb; stem very short, erect, simple. *Roots* many, silvery-green flattened, 1.5–2 mm wide, addressed to the host tree bark. *Inflorescence* axillary, pendulous raceme 13-30 cm long, arising near the stem apex; scape and rachis dull pale green, densely tomentose; scape 2.3-8 cm long, terete, with 3–4 distant triangular whitish scarious sterile bracts 2–2.5 mm long, 1-1.5 mm wide; rachis rather straight, with 7–8 lax, spirally arranged flowers. *Flowers* widely opening, 1-1.2 cm across; sepals and petals whitish cream, without any marking or tinct with pale purple at base; lip yellowish white or white; side lobes cream, its base with yellow base (inside); column whitish, column foot with dark purple variegated base; anther cap pale yellow; pollinia dull yellowish; pedicel and ovary terete, dull olive-green, straight or slightly curved, 4-4.5 mm long, densely tomentose with short hairs; floral bracts whitish, scarious, triangular, acute, 1.8-3 mm long, 1-2 mm. *Dorsal sepal* oblanceolate, 5-7 mm long, 3.5-4 mm, recurved, rounded apex; entire not ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Lateral sepals* oblanceolate or obovate, slightly oblique, 5–7 mm long, 3.5–4 mm wide, spreading, rounded at apex, entire not ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Petals* ovate almost rounded or lanceolate oblong, 6-6.2 mm long, 3.7-4.2 mm wide, rounded at apex, entire without ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Labellum* erect, 2.2-2.6 mm long, 2.3-2.5 mm wide, 4.5 mm high, movably attached to the column foot apex, obscurely divided into a saccate hypochile and 3-lobbed epichile; hypochile concave to saccate with rounded and grooved tip, 2-3 mm long, 2.3-2.5 mm wide, with a longitudinal median keel covered by thick and short hairs about half of the keel; side lobes oblong, *ca.* 2 mm long, 2-3 mm, slightly recurved; mid-lobe very small, *ca.* 0.5 mm long, *ca.* 1 mm wide. *Column* 4.5-5 mm long including column foot; stigma concave, transversely lunate. *Anther cap* simple, helmet-shaped, 1-1.7 mm long, 1-2 mm wide, at front with broadly triangular, obtuse, *ca.* 0.2 mm long, with thread like appendages 0.6-1.2 mm long on each side; pollinia 2, globular, *ca.* 0.5 mm in diameter, each almost completely split into 2 unequal hemispheric halves; stipe (tegula) in the form of simple, oblong-lanceolate, *ca.* 1 mm long; viscidium attached distally, simple thin plate, quadrate, 0.5 mm long and wide. *Capsule* unknown.

Vernacular name: Ueang Phaya Raibai (เอื้องพญาไร้ใบ)

Ecology: On tree trunk or branches in hill evergreen forest, alt. 800-1,200 m. Flowering: February-March.

Distribution: East Himalaya, Myanmar, Nepal, West Himalaya and Thailand.

Specimens examined: NORTHERN: Chiang Mai, Mae Taman, Chiang Dao, 21.25.2004, *Th. Wongprasert et al.* 045-15 (BKF), Sa Loung, Mae Rim, 01.04.2008, *A. Keratikorkol* 362 (QBG!), Mae Hong Son Province, Pang Oung, 19.04.2021, *T.Chanokkhun* 325 (QBG!); SOUTH-WESTERN: Phetchaburi, Kaeng Krachan National Park, Phanoen Thung, elevation 1,000 m., 29.03.2008, *S. Suddee* et al. 3727 (BKF!), Uthai Thani, Huay Kha Khaeng Wildlife Sanctuary, 09.04.2013, *P. Srisom* 21 (BKF!).

Conservation status in Thailand: Near Threatened. There are 6 populations from herbarium information, including the population from Doi Inthanon National Park (W. Phumsaringkharm facebook accessed on 13 March 2023). The EOO of this species in Thailand is 20,561 km^2, which does not match with threatened status but it has rather few populations (Figure 4.20).

Notes: Seidenfaden (1988) indicated that type specimen of *Epidendrum useneoides* G. Don may be disappeared, then he selected specimen of Wallich (Wall. 7330) as a neotype.

7. *Chiloschista viridiflava* Seidenf., Opera Bot. 95: 175. Type specimen: *G. Seidenfaden* & *T. Smitinand* 9616 (holotype-C!) (Figure 4.21-4.22).

Epiphytic herb; stem very short, erect, simple. *Roots* many, silvery-green flattened, 1–2 mm wide, addressed to the host tree bark. *Inflorescence* axillary, pendulous raceme 5-11 cm long, arising near the stem apex; scape and rachis dull pale green or dull purple, densely tomentose; scape 0.5-5 cm long, terete, with 3–4 distant triangular whitish scarious sterile bracts 2-2.5 mm long, 1-1.5 mm wide; rachis rather straight, with 7–8 lax to dense, spirally arranged flowers. *Flowers* widely opening, 1-1.4 cm across; sepals and petals greenish white or yellowish green, without any marking sometime faint with brown tinct; lip white to cream; side lobes cream, its base

with pale yellow base (inside); column whitish, column foot with dark purple variegated base; anther cap pale yellow; pollinia dull yellowish; pedicel and ovary terete, dull olive-green, straight or slightly curved, 2.5-5 mm long, densely tomentose with short hairs; floral bracts whitish, scarious, triangular, acute, 1.5-3 mm long, 1.5-2 mm. *Dorsal sepal* broadly obovate to almost round, 5-7 mm long, 3.5-.5 mm, recurved, rounded apex or subobtuse; entire without or ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Lateral sepals* obovate, 5.5–7 mm long, 4–4.5 mm wide, spreading, rounded to obtuse at apex, entire not ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Petals* obovate or ovate oblong, 5-6 mm long, 3-5-5 mm wide, rounded at apex, entire (without-)with sparsely ciliate margin, abaxially sparsely hairy, adaxially glabrous. *Labellum* erect, 1.5-2.5 mm long, 1.5-3 mm wide, 5-6.5 mm high, movably attached to the column foot apex, obscurely divided into a saccate hypochile and 3-lobbed epichile; hypochile concave to saccate with rounded tip, 2-3 mm long, 1.5-3 mm wide, with a longitudinal median keel covered by thick and short hairs along or almost of the keel; side lobes oblong, 3-3.5 mm long, 1.5-2 mm, erect or recurve, obtuse; mid-lobe very small, oblong, *ca.* 0.5 mm long, *ca.* 1 mm wide. *Column* 4-5 mm long including column foot; stigma concave, transversely lunate. *Anther cap* simple, helmet-shaped, 1.3-1.5 mm long, 1.4-1.5 mm wide, at front with broadly triangular, with thread like appendages 1.6-2.3 mm long on each side, obtuse, *ca.* 0.2 mm long; pollinia 2, globular, 0.4-0.5 mm in diameter, each almost completely split into 2 unequal hemispheric halves; stipe (tegula) in the form of simple, oblong-lanceolate, 1-1.5 mm long; viscidium attached distally, simple thin plate, quadrate, 0.5 mm long and wide. *Capsule* unknown.

Vernacular name: Ueang Phaya Raibai Dok Khiew (เอื้องพญาไร้ใบดอกเขียว)

Ecology: On tree or branches in mixed deciduous forest and dry dipterocarp forest, alt. 250-800 m. Flowering: March-May.

Distribution: Nepal and Thailand.

Specimens examined: NORTHERN: Chiang Mai, Doi Saked, 10. 04. 1987, *G. Seidenfaden & T. Smittinand* 9616 (C!), *Ibid., G. Seidenfaden & T. Smittinand* 9617-23 (C), Huai Bong, Tambon Ta Nuea, Mae on District, elevation 480 m., 30. 05. 2016,

W. La-ongsri, P. Panyachan, T. Pingyat & S. Satata 4869 (QBG!), Mae Sa elephant camp, Mae Rim, elevation 580 m., 01.06.2016, *W. La-ngsri, P. Panyachan, T. Pingyat & S. Satata* 4875 (QBG!), Suan Pha Luang Sankham Pang, Tambon Ta Nuea, Mae on District, elevation 480 m., 30.05.2016, *W. La-ongsri, P. Panyachan, T. Pingyat & S. Satata* 4868 (QBG!), *Ibid., T. Chanokkhun* 469 (QBG!), Mae Rim, Doi Sutep-Pui National Park, 03.05.1989, *J. F. Maxwell* 89-550 (BKF!), Phitsanulok, Plant brought from Khwae Noi, Flower at Rom Klao Garden, 31.03.2005, *S. Watthana* 1833 (BKF!); NORTH-EASTERN: Loei, Phu Kradueng National Park, elevation 316 m., K. Duangdee *s.n.* (BKF!), *Ibid.*, *K. Duangdee* 82 (BKF!); *Ibid.,* elevation 300 m., 14.06.1992, *C. Niyomdham* 2954 (BKF!); SOUTH-WESTERN: Phetchaburi, Kaeng Krachan National Park, Ban Krang Camp, elevation 403 m., 22.01.2011, *N. Toolmal, Ch. Chunmngoen & W. Somprasong* 73 (BKF!); Pong Phrom, 16.04.2008, *S. Ruksue* 81 (BKF!).

Conservation status in Thailand: Least Concern. This species is found many populations, more than 20. (Each population usually found many individuals (Figure 4.23).

Notes: This species can be recognized by having pure green sepals and petals but sometime with weak brown tinct at base of sepals and petals.

4.1.3 Discussion on taxonomic revision and conservation status

Based on morphological characteristic of the genus *Chiloschista* in Thailand, *Chiloschista exuperei*, *C. extinctoriformis* and *C. rodiguezii* have their unique characteristics and well separate from other species (see notes of each species). The group which has short saccate and round tip labellum is so difficult to separate into distinct species i.e., *C. lunifera, C. parishii*, *C. usneoides* and *C. viridiflava.* Only the color pattern can be used for taxonomic character. The shape and hairiness on margin of sepals and petals, presenting thread appendage of operculum, callus shape and hairiness on callus and mid-lobe shape which were proposed from previous authors are variable characters. Thus, the identification key to species which I provided forced

me to use the color pattern only in some group. It should be noted that with little taxonomic characters may led to classify Thai *Chiloschista* into varieties level. However, the phylogenetic analysis result (see below) based on ITS showed that each species proposed by Seidenfaden (1988) is well separated. I do strongly recommend that it needs more genes and samples which related the floral morphology for phylogenetic analysis.

For the conservation status of Thai *Chiloschista*, I followed the IUCN Standards and Petitions Committee's 2019 version of the Guidelines for Applying the IUCN Red List Categories and Criteria, Version 14, to evaluate the national level of each species. Previous reports by Chamchumroon, V., Suphuntee, N., Tetsana, N., Poopath, M. and Tanikkool, S. (2017) indicated that *C. extinctoriformis*, *C. ramifera* and *C. viridiflava* were endangered species (EN) but not indicated the criteria reason (Table 2.1). The authors seem to use the number of the occurrent population to evaluate the conservation status. From my evaluation based on the area of their distribution and population qualities, I found that *C. extinctoriformis*, *C. exuperei* and *C. rodriguezii* are vulnerable (VU) and indicated the criteria reason.

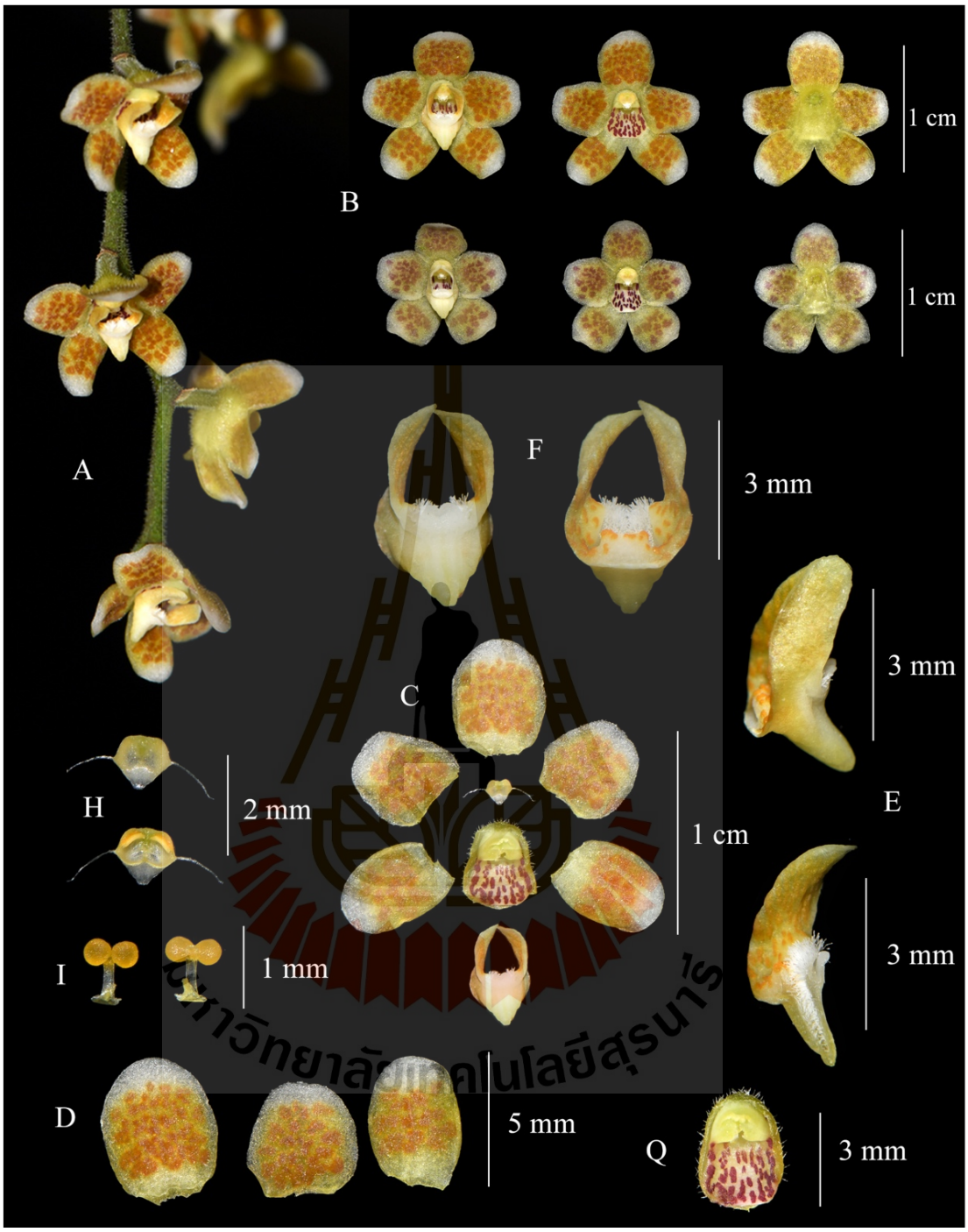

Figure 4.3 *Chiloschista extinctoriformis* Seidenf.: A. inflorescence, B. flowers showing with and without the labellum and front and back sides, C. dissected part of a flower, D. dorsal sepal, petal and lateral sepal from left to right, E. labellum side view and longitudinal half cut showing inside, F. column (Pictured by T.Chanokkhun).

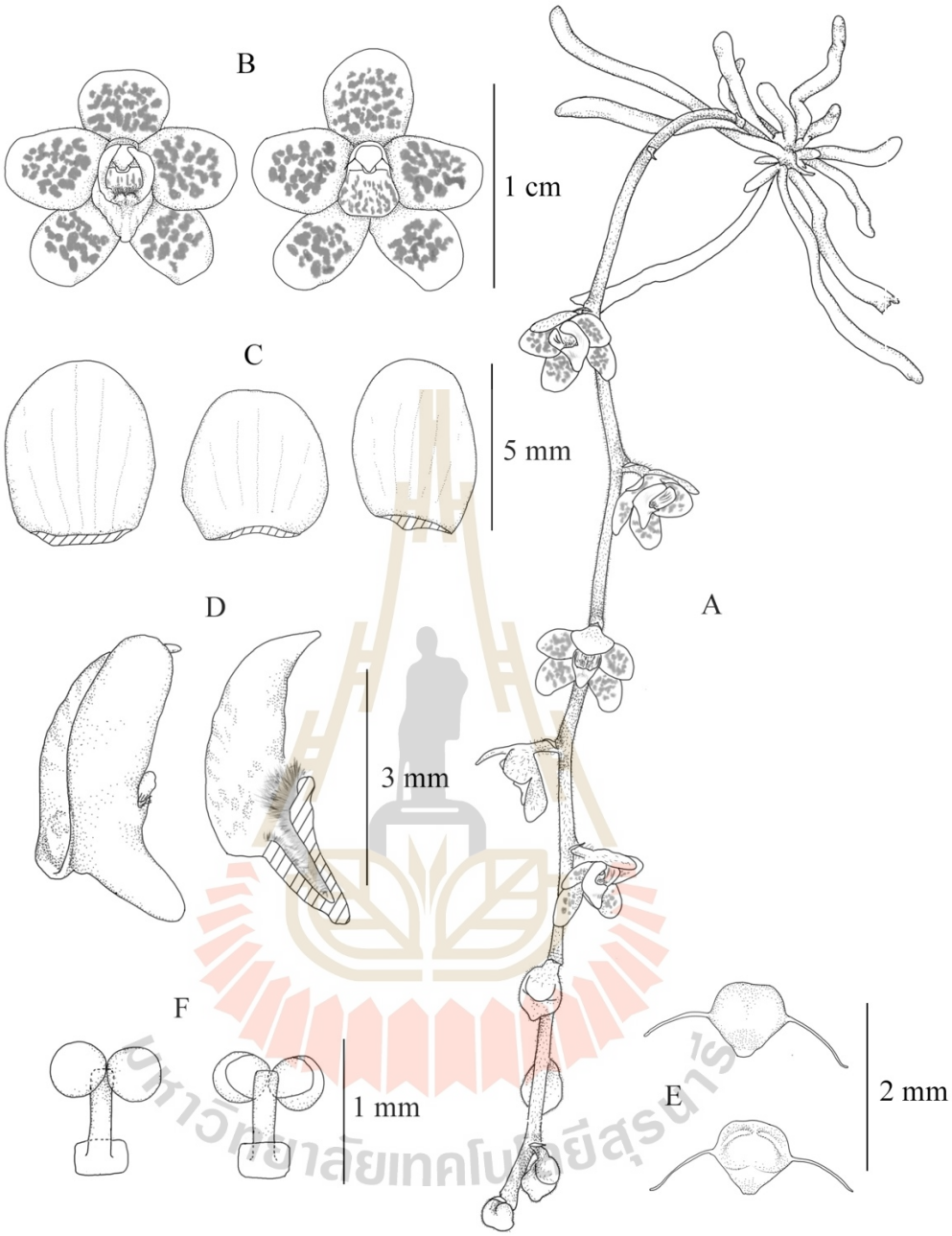

Figure 4.4 *Chiloschista extinctoriformis* Seidenf.: A. habit, B. flowers, C. dorsal sepal, petal and lateral sepal (from left to right) , D. labellum side view, E. operculum, F. pollinaria (Drawn by T.Chanokkhun).

Figure 4.5 Distribution map of *Chiloschista extinctoriformis* Seidenf. with available location (The map was received from google map).

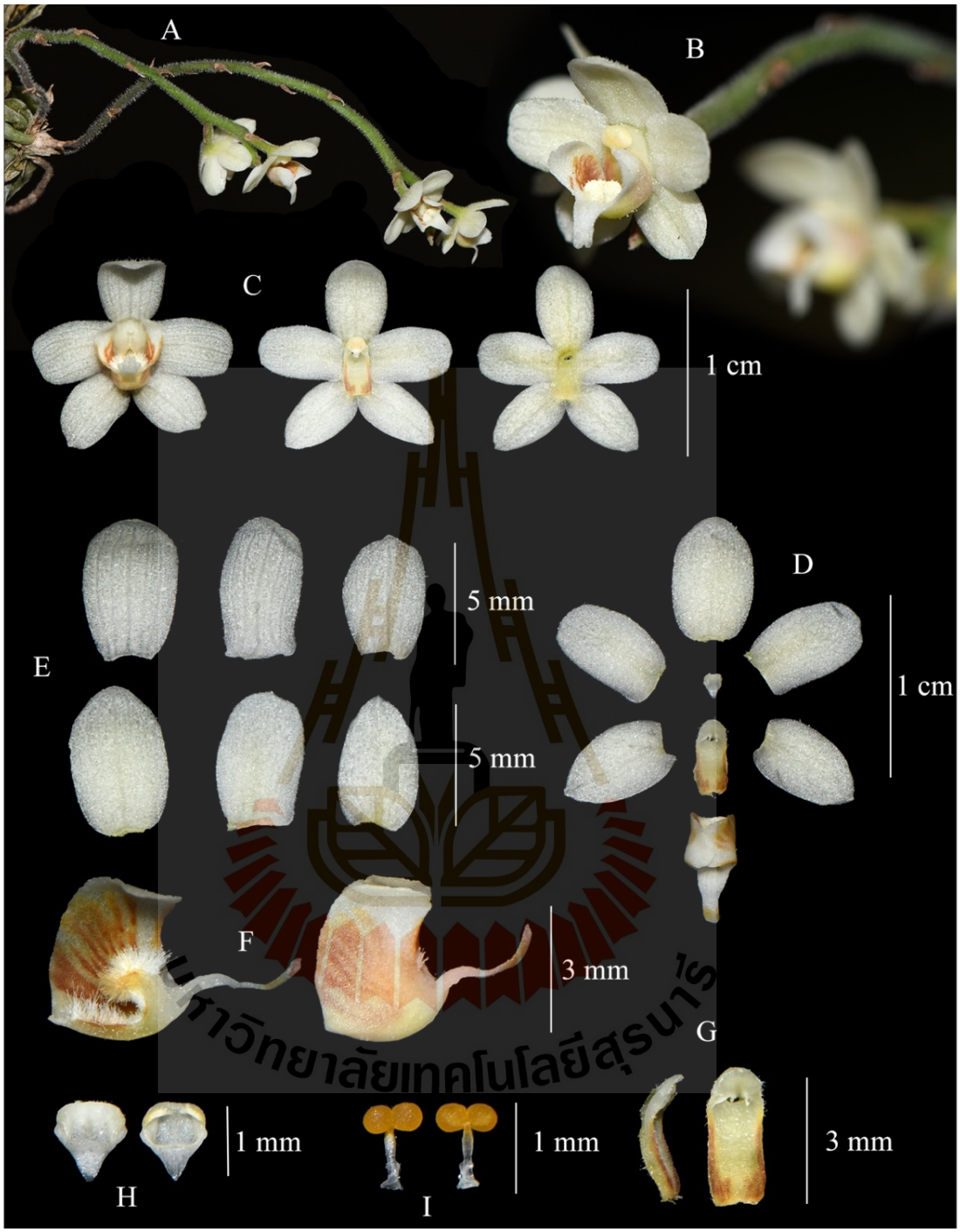

Figure 4. 6 *Chiloschista exuperei* (Guillaumin) Garey: A. habit, B. closed up inflorescence, C. flowers, with and without labellum and front and back views, D. dissected flower parts, E. dorsal sepal, petal and lateral sepal, from 2 flower, F. labellum side view, with longitudinal half cut showing inside, G. column, H. operculum, I. pollinaria (Pictured by T.Chanokkhun).

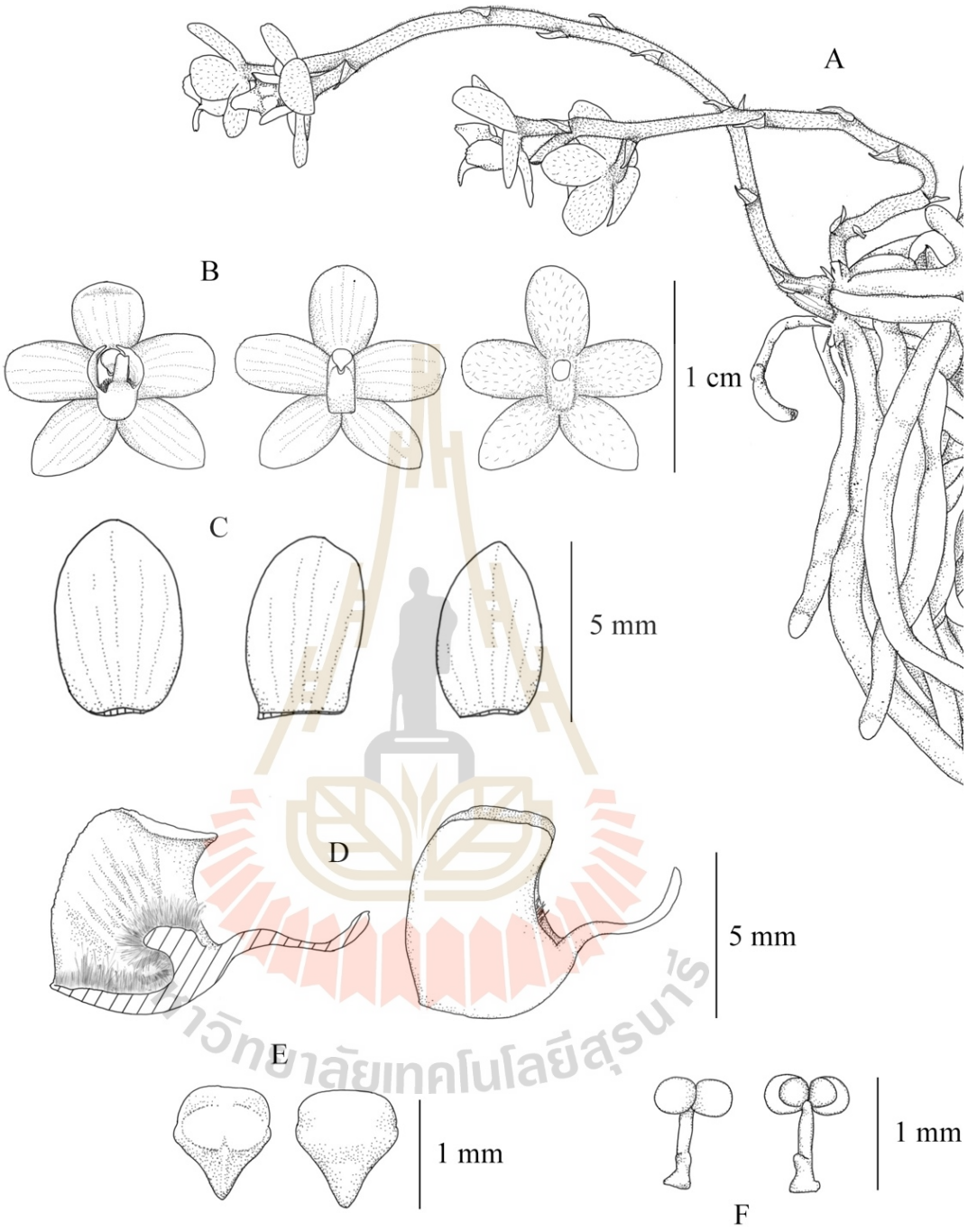

Figure 4.7 *Chiloschista exuperei* (Guillaumin) Garey: A. habit, B. flowers, with and without labellum and front and back views, C. dorsal sepal, petal and lateral sepal (from left to right), D. labellum side view, E. operculum, F. pollinaria (Drawn by T.Chanokkhun).

Figure 4.8 Distribution map of *Chiloschista exuperei* (Guillaumin) Garey with available location (The map was received from google map).

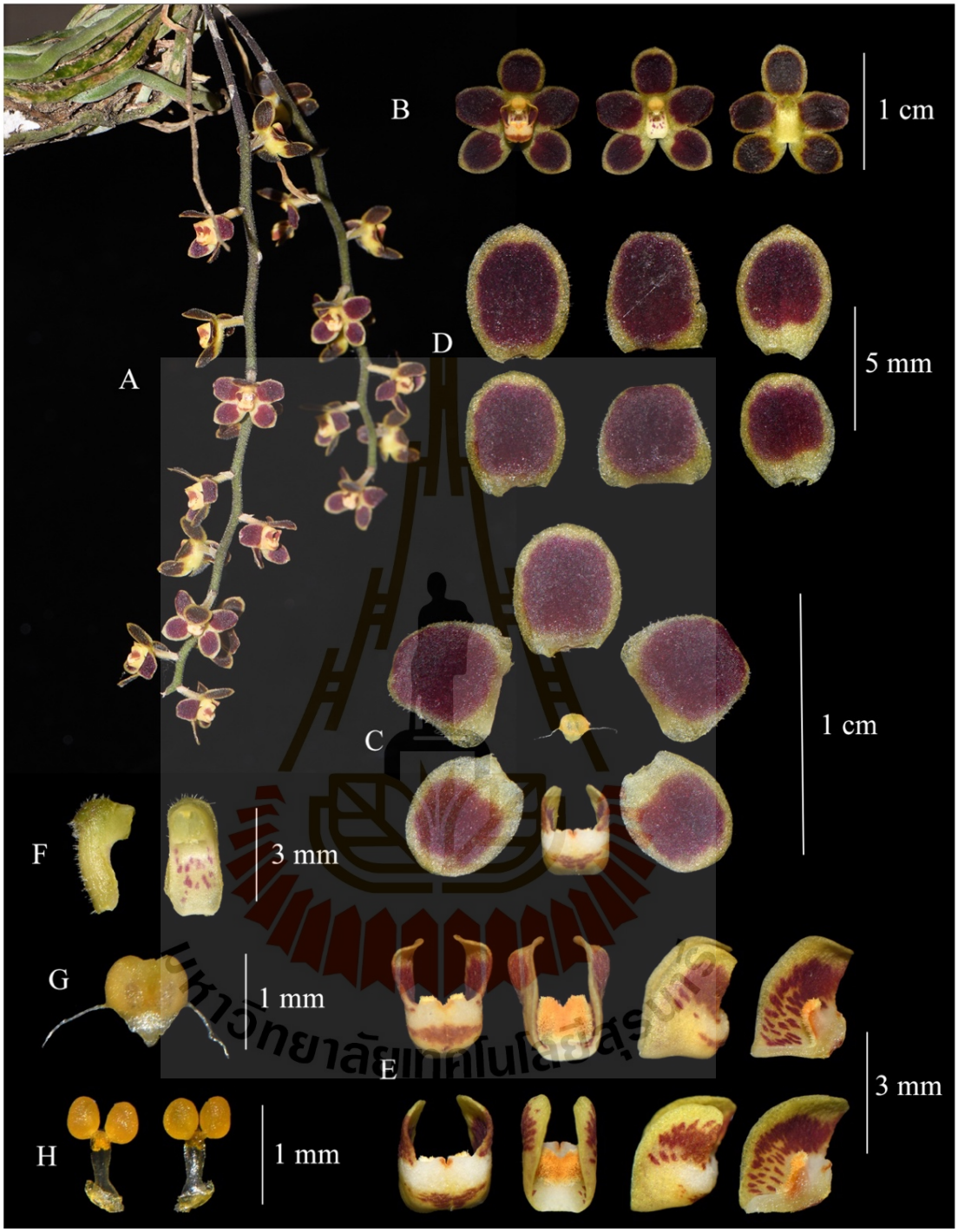

Figure 4.9 *Chiloschista lunifera* (Rchb.f.) J.J.Sm.: A. habit, B. flowers, with and without labellum and front and back views, C. dissected flower parts, D. dorsal sepal, petal and lateral sepal (from left to right), from 2 flower, E. labellum from two flowers, showing front and back view and side view, with longitudinal half cut showing inside, F. column, G. operculum, H. pollinaria (Pictured by T.Chanokkhun).

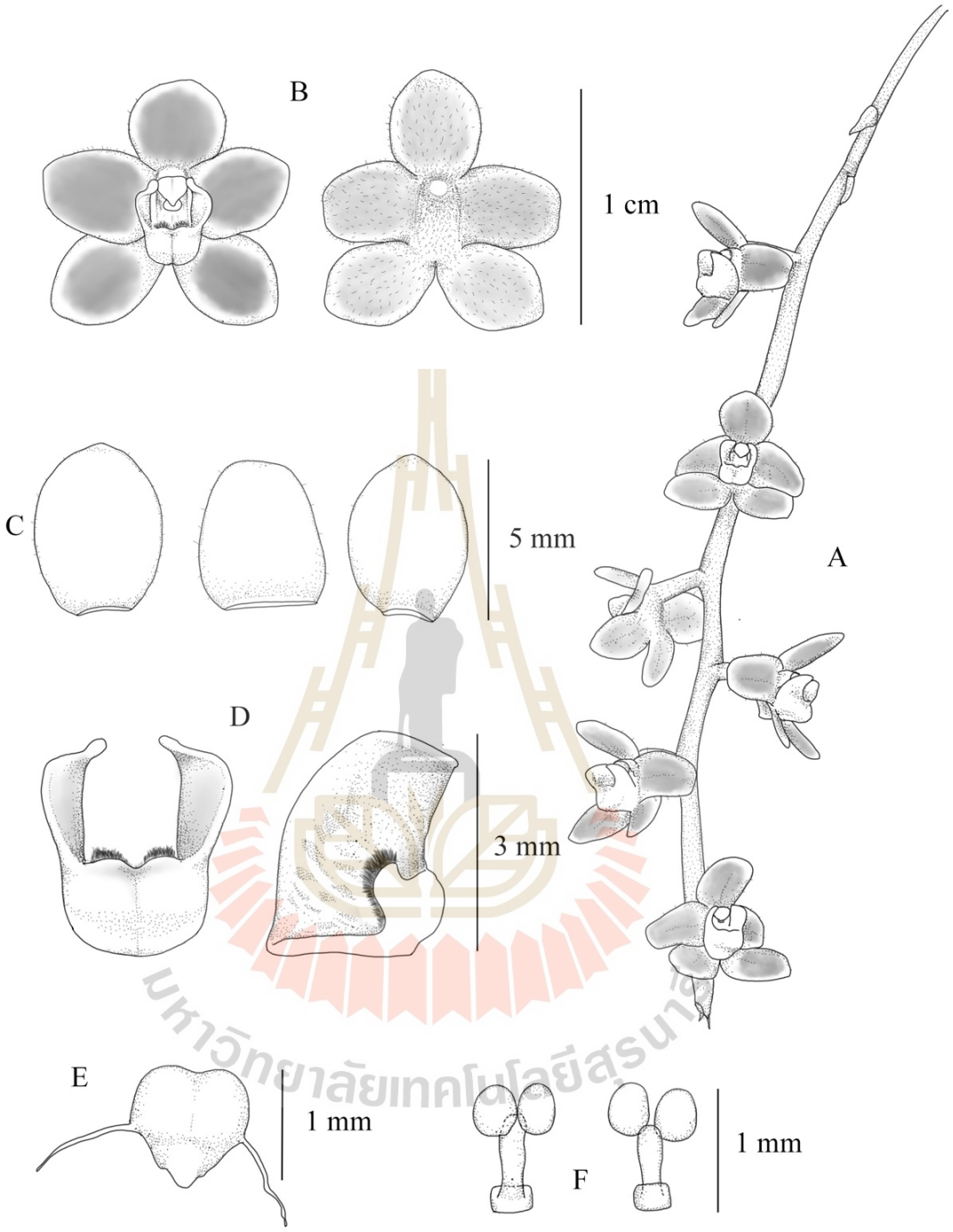

Figure 4.10 *Chiloschista lunifera* (Rchb.f.) J.J.Sm.: A. habit, B. flowers, front and back vie, C. dorsal sepal, petal and lateral sepal (from left to right), D. labellum, front and side view, with longitudinal half cut showing inside, E. operculum, F. pollinaria (Drawn by T.Chanokkhun).

Figure 4.11 Distribution map of *Chiloschista lunifera* (Rchb.f.) J.J.Sm. with available location (The map was received from google map).

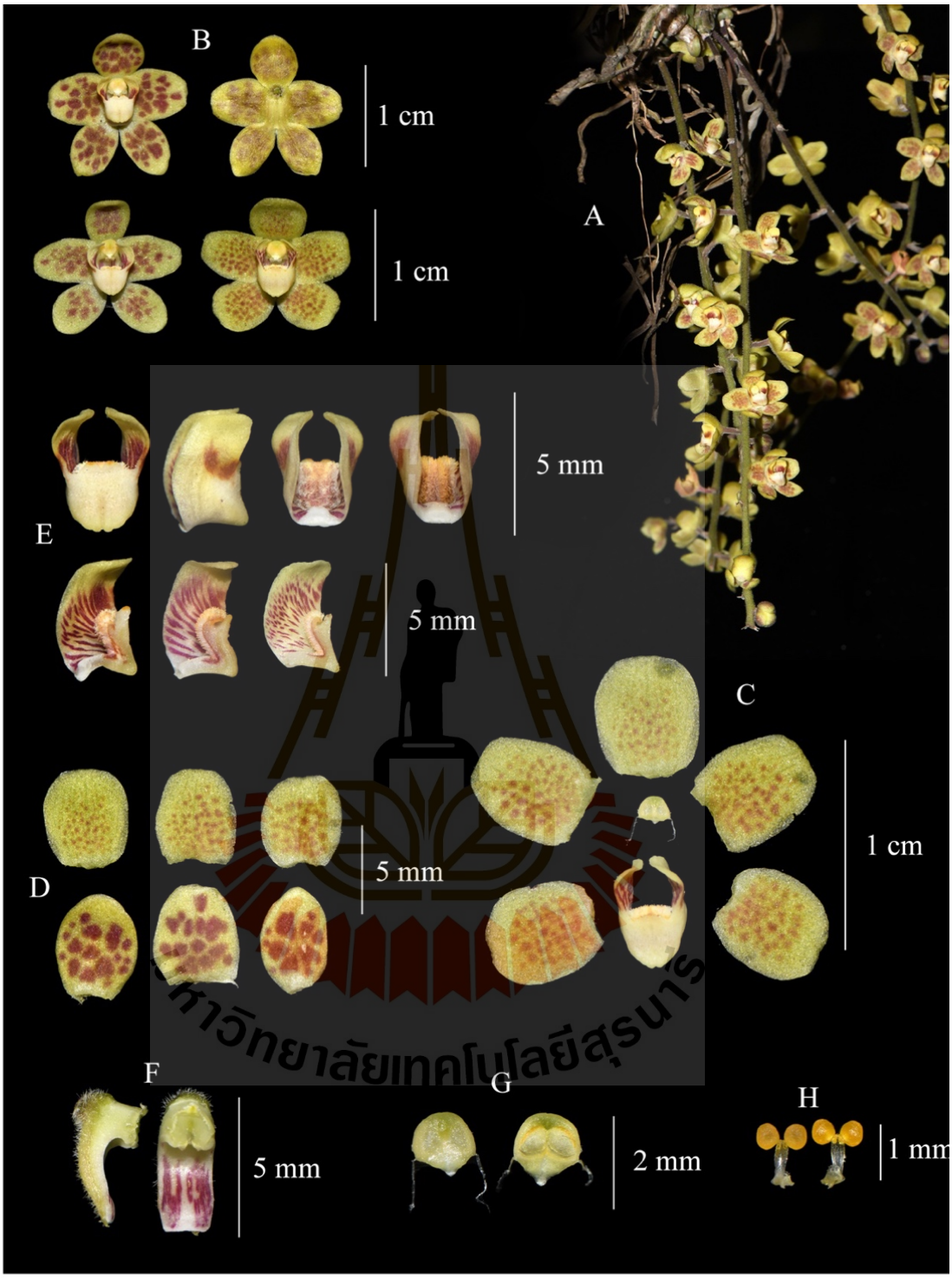

Figure 4.12 *Chiloschista parishii* Seidenf.: A. habit, B. flowers, front and back view, C. dissected flower parts, D. dorsal sepal, petal and lateral sepal, from 2 flower, E. labellum from two flowers, showing front and back view and side view, with longitudinal half cut showing inside, F. column, G. operculum, H. pollinaria (Pictured by T.Chanokkhun).

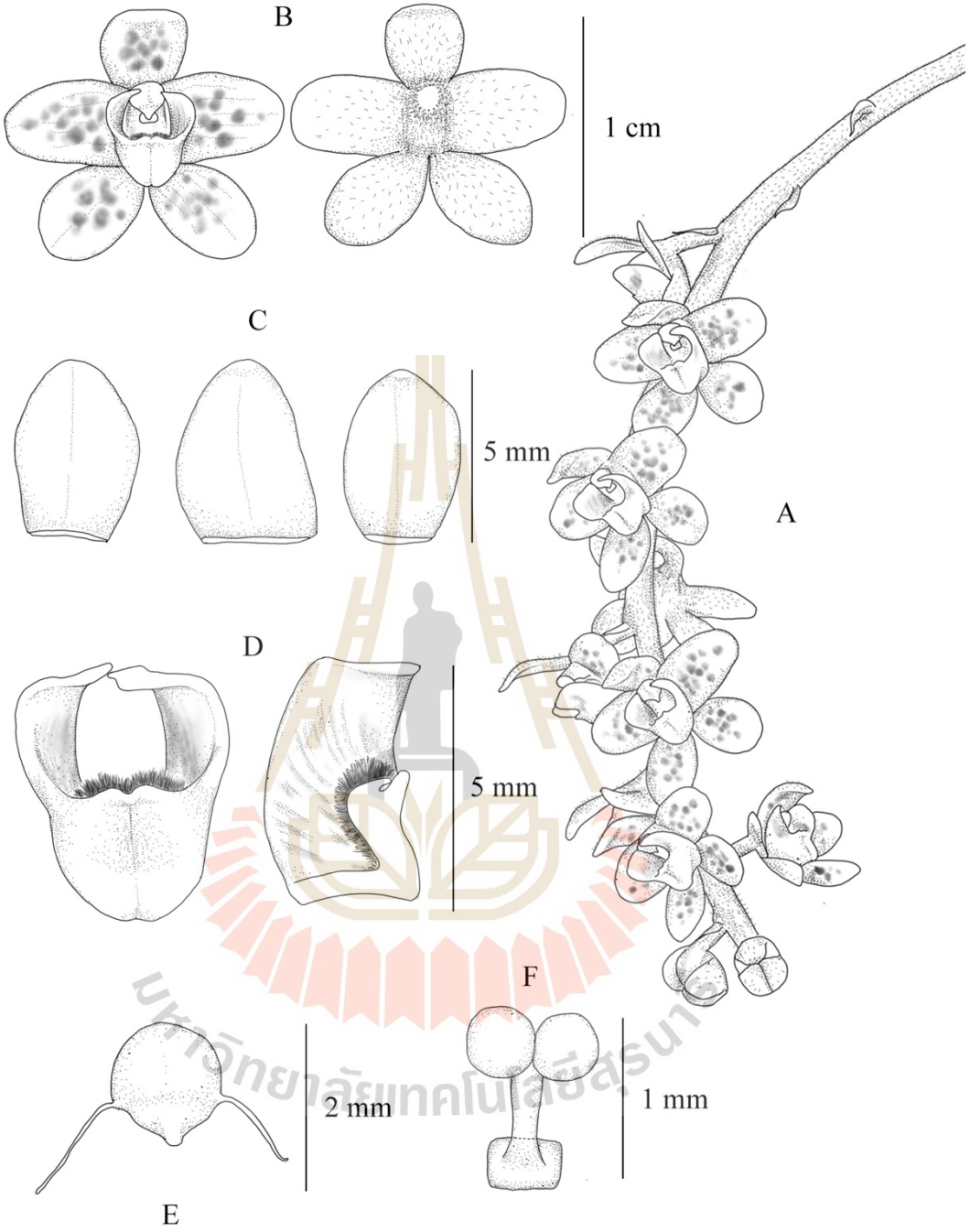

Figure 4.13 *Chiloschista parishii* Seidenf.: A. habit, B. flowers, front and back view, C. dorsal sepal, petal and lateral sepal (from left to right), D. labellum, front and side view, with longitudinal half cut showing inside, E. operculum, F. pollinaria (Drawn by T.Chanokkhun).

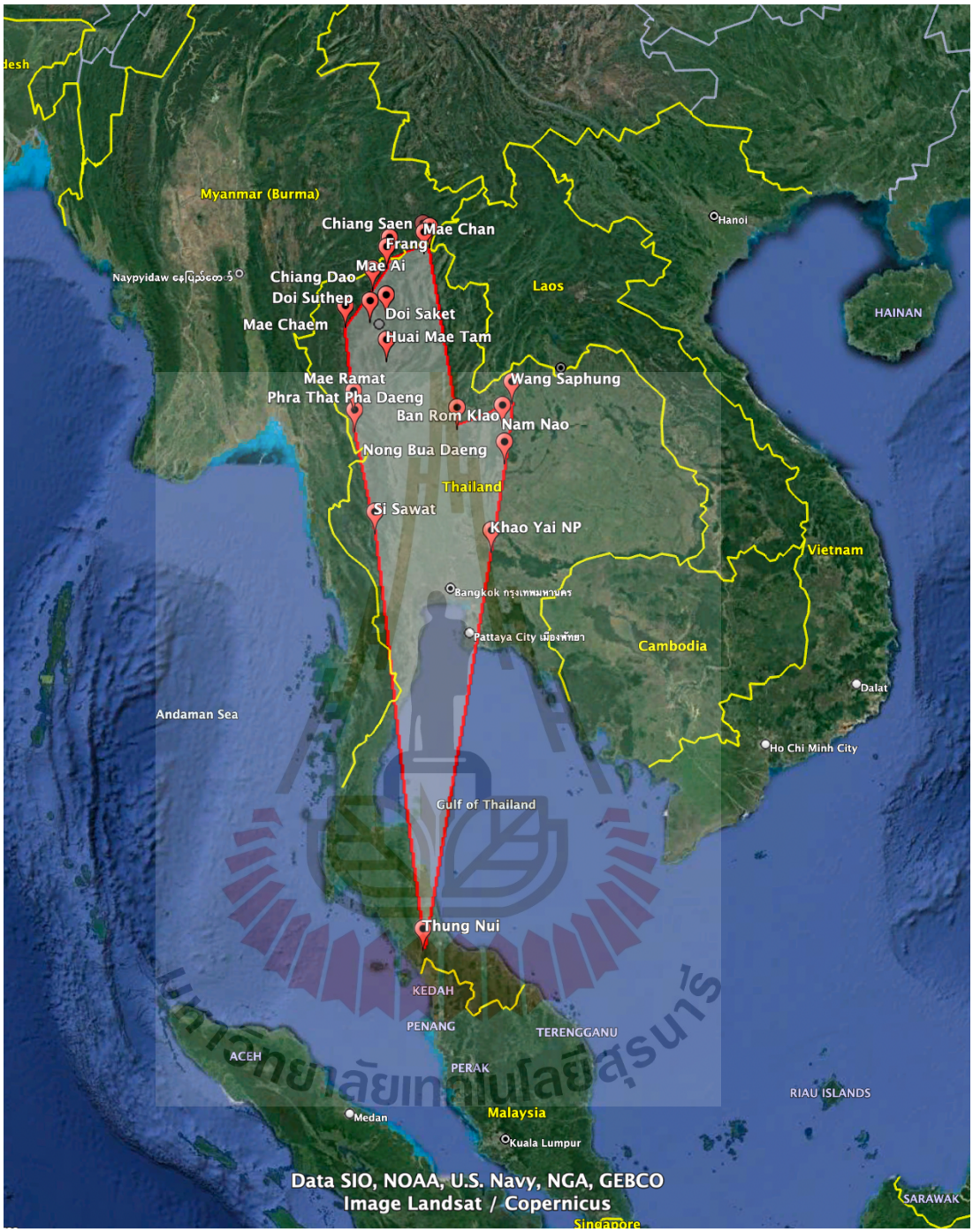

Figure 4.14 Distribution map of *Chiloschista parishii* Seidenf. with available location (The map was received from google map).

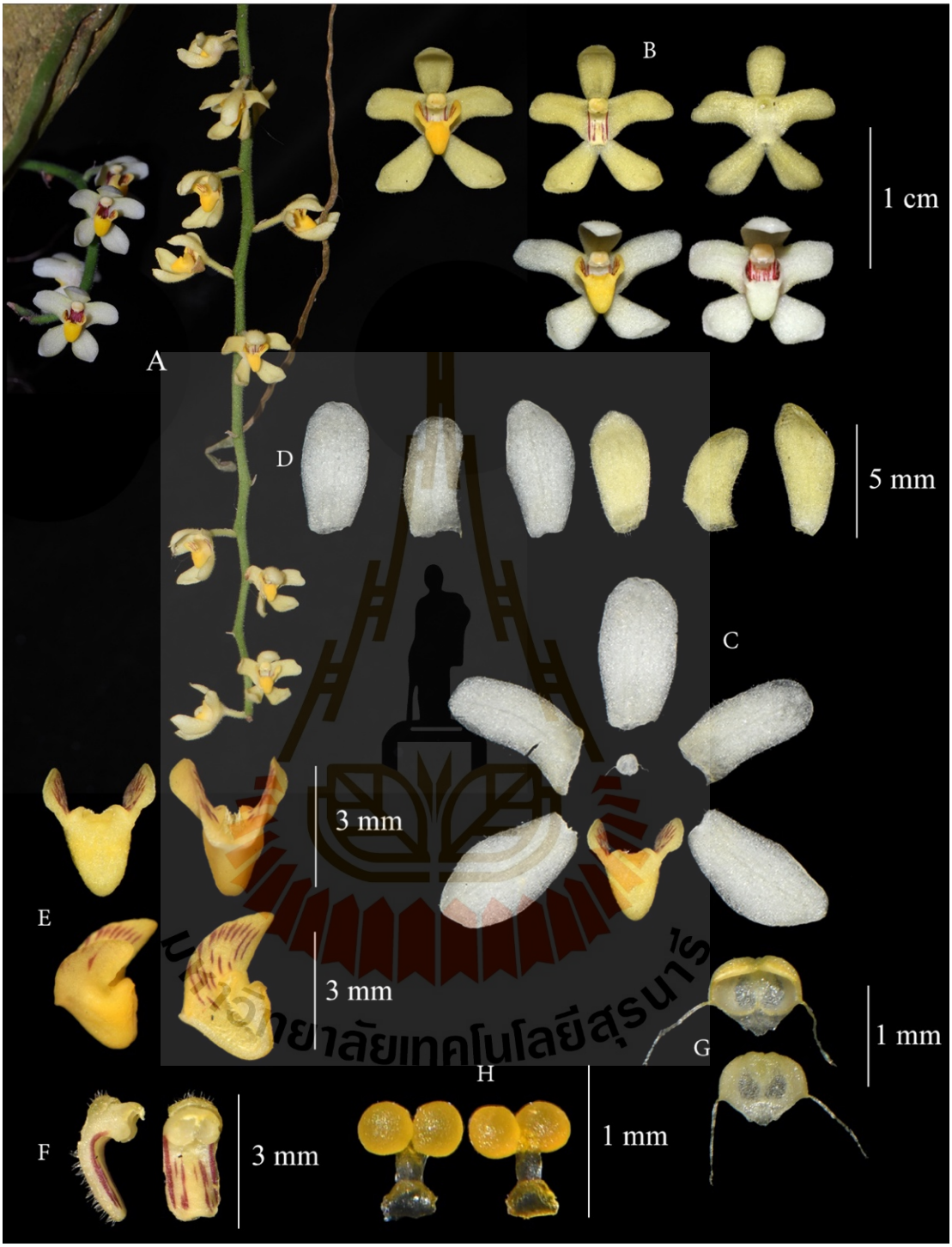

Figure 4.15 *Chiloschista rodiguezii* Casvestro & Ormerod: A. habit, B. flowers, with and without labellum and front and back views, C. dissected flower parts, D. dorsal sepal, petal and lateral sepal, from 2 flower, E. labellum, showing front and back view and side view, with longitudinal half cut showing inside, F. column, G. operculum, H. pollinaria (Pictured by T.Chanokkhun).

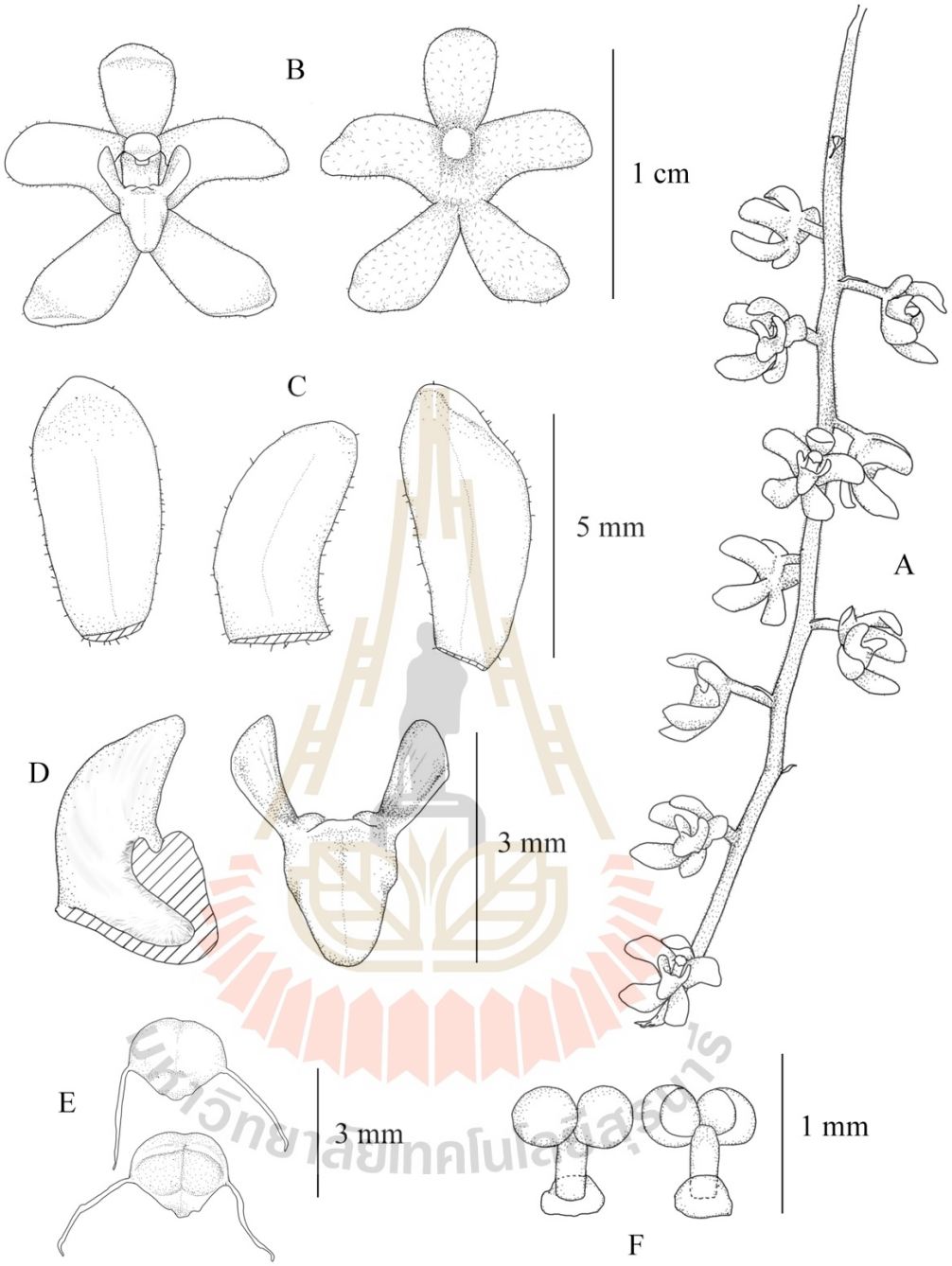

Figure 4.16 *Chiloschista rodiguezii* Casvestro & Ormerod: A. habit, B. flowers, front and back view, C. dorsal sepal, petal and lateral sepal (from left to right), D. labellum, front and side view, with longitudinal half cut showing inside, E. operculum, F. pollinaria (Drawn by T.Chanokkhun).

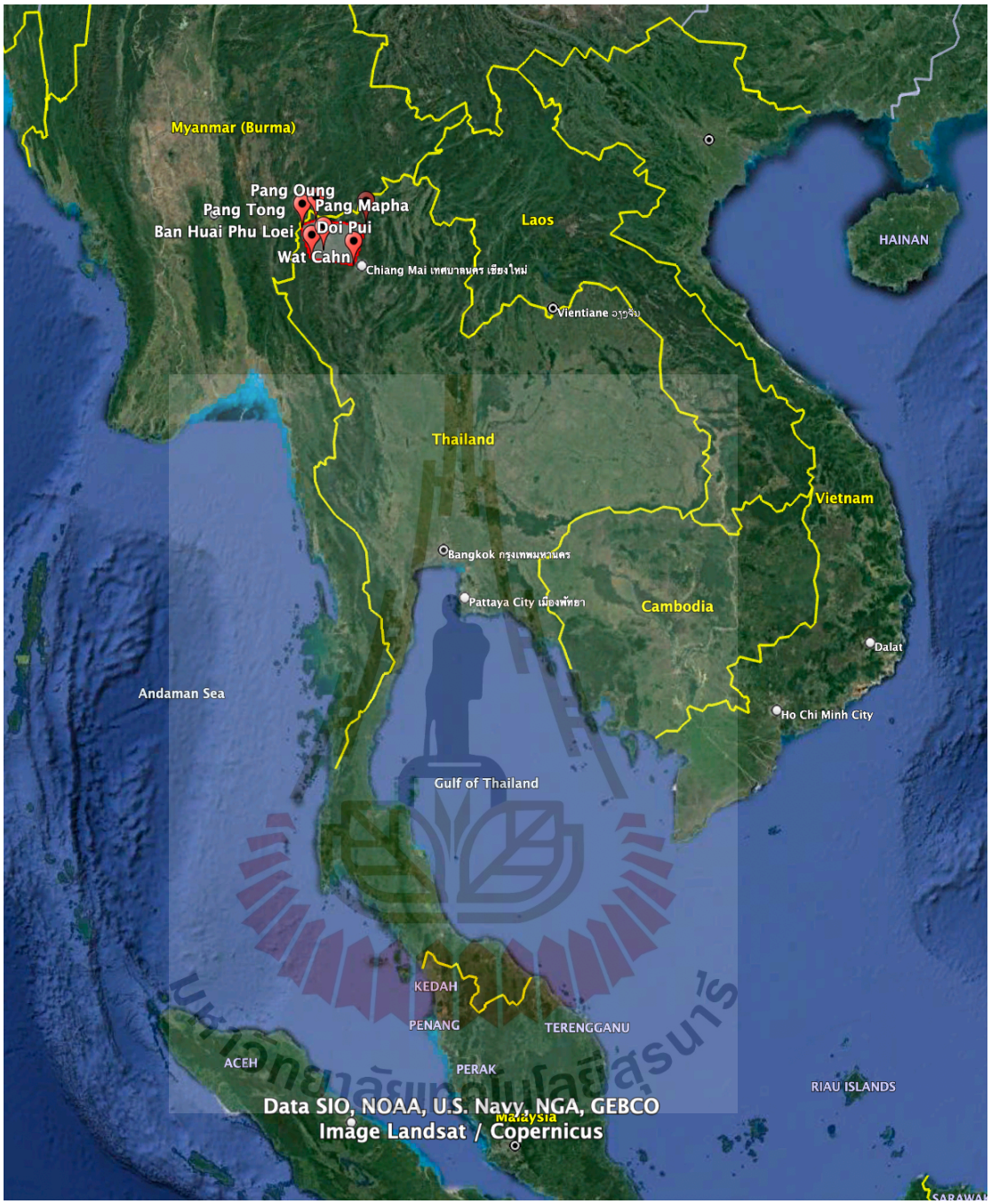

Figure 4. 17 Distribution map of *Chiloschista rodiguezii* Casvestro & Ormerod with available location (The map was received from google map).

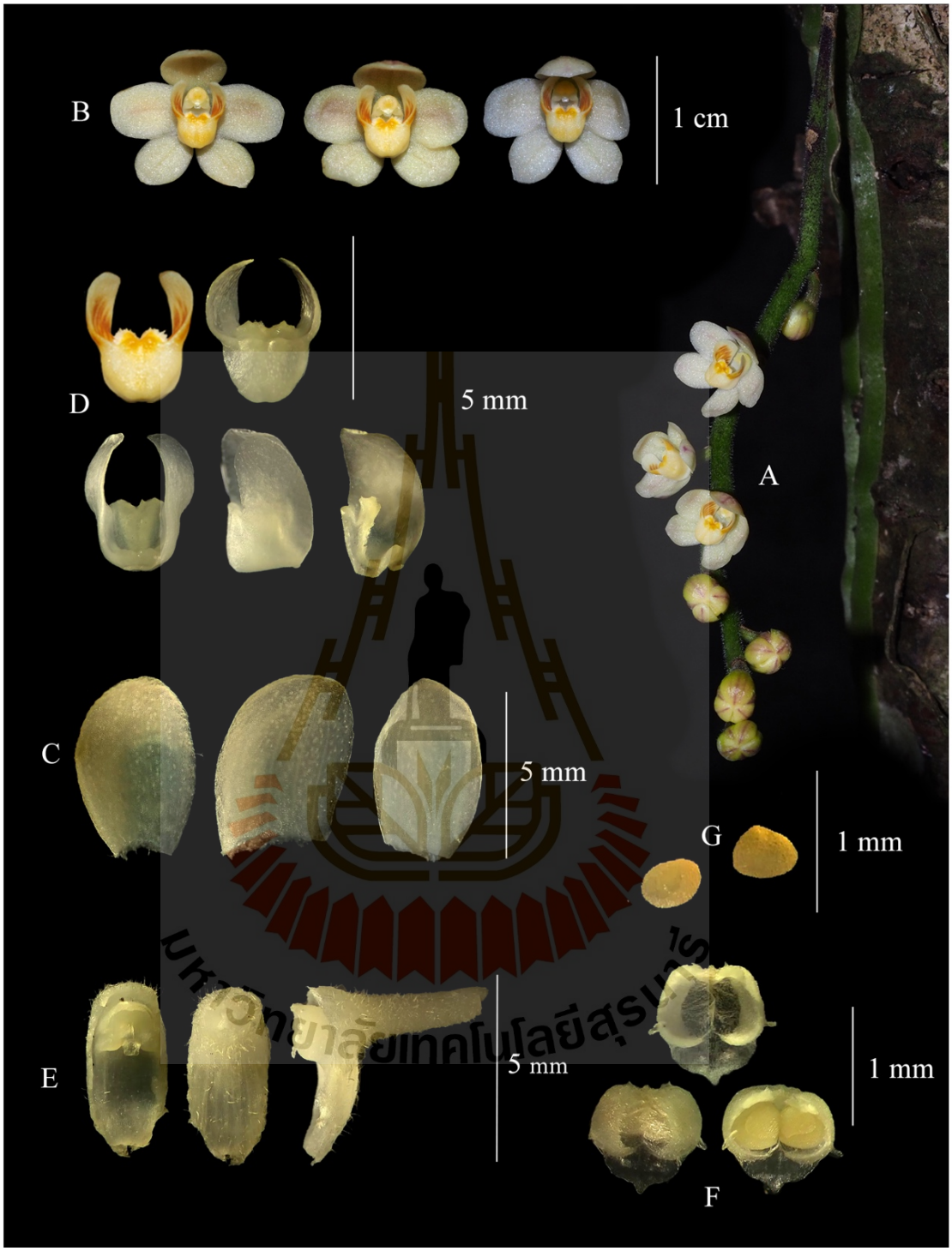

Figure 4.18 *Chiloschista usenoides* (D.Don) Lindl.: A. habit, B. flowers, from three flower, C. dorsal sepal, petal and lateral sepal (from left to right), D. labellum, showing front and back view and side view, with longitudinal half cut showing inside, E. column, F. operculum, G. pollen (Pictured by T.Chanokkhun).

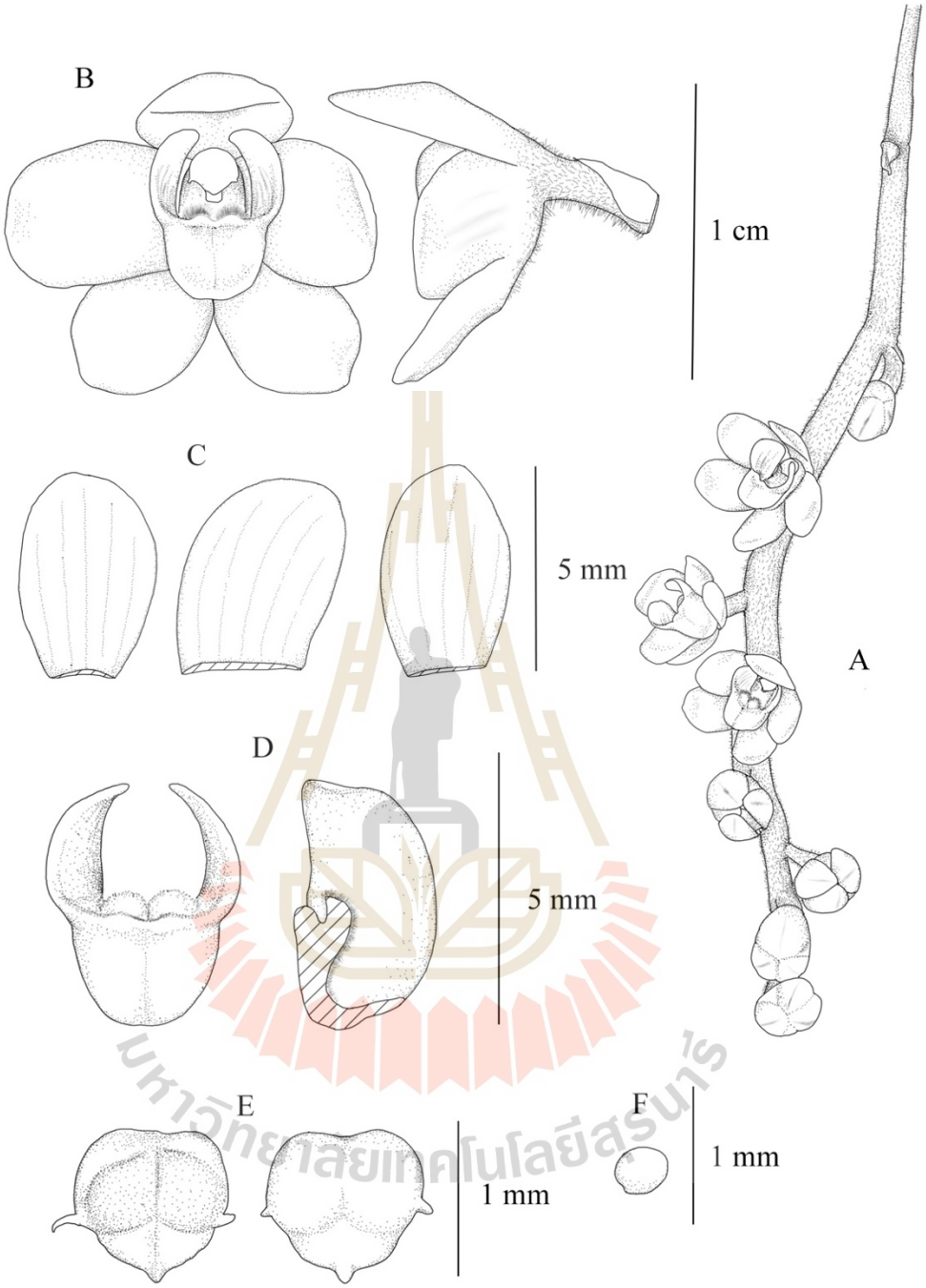

Figure 4.19 *Chiloschista usenoides* (D.Don) Lindl.: A. habit, B. flowers, front and side view, C. dorsal sepal, petal and lateral sepal (from left to right), D. labellum, front and side view, with longitudinal half cut showing inside, E. operculum, F. pollen (Drawn by T.Chanokkhun).

Figure 4.20 Distribution map of *Chiloschista usenoides* (D.Don) Lindl. with available location (The map was received from google map).

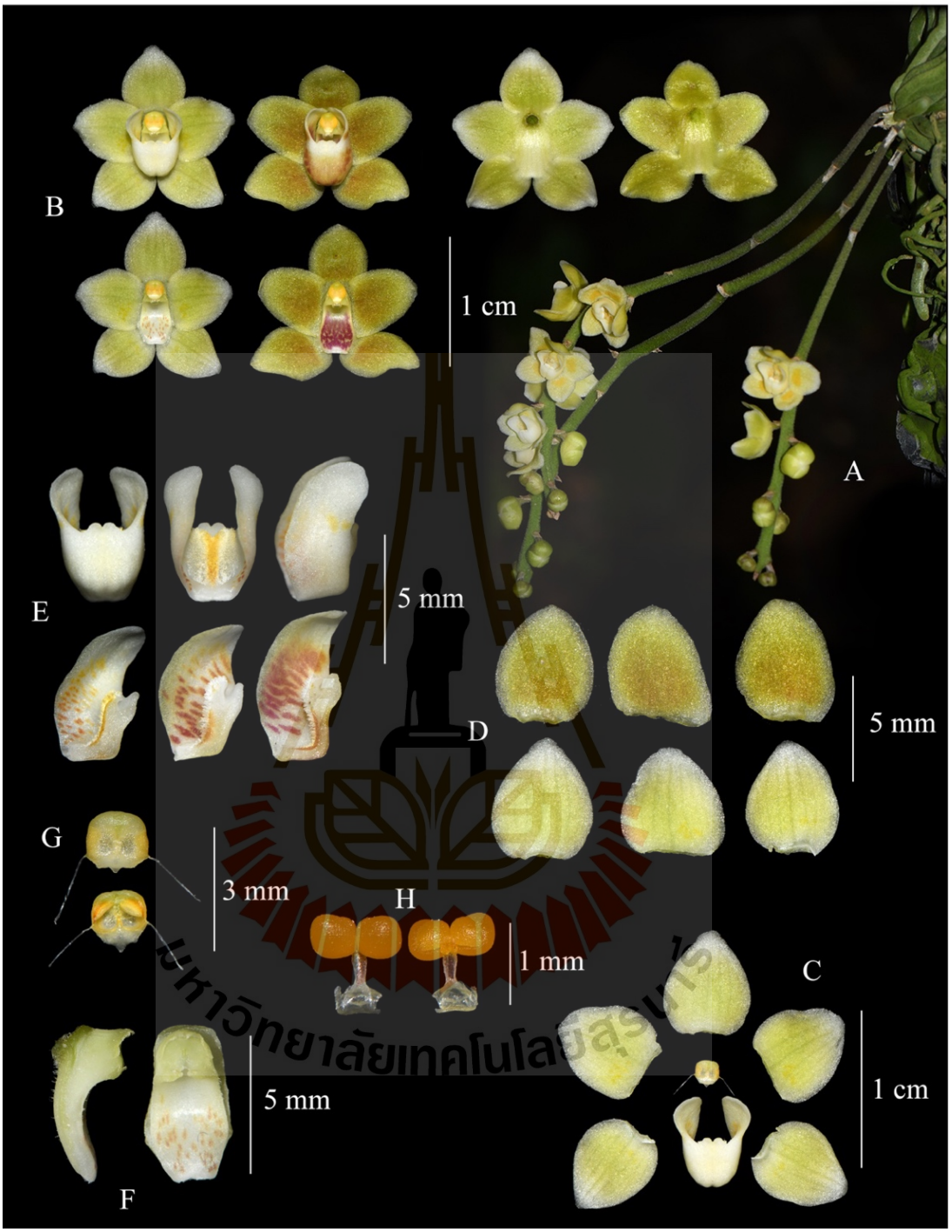

Figure 4.21 *Chiloschista viridiflava* Seidenf.: A. habit, B. flowers, with and without labellum and front and back views, C. dissected flower parts, D. dorsal sepal, petal and lateral sepal, from 2 flower, E. labellum from two flowers, showing front and back view and side view, with longitudinal half cut showing inside, F. column, G. operculum, H. pollinaria (Pictured by T.Chanokkhun).

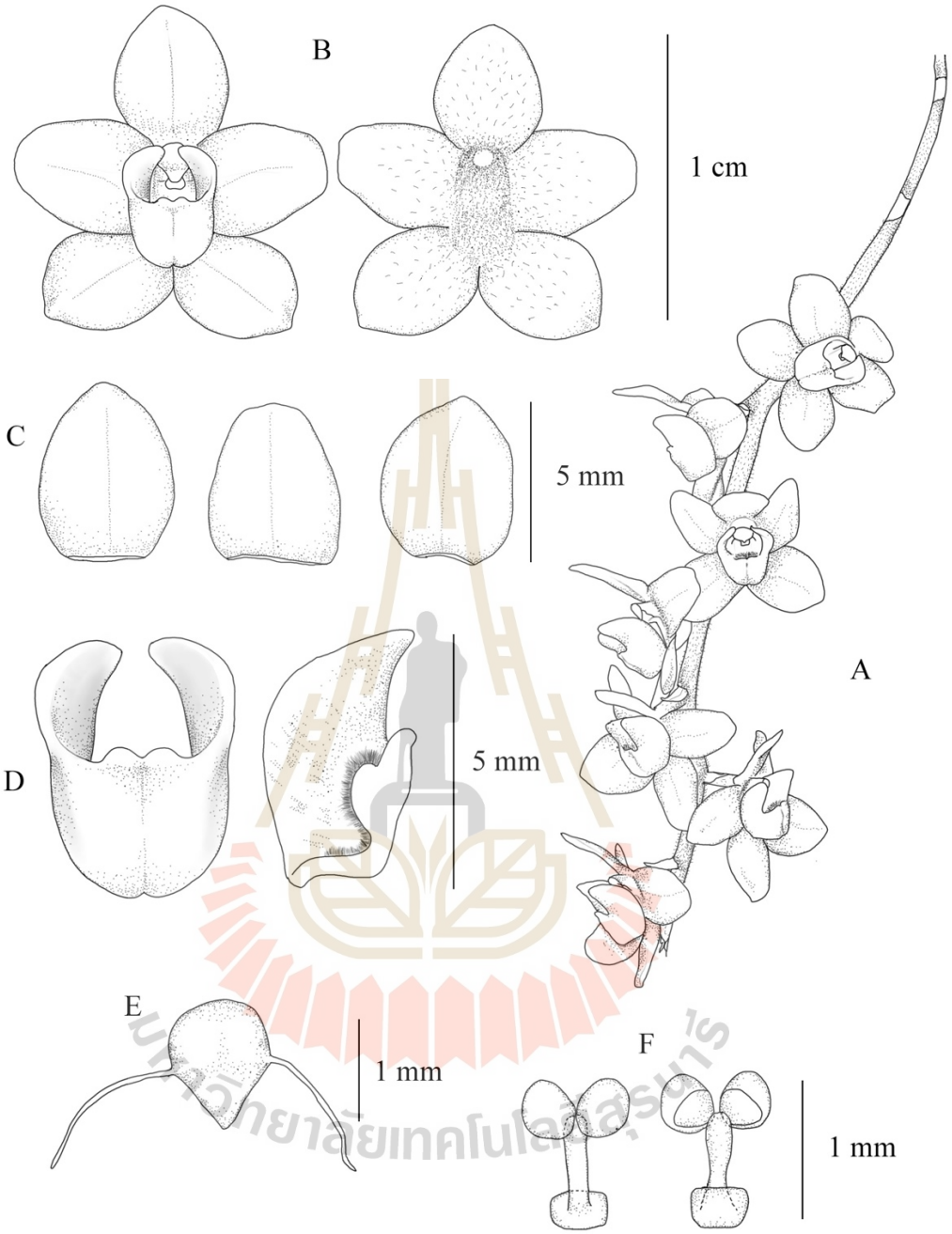

Figure 4.22 *Chiloschista viridiflava* Seidenf.: A. habit, B. flowers, front and back view, C. dorsal sepal, petal and lateral sepal (from left to right), D. labellum, front and side view, with longitudinal half cut showing inside, E. operculum, F. pollinaria (Drawn by T.Chanokkhun).

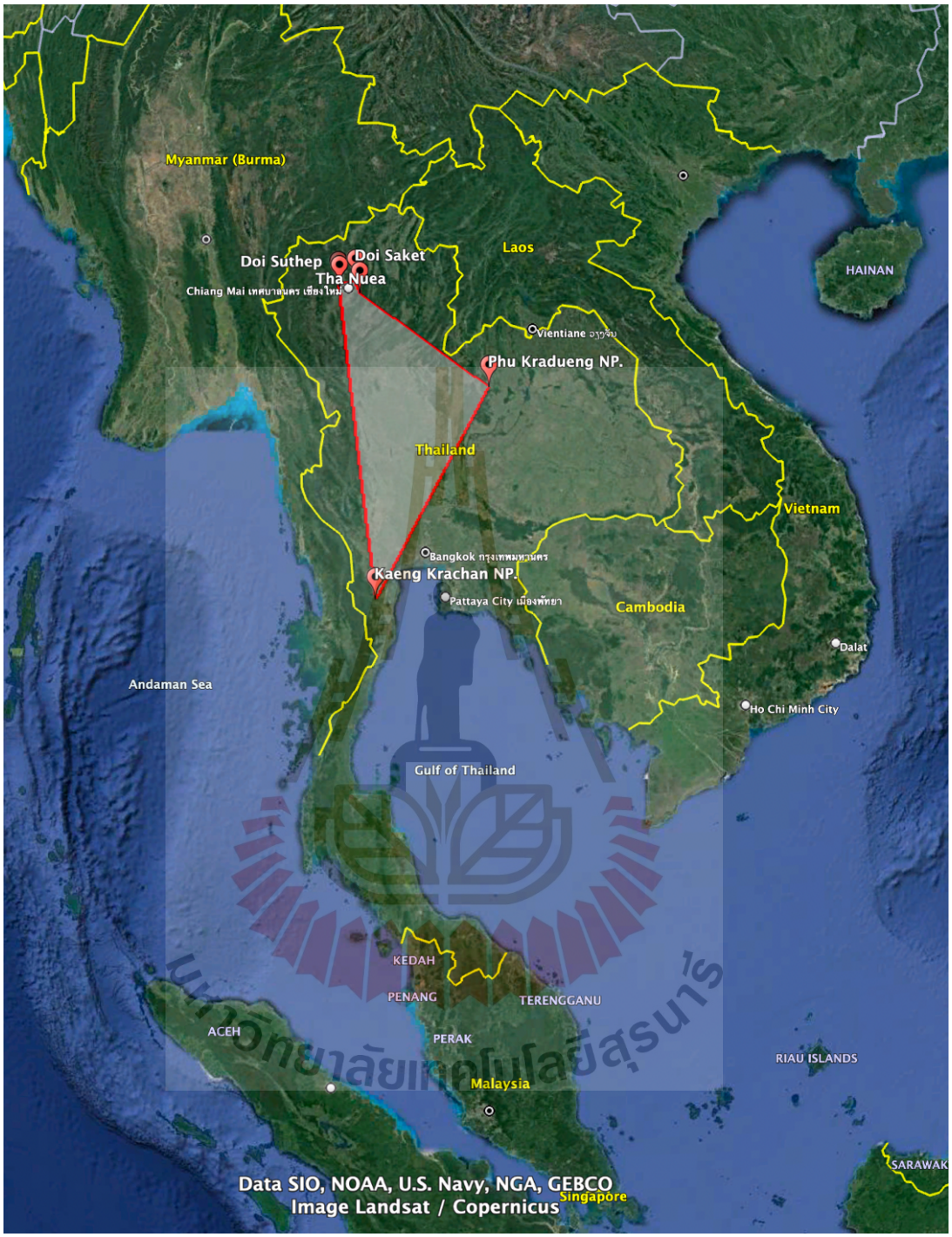

Figure 4.23 Distribution map of *Chiloschista viridiflava* Seidenf. with available location (The map was received from google map).

4.2 Phylogenetic analysis

In this study, I collected *C. extinctoriformis* (2 samples), *C. exuperei* (2 samples), *C. lunifera* (2 samples), *C. parishii* (3 smaples), *C. rodriguezii* (1 sample), *C. usneoides* (2 samples), *C. viridiflava* (2 samples) and *Chiloschista.* sp. (1 sample) for constructing the phylogenetic tree. The last unknown species, it was a vegetative specimen from Khao Yai Nation Park, aimed to use the phylogenetic analysis to test what it is. For *C. yunnanensis,* I employed the sequence from GeneBank. In this study, 2 endemic species, *C. extinctoriformis* and *C. rodriguezii* were extracted to new *mat*K and ITS sequences based on specimens collected from Thailand and for example agarose gel electrophoresis for amplified *mat*K and ITS gene test (Figure 4.24, 4.25). They could be used for further studies on systematics in the future, including species identification.

Before using all sequence of *Chiloschista* received from this study, I blasted to confirm that they belong to Orchidaceae with GenBank database sequences by using NCBI nucleotide BLAST (blastn) (http://blast.ncbi.nlm.nih.gov). As expect, the results confirmed that they are orchids. The aligned with Bioedit program and manual correction of *mat*K, ITS, and combined *mat*K and ITS matrices with gaps were 1,633, 1422 and 3,052 bases long, respectively. The number of parsimony-informative sites was performed by Paup program showed that *mat*K, ITS and combined *mat*K and ITS were 72, 153 and 225, respectively.

Based on *mat*K sequences, the phylogenetic analysis revealed that there is high statistic support of 2 clades between ingroup and outgroup, congruent with previous study (Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y., 2015). From this study, the genus *Choloschista* form a monophyletic group with strong bootstrap percentage (BP= 100) and Bayesian posterior probabilities (PP=1), but the resolution infrageneric level is very poor (Figure 4.26). The samples of each species from different populations according to Seidenfaden's species circumscription (1988) showed that *C. exuperei*, *C. lunifera*, *C. parishii* and *C. viridiflava* form a monophyletic group. While *C. extinctoriformis* and *C. usneoides* are not form a monophyletic group based on *mat*K, may cause of low informative site. For *C. yunnanensis* and *C. rodriguezii* which have only one sample included in this analysis are needed more sample to prove the monophyletic at the species level. The phylogenetic analysis based on *mat*K evident

is often not showed the high resolution inside the genus. For example, the phylogenetic analysis based on *mat*K on the genus *Pomatocalpa* (Watthana *et al.*, 2006).

Based on ITS, the result is rather congruent with of *mat*K but it is quite better than of *mat*K. *Chiloschista* spp. form a clade with high BP (100) and PP (1) which indicating a good genus (Figure 4.27). They are separated clade from the outgroup as same as of *mat*K result. All species according to Seidenfaden's species circumscription (1988) which have more than one sample form a monophyletic group. One unknown species was a group of *C. useneoides* showing that ITS information is able to identify the species level which shall be useful for molecular identification of this genus.

The combined genes showed the result almost congruent with of ITS result. However, there is no distinctly subclade formation inside the *Chiloschista* clade, but specimen of each species forms a clade like of ITS, indicating a monophyletic group at the species level (Figure 4.28).

From a previous report, Topik, H., Peter, W., Tomohisa, Y., and Motomi, I. (2005) and Topik, H., Peter, W., Tomohisa, Y., Motomi, I., and Rod, R. (2012) analyzed the phylogenetic of subtribe Aeridinae. They employed only *Chiloschista viridiflava* representative on thier study. It was show that that *Chiloschista* was a sister group with *Ornithochilus fifformis*. The last update on *Chiloschista* phylogenetic position involvement was reported by Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y. (2015), showed that the *Chiloschista* clade is a sister group with the clade of *Phalaenopsis*, *Thrixspermum*, *Vanda*, *Aerides*, *Trichoglottis*, *Abdominea*, *Gastrochillus* and *Cleisostoma.* It should be noted that another genus of leafless orchid, *Taeniophyllum* which having a vegetative morphological characteristic like to *Chiloschista*, is in the different clade with *Chiloschista* clade as same as the previous studies (Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y., 2015; Carlsward, B. S., Whitten, W. M., Williams, N. H., and Bytebier, B., 2006), indicating the leafless character evolved more than one time in the subtribe Aeridinae. Thus, the information of my study and other previous studies supports the monophyly of the genus *Chiloschista*. However, the genus-level relationship based on combined ITS and *mat*K data revealed that Thai *Chiloschista* does not reveal any subgroups.

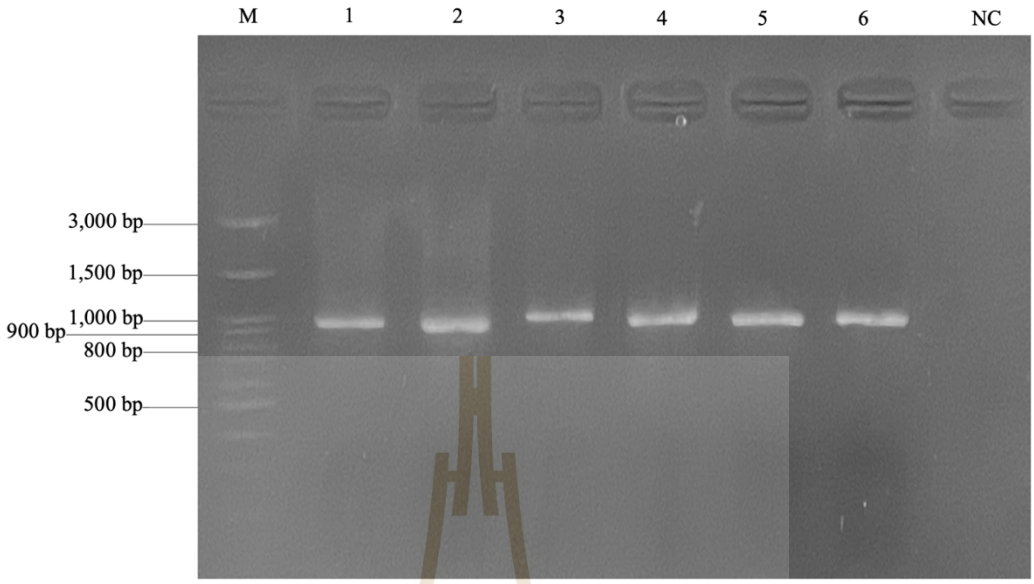

Figure 4.24 Example of agarose gel electrophoresis for amplified *mat*K gene test. Bands were fractionated by 1.5 % TBE agarose gel (25 min., 100 V/cm); Lane: **M** = One Mark B DNA Ladder, **1** = *Vanda* sp.1, **2** = *Vanda* sp.1, **3** = *C. lunifera*, **4** = *C. extinctoriformis*, **5** = *C. exuperei*, **6** = *C. rodriguezii* and **NC** = Negative control.

Several species with comparable floral morphology, including *C. parishii*, *C. viridiflava*, and *C. usneoides*, share morphological traits such as the characteristic of the labellum. Although they only differ in color pattern, it can be hard to distinguish them from herbarium and spirit specimens. The phylogenetic tree produced by this analysis, however, revealed that each species belonged to a monophyletic group. In case of *C. parishii* has color marking on sepals and petals varied from small dots to rather large dots but the result from phylogenetic analysis they form in the same clade. The molecular region, which is based on the *mat*K and ITS sections, is thus consistent with Seidenfaden species delimitation. (Seidenfaden, G., 1988). Nevertheless, more samples from more population should be added as well as more gene region to improve the phylogenetic tree of this genus.

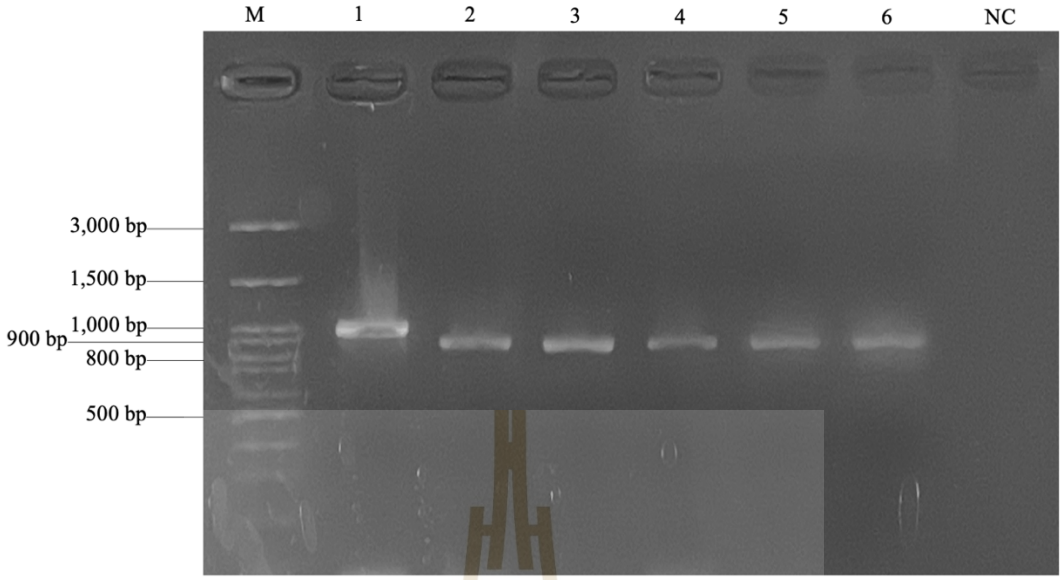

Figure 4.25 Example of agarose gel electrophoresis for amplified ITS gene test. Bands were fractionated by 1.5 % TBE agarose gel (25 min., 100 V/cm); Lane: **M** = One Mark B DNA Ladder, **1** = *Vanda* sp.1, **2** = *Vanda* sp.1, **3** = *C. lunifera*, **4** = *C. extinctoriformis*, **5** = *C. exuperei*, **6** = *C. rodriguezii* and **NC** = Negative control.

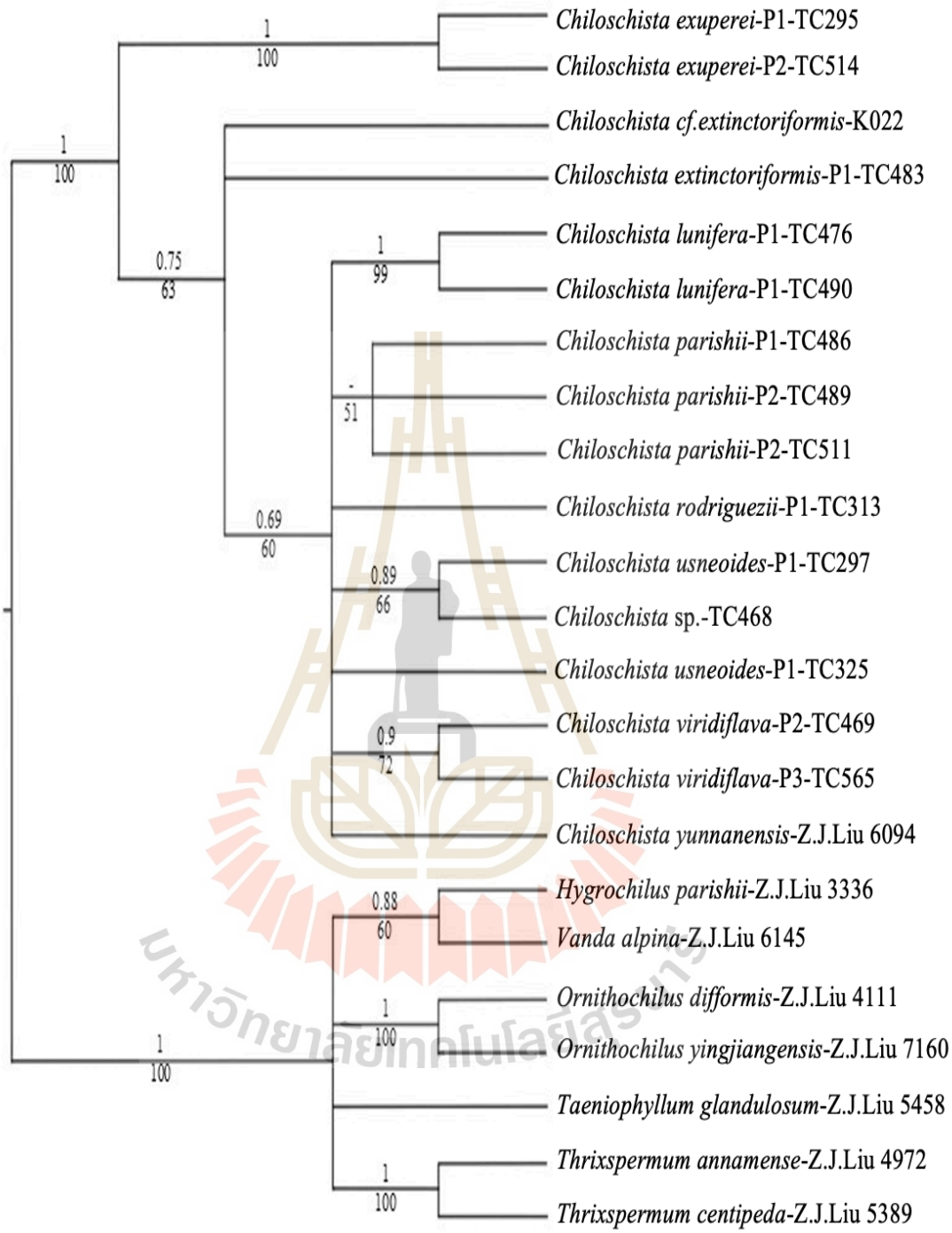

Figure 4.26 Phylogenetic tree based on *mat*K, showing bootstrap percentage (BP) and Bayesian posterior probabilities (PP). The number indicated above the branch is the Bayesian posterior probabilities (PP) and the lower one is the bootstrap percentage (BP).

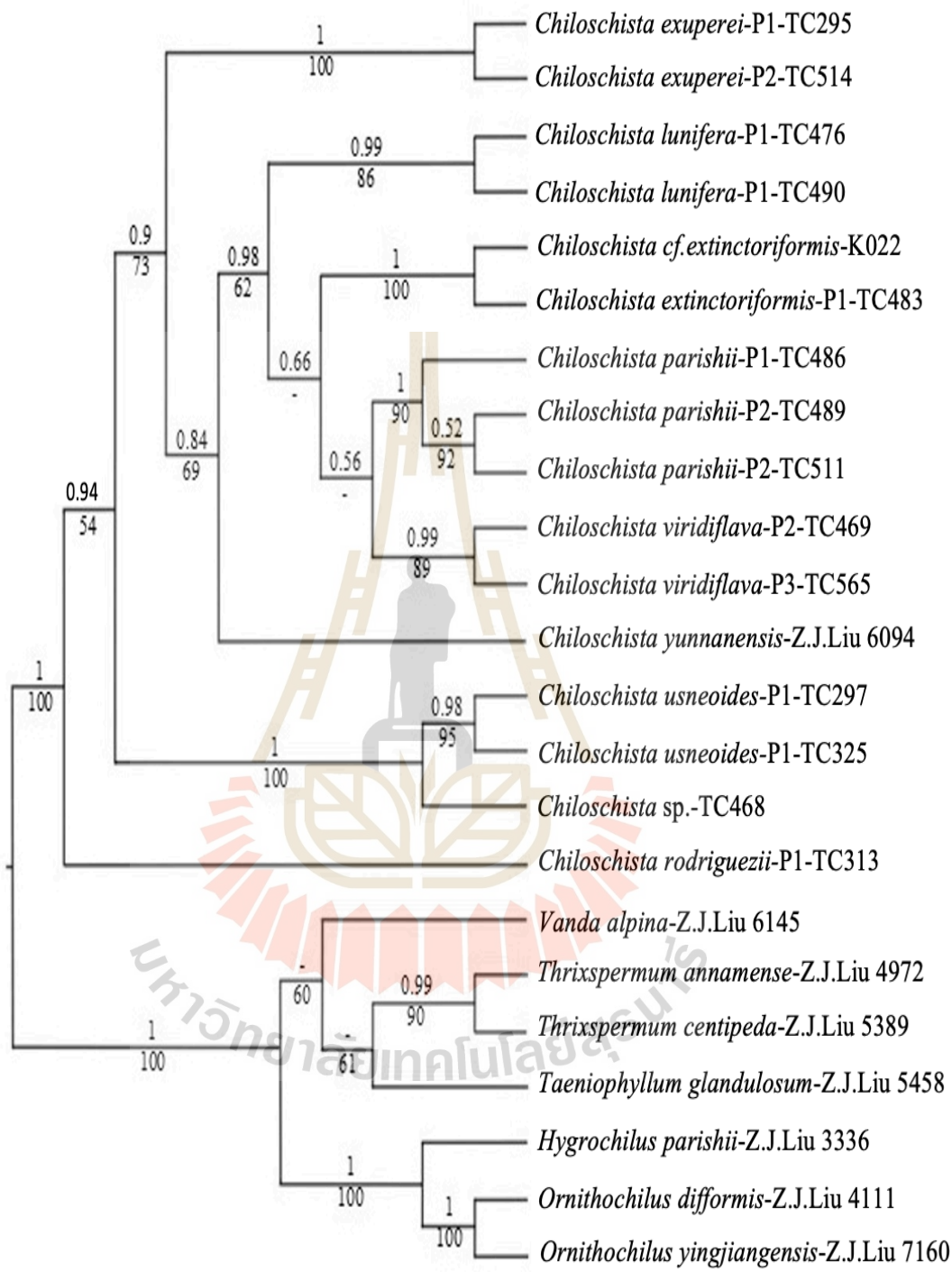

Figure 4.27 Phylogenetic tree based on ITS, showing bootstrap percentage (BP) and Bayesian posterior probabilities (PP). The number indicated above the branch is the Bayesian posterior probabilities (PP) and the lower one is the bootstrap percentage (BP).

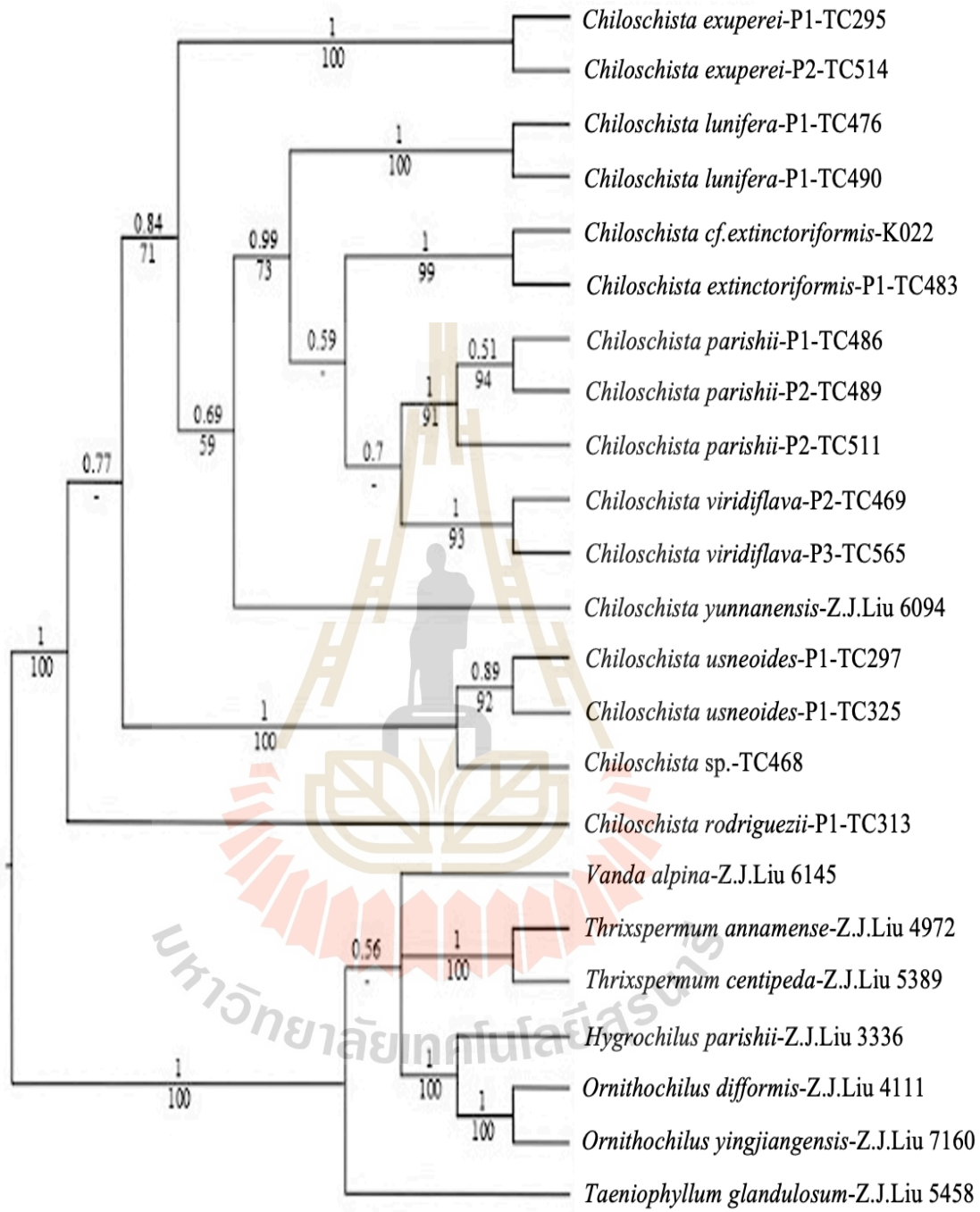

Figure 4.28 Phylogenetic tree of *Chiloschista* based on the combined nuclear (ITS) and plastid (*mat*K) markers, showing bootstrap percentage (BP) and Bayesian posterior probabilities (PP). The number indicated above the branch is the Bayesian posterior probabilities (PP) and the lower one is the bootstrap percentage (BP).

CHAPTER V
CONCLUSION

5.1 Taxonomy

Based on morphology and molecular phylogeny evident from this study, there are totally 7 species of *Chiloschista* in Thailand, which are *C. extinctoriformis*, *C. exuperei*, *C. lunifera*, *C. parishii*, *C. rodiguezii*, *C. usneoides* and *C. viridiflava.* There are 2 new synonyms, which are *C. ramifera* Seidenf. (syn. of *C. lunifera*), *C. trudelii* Seidenf. (syn. of *C. viridiflava*). While *C. lindstroemii* Dalstrom & Kolan. is likely to be a synonym fo *C. parishii*, unfortunately, I have not seen the type specimen yet. Another species name reported by Seidenfaden (1988), *C. pullisa* (Koen.) Schltr. is a synonym of *Taeniophyllum pusillum* (Wild.) Seidenf. & Ormerod, previously reported. Thus, the member of species in the genus *Chiloschista* in Thailand were reduced from 11 species to 7 species. There is no new record and new species found from recently.

A key to species of recently accepted was conducted. Each accepted species of *Chiloschista* in Thailand has been reported the nomenclature, including taxonomic literatures and type specimens, description, ecology, distribution and conservation status.

I have followed the IUCN Red List Categories and Criteria, version 14 (The Standards and Petitions Committee of the IUCN Species Survival Commission, 2019) to evaluate the conservation status of each species in Thailand. According to available localities and population information, three species are vulnerable which are *C. extinctoriformis, C. exuperei and C. rodriguezii.* The other 4 species are lest concern (LC) due to being high number of existing individuals and populations. The result of the conservation status from this study has updated from the previous report (Chamchumroon, V., Suphuntee, N., Tetsana, N., Poopath, M. and Tanikkool, S., 2017), reducing the status of *C. extinctoriformis* as vulnerable (VU), and reducing *C. ramifera* (syn. of *C. lunifera*) and *C. viridiflava* as lest concern (LC).

5.2 Phylogeny

In this study, the totally 7 species with accepted based on morphology were employed to analyze the phylogenetic tree. I have been presented the species in the *Chiloschista* which have never been sequenced on *mat*K and ITS which are *C. extinctoriformis* and *C. rodriguezii* based on specimens collected from Thailand. Both are the endemic species to Thailand.

The phylogenetic analysis of the genus *Chiloschista* based on molecular data, *mat*K and ITS from this study has confirmed that this genus is monophyletic group. Each species according to Seidenfaden' s species circumscription (Seidenfaden, 1988) form a clade indicating that they are good species based on phylogenetic species concept. However, the relationship among the species is not grouped together and not being any subclade. To reveal a more intra-specific relationship of *Chiloschista*, it should be added more gene regions for the phylogenetic analysis.

The result based on phylogenetic analysis from this study showed that the closed morphology species group which were *C. parishii*, *C. usneoides* and *C. viridiflava* which I thought that they could be the same species, because they were difficult to separate from each other when they were preserve in alcohol collection. However, based on the phylogenetic tree of this study, the color pattern varied from small dots to rather large dots in *C. parishii* from different plants showed that they were the same species. It is indicating that there is broad variation. More samples which are related with morphological characteristic in different populations may reveal different result on phylogenetic tree.

For identification of the orchid by using the molecular evident of the genus *Chiloschista*, the ITS gene can be a good evident to identify at species level, better than *mat*K. This shall be useful for identifying vegetative specimens without flower as well.

REFERENCES

REFERENCES

Brown, R. (1810). Prodromus florae Novae Hollandiae. *Forgotten Books*. London.

Cavestro, W., and Ormerod, P. (2005). Une nouvelle espèce de Chiloschista de Thaïlande: Chiloschista rodriguezii Cavestro & Ormd. *Orchidées exotiques-Culture*. *36*(3), 166-179.

Chamchumroon, V., Suphuntee, N., Tetsana, N., Poopath, M., and Tanikkool, S. (2017). Threatened plants in Thailand. *Omega Printing Co., Ltd.* Bangkok.

Carlsward, B. S., Whitten, W. M., Williams, N. H., and Bytebier, B. (2006). Molecular phylogenetics of Vandeae (Orchidaceae) and the evolution of leaflessness. *American Journal of Botany*, *93*(5), 770-786.

Chase, M. W., Cameron, K. M., Barrett, R. L., and Freudenstein, J. V. (2003). DNA data and Orchidaceae systematics: a new phylogenetic classification. *Orchid Conservation. 69*, 69-89.

Chase, M. W., Cameron, K. M., Barrett, R. L., and Freudenstein, J. V. (2015). An updated classification of Orchidaceae. *Botanical Journal of the Linnean Society*. *177*(2), 151-174.

Christenhusz, M. J. M., and Byng, J. W. (2016). The number of known plants species in the world and its annual increase. *Phytotaxa*. *261*(3), 201-217.

Cuénoud, P., Savolainen, V., Chatrou, L. W., Powell, M., Grayer, R. J., and Chase, M. W. (2002). Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid rbcL, atpB, and matK DNA sequences. *Amer. J. Bot*. *89*(1), 132–144.

Dalstrom, S., and Kolanowska, M. (2020). A new yellow-flowered *Chiloschista* (Orchidaceae: Aeridinae) from Thailand. *Lankesteriana. 20*(2), 241-248.

Dassanayake, M. D., and Fosberg, F. R. (1981). A Revised Handbook to the Flora of Ceylon. *Amerind Publ.* New Dhelhi., 108-194.

Davis, P. H., and Heywood, V. H. (1963). Principle of angiosperm taxonomy. Edinburgh, Oliver and Boyd

Doyle, J. J., and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin*. *19*(1), 11-15.

Givnish, T. J., Spalink, D., Amess, M., Lyon, S., Hunter, S., Zuluaga, A., Iles, W., Clements, M., Kalin, M., Leebens-Mack, J., Endara, L., Kriebel, R., Neubig, K., Whitten, W., Williams, N., and Cameron, K. (2015). Orchid phylogenomics and multiple drivers of their extraordinary diversification. Proceeding. *Biological Sciences*. *282*, 1-10.

Gyeltshen, N., Gyeltshen, C., Tobgay, K., Dalstrom, S., Gurung, D. B., Gyeltshen, N., and Ghalley, B. B. (2020). Two new spotted *Chiloschista* species (Orchidaceae: Aeridinae) from Bhutan. Lankesteriana*. 19*(1), 23-29.

Judd, W. S., Campbell, C. S., Kellogg E. A., Stevens, P. F., and Donoghue, M. J. (2016). Plant systematics: a phylogenetic approach. *Rhodora. 118*, 418-420.

Kew Science. Plants of the world online.org (Online). Available: http://www.plant softheworldonline.org/taxon/urn:lsid:ipni.org:names: 29027-1#sources.

Lindley, J. (1840). The genera and species of orchidaceous plants. *Ridgways*. London.

Pedersen, H.Æ. (2010). Species delimitation and recognition in the *Brachycorythis helferi* complex (Orchidaceae) resolved by multivariate morphometric analysis. *Botanical Journal of the Linnean Society. 162*, 64-76.

Pedersen, H.Æ., Kurzweil, H., Suddee, S., and Cribb, P. J. (2011). Flora of Thailand. Vol. 12, Part 1. *Phachachon Co.Ltd*. Bangkok.

Pfitzer, E. (1887). Entwurf einer natürlichen Anordnung der Orchideen. *Kessinger Publishing, LLC*. Carl Winter's Universitätsbuchhandlung. Heidelburg.

Posada, D., and Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics*. *14*, 817-818.

Pridgeon, A. M., Cribb, P. J., Chase, M. W., and Rasmussen, F. N. (1999). Genera Orchidacearum, Vol. 1. *Oxford University Press*. United Kingdom.

Reece, J. B., Urty, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., and Jackson, R. B. (2014). Campbell Biology (10th ed.). *Pearson*. New York.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., and Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient

Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*. *61*, 539-542.

Schlecter, R. (1926). Das system der orchidaceen. *Notizblatt des Botanischen Gaı und Museums zu BerlinDahlem. 9*, 563-591.

Seidenfaden, G. (1988). *Chiloschista* Lindl. Orchid genera in Thailand 14. Fifty-nine vandoid Genera. *Opera Botanica*. *95*, 168-181.

Simpson, M. G. (2010). Plant systematics Second Edition. *Acadimic Press is an imprint of Elsevier*. China.

Smitinand, T. (2014). Thai plant names (Revised edition 2014). *Royal Forest Department*. Bangkok.

Stevens, P. F. (2020). Angiosperm Phylogeny Website (Online). Available: http://www.mobot.org/MOBOT/research/APweb/.

Sun, Y., Skinner, D. Z., Liang, G. H., and Hulbert, S. H. (1994). Phylogenetic analysis of Sorghum and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics. 89*(1), 26-32.

Swartz, O. (1800). Afhandling on orchidemes slaegter och deras systematiska indelning. *Kongl Vetenskaps Academiens Nya Handlingar. 21*, 115-138.

Thiers, B. (2010). Index Herbariorum: A global directory of public herbaria and associatedstaff. New York Botanical Garden's Virtual Herbarium. Available Source: http://sweetgum.nybg.org/ih/. November 11, 2010

Topik, H., Peter, W., Tomohisa, Y., and Motomi, I. (2005). Molecular phylogenetics of subtribe Aeridinae (Orchidaceae): insights from plastid *mat*K and nuclear ribosomal ITS sequences. *Journal of Plant Research*. *118*, 271-284.

Topik, H., Peter, W., Tomohisa, Y., Motomi, I., and Rod, R. (2012). Phylogeny of Subtribe Aeridinae (Orchidaceae) inferred from DNA sequences Data: Advanced Analyses Including Australasian Genera. *Jurnal Teknologi*. *59*, 87-95.

Utteridge, T., and Bramley, G. (2015). The kew tropical plant families identification handbook 2ed. *Kew Publishing*, Royal Botanic Garden, Kew.

Van Steenis, C. G. G. J. (1957). Flora Malesiana ser. I, Volume 5. In: Van Steenis, C. G. G. J. (ed) 1955-1958. Specific and infraspecific delimitation., CLXVII-CCXXXIV.

Watthana, S., Topik, H., Ito, m., and Yukawa, T. (2006). Phylogeny of the genus *Pomatocalpa* Breda (Orchidaceae). *Gardens' Bulletin Singapore. 58*, 55-80.

Wood, J. (2014). Chiloschista. In: Pridgeon, A. M., Cribb, P. J., Chase, M. W., and Rasmussen, F. N. (eds.). Genera Orchidacearum, Volume 6, Epidendroideae (Part 3). *Oxford University Press*. United Kingdom., 152-156.

Wu, Z. Y., Raven, P. H., and Hong, D. Y. (2009). Flora of China. Vol. 25 (Orchidaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

Xu, Q., Zhang, G., Liu, Z., and Luo, Y. (2014). Two new species of *Dendrobium* (Orchidaceae: Epidendroideae) from China: evidence from morphology DNA. *Phytotaxa*. *174*(3), 129-143.

Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y. (2015). A molecular phylogeny of Aeridinae (Orchidaceae: Epidendroideae) inferred from multiple nuclear and chloroplast regions. *Molecular Phylogenetics and Evolution*. *85*, 247-254.

CURRICULUM VITAE

Name	Mr. Thotsaporn Chanokkhun
E-mail	thotsaporn.csc56@gmail.com
Phone	091-3487717
Date of birth	17 May 1994
Place of birth	Yasothon, Thailand
Education	B.Sc. degree in Biology from Ubon Ratchathani Rajabhat University.

Publishcations

1 **Chanokkhun, T.**, Jitpromma, T., Muangsan, N., and Watthana, S. (2023). Notes on Phylogeny of the Genus *Chiloschista* Lindl. (Orchidaceae) in Thailand. *Proceedings of the 17th South East Asian Technical University Consortium (SEATUC) Symposium.*, 393-396.

2 Jitpromma, T., **Chanokkhun, T.**, Muangsan, N., and Watthana, S. (2023). Notes on Phylogeny of the Genus *Micropera* Lindl. (Orchidaceae) in Thailand. *Proceedings of the 17th South East Asian Technical University Consortium (SEATUC) Symposium.*, 402-405.

3 Muangsan, N., Saensouk, P., **Chanokkhun, T.**, Watthana, S. (2015). Comparative leaf epidermis study in *Habenaria* spp. (Orchidaceae) from Thailand. *Biodiversitas Journal of Biological Diversity*. *23*, 4159-4168.