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

TAMMANOON JITPROMMA



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 2021 การทบทวนกล้วยไม้สกุลเอื้องช่อม่วงในประเทศไทย



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต สาขาวิชาชีววิทยาสิ่งแวดล้อม มหาวิทยาลัยเทคโนโลยีสุรนารี ปีการศึกษา 2564

## TAXONOMIC REVISION OF THE GENUS *MICROPERA* 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

(A<mark>sst.</mark> Prof. Dr. Santi Watthana) Member (Thesis Advisor)

Kyder Joons

(Assoc. Prof. Dr. Nooduan Muangsan) Member

P. Suna

(Asst. Prof. Dr. Pongthep Suwanwaree) Member

Matchai

5-515n

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

(Dr. Sirilak Chumkiew)

Member

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

นายธรรมนูญ จิตพรมมา : การทบทวนอนุกรมวิธานกล้วยไม้สกุลเอื้องช่อม่วงในประเทศไทย (TAXONOMIC REVISION OF THE GENUS *MICROPERA* LINDL. (ORCHIDACEAE) IN THAILAND) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.สันติ วัฒฐานะ, 85 หน้า.

## คำสำคัญ: เอื้องช่อม่วง, แมทเค, ไอทีเอส

E 475NET

สกุลซ่อม่วง จัดอยู่ในวงศ์กล้วยไม้ ซึ่งเป็นสกุลที่ยังไม่แน่ใจว่าเป็นสกุลที่เป็นโมโนไฟเลติก หรือไม่ โดยก่อนหน้านี้ได้มีการวิเคราะห์ไฟโลเจนี แต่ใช้เพียงชนิดเดียวเป็นตัวแทนในการวิเคราะห์ ซึ่ง การเพิ่มจำนวนชนิดจะเป็นการพิสูจน์ว่าสกุลนี้เป็นกลุ่มที่เป็นโมโนไฟเลติกส์หรือไม่ ดังนั้นการศึกษา ครั้งนี้มีวัตถุประสงค์เพื่อ วิเคราะห์ไฟโลเจนี และทบทวนทางอนุกรมวิธานสกุลช่อม่วงในประเทศไทย ซึ่งการวิเคราะห์ไฟโลเจนีจากข้อมูลทางสัณฐานวิทยาจำนวน 27 ลักษณะและอณูชีววิทยาด้วยยีนไอที เอส (ITS) และแมทเค (*mat*K) โดยการเลือกชนิดที่อยู่ในเคลดของสกุลไตรโคกลอสติสตามผล การศึกษาก่อนหน้านี้ และเพิ่มชนิดในสกุลซ่อม่วงที่พบในประเทศไทยเข้าไปวิเคราะห์ 4 ชนิด พบว่า สกุลช่อม่วง เป็นกลุ่มที่เป็นโมโนไฟเลติกส์ ผลการทบทวนทางอนุกรมวิธานจากการศึกษาตัวอย่างที่หอ พรรณไม้กรมป่าไม้ มหาวิทยาลัยโคเปนฮาเกน สวนพฤกษศาสตร์คิว และสวนพฤกษศาสตร์สมเด็จ พระนางเจ้าสิริกิติ์ พบว่ามี 4 ชนิด ในประเทศไทย ซึ่งการศึกษาครั้งนี้ได้นำเสนอรูปวิธานแยกชนิด คำ บรรยายลักษณะแต่ละชนิด ข้อมูลทางนิเวศวิทยา และการกระจายพันธุ์ รวมทั้ง ภาพวาด

ลายมือชื่อนักศึกษา ลายมือชื่ออาจารย์ที่ปรึกษา 🧹

โนโลยีสุรมา

สาขาวิชาชีววิทยา ปีการศึกษา 2564 TAMMANOON JITPROMMA : TAXONOMIC REVISION OF THE GENUS *MICROPERA* LINDL. (ORCHIDACEAE) IN THAILAND. THESIS ADVISOR : ASST. PROF. SANTI WATTHANA, Ph.D. 85 PP.

Keyword: Micropera, matK, ITS

The genus *Micropera* Lindl. belongs to the family Orchidaceae. It is one of the uncertainty monophyletic groups. The previous phylogenetic analysis employed only one species, needed more species to reveal whether this genus forms a monophyletic group or not. Thus, this study was conducted to construct the phylogeny for genus *Micropera* based on molecular and morphological data and to revise the genus *Micropera* in Thailand. The phylogenetic analysis was performed by using 27 morphological characters and ITS and *mat*K genes. The taxa in Trichoglottis clade from previous study were selected with adding 4 taxa of genus *Micropera* from Thailand. It was shown that *Micropera* is a monophyletic group based on morphological and molecular evidence. The taxonomic treatment based on specimens from Thai Forest Herbarium (BKF), Copenhagen Herbarium (C), Kew Herbarium (K), and Queen Sirikit Botanic Garden Herbarium (QBG) revealed 4 species found in Thailand. A key to species, description, ecology and distribution, as well as illustration were provided.

รั<sub>้ว้าอักยาลัยเทคโนโลยีสุร</sub>บ

School of Biology Academic Year 2021

Student's Signature	2
Advisor's Signature_	d-int

## ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my advisor, Assistant Professor Dr.Santi Watthana 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.Thotsaporn Chanokkhun 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.

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

ะ

าคโนโลยีสุรบาว

Tammanoon Jitpromma

## CONTENTS

		Page
ABST	RACT IN THAI	I
ABST	RACT IN ENGLISH	
ACKN	IOWLEDGEMENTS	
CONT	rents	IV
LIST (	OF TABLES	VI
LIST (	OF FIGURES	VII
LIST (	OF ABBREVIATIONS	IX
CHAP	PTER	
I		1
	1.1 Research objectives	2
	1.2 Research hypothesis	2
II	LITERATURE REVIEWS	3
	2.1 Classification of family Orchidaceae	3
	2.2 The genus <i>Micropera</i> Lindl	6
	2.3 Taxonomy history of <i>Micropera</i> in Thailand	8
	2.4 Phylogeny of <i>Micropera</i>	9
III	MATERIALS AND METHODS	11
	3.1 Morphological phylogenetic analysis	
	3.1.1 Taxon sampling	
	3.1.2 Morphological Dataset	
	3.1.3 Morphological phylogenetic analyses	
	3.2 Molecular Phylogeny	
	3.2.1 Taxon selection	
	3.2.2 DNA extraction	15
	3.2.3 PCR Amplification and Sequencing	
	3.2.4 Phylogenetic analysis	

# CONTENTS (Continued)

		Page
	3.3 Taxonomic revision	
	3.3.1 Materials	
	3.3.2 Morphological dat <mark>a c</mark> ollection	
	3.3.3 Taxonomic proble <mark>m</mark> solution	
	3.3.4 Nomenclature problem solution	
	3.3.5 Documentation	
IV	RESULT	20
	4.1 General morphology of the genus <i>Micropera</i>	20
	4.2 Phylogeny of <i>Microp<mark>era</mark></i> based on molecular data	
	4.3 Phylogeny of <i>Micropera</i> based on molecular data	
	4.3.1 Polymerase Chain Reaction	
	4.3.2 Sequences and alignment	
	4.4 Taxonomic Treatment	
V	CONCLUSION	56
	5.1 Conclusion	
	5.2 Suggestion	
REFFE	RENTES	57
APPEN	DICES	
	APPENDIX A DNA MARKER STANDARD CURVE	63
	APPENDIX B DNA SEQUENCES	64
	APPENDIX C PARSIMONOUS TREES FROM THIS ANALYSIS	
CURRIC	CULUM VITAE	85

## LIST OF TABLES

Table	Pag	e	
2.1	Micropera species and distribution in Thailand	8	
3.1	List of morphological character <mark>s a</mark> nd character states used for phylogeny		
	analysis1	2	
3.2	Species and DNA regions used in this study1	4	
3.3	The amplification reaction of gene sequences	6	
4.1	The data metric is based on morphological characters	3	
4.2	The information derived from the parsimony analysis of the <i>mat</i> K, ITS and		
	combined genes	7	
B.1	DNA sequences ITS	9	
B.2	DNA sequences matk	1	



## LIST OF FIGURES

Figure	P	age
2.1	General characters of the <i>Micropera</i> Lindl	7
2.2	The Bayesian tree obtained fro <mark>m</mark> analysis of the combined dataset of	
	subtribe Aeridina	. 10
3.1	Floral diagram for measuring	. 19
4.1	The vegetative and reproductive of <i>Micropera</i> Lindl	. 21
4.2	Tree based on morphological characters of a consensus tree	. 24
4.3	Agarose gel electrophoresis for amplified matK gene of Micropera	. 25
4.4	Agarose gel electrophoresis for amplified ITS gene of <i>Micropera</i>	. 26
4.5	One of the original trees of Trichoglottis clade based on the ITS marker	. 28
4.6	Bootstrap consensus tree of Trichoglottis clade based on the <i>mat</i> K	
	markers	. 29
4.7	Original tree of Trichoglottis clade based on the <i>mat</i> K markers	. 30
4.8	Bootstrap consensus tree of Trichoglottis clade based on the matK	
	markers	. 31
4.9	Original tree of Trichoglottis complex based on the combined nuclear	
	(ITS), and plastid ( <i>mat</i> K) markers	. 33
4.10	Bootstrap consensus t <mark>ree o</mark> f Tri <mark>choglottis</mark> complex based on the	
	combined nuclear (ITS), and plastid ( <i>mat</i> K) markers	. 34
4.11	Micropera pallida (Roxb.) Lindl	. 39
4.12	Micropera pallida (Roxb.) Lindl	. 40
4.13	Distribution map of <i>Micropera pallida</i> (Roxb.) Lindl	. 41
4.14	Micropera thailandica (Seidenf. & Smitinand) Garay	. 44
4.15	Micropera thailandica (Seidenf. & Smitinand) Garay	. 45
4.16	Distribution map of Micropera thailandica	
	(Seidenf. & Smitinand) Garay	. 46
4.17	Micropera obtusa (Lindl.) Tang & Wang	. 49

# LIST OF FIGURES (Continued)

Figure		Page
4.18	Distribution map of Micropera obtusa (Lindl.) Tang & Wang	50
4.19	Micropera rostrata (Roxb.) Balakrishnan	53
4.20	<i>Micropera rostrata</i> (Roxb.) Balakrishnan	54
4.21	Distribution map of <i>Micropera <mark>ros</mark>trata</i> (Roxb.) Balakrishnan	55
A.1	DNA marker standard curve	58
C.1	All shortest tree from maxim <mark>um</mark> parsimony analysis, based on the ITS	
	gene	78
C.2	All shortest tree from maximum parsimony analysis, based on the matK	
	gene	80
C.3	All shortest tree from maximum parsimony analysis, based on the	
	combined nuclear (ITS) and plastid ( <i>mat</i> K) gene	83



## LIST OF ABBREVIATIONS

bpbase pairCTABCetyltrimethylammonium bromide

DMSO Dimethyl sulfoxide

DNA Deoxy-ribonucleic acid

mM millimolar

PCR Polymerase chain reaction

pmol/µL picomole per micro<mark>li</mark>ter

µL microliter

°C degree Celsius

รัฐมาย เป็นสุราย เราเวิกยาลัยเทคโนโลยีสุราย

# CHAPTER I

The orchid is one of the most beautiful plants and popular for ornamental purposes. There are about 27,000 species, distributed in almost every region in the world. Taxonomists are trying to identify plant species and classify them correctly for biological concepts. Southeast Asia is one area which has so diverse orchid species, especially Thailand. Previous study by taxonomists estimated that there are around 1,200 orchid species in Thailand (Boonkorkaew, 2010). There are also plenty of beautiful wild orchid species and used as an ornamental plant.

Name of the orchid species, scientific or botanical name, is so important for worldwide communication. Taxonomy is a subject of biology, covering description, identification, nomenclature, and classification (Simpson, M. G., 2010). Botanists use biological characters such as morphology, anatomy, DNA sequence to group the taxonomic unit such as species, genus, family, etc. In the past, we used only morphological character to be the taxonomic character to distinguish the taxa. After evolution theory, proposed by Charles Darwin, the organism classification has been reflected by evolutionary concepts. Thus, the phylogenetic classification is a more popular use for systematics, a covered task of taxonomy. The taxonomic group must be a monophyletic group. However, some taxonomic groups have not shown the clearly monophyletic or natural group, due to few samples for phylogenetic analysis.

The genus *Micropera* (Roxb.) Lindl. is one of the uncertain generic groups, showing low phylogenetic statistical value support (Pridgeon, A. M., Cribb, P. J., Chase, M. W., and Rasmussen, F. N., 2014) So far, there is only one species used for phylogenetic analysis. In Thailand, Seidenfaden (1988) reported this genus 4 species. However, he has never provided any description based on specimens found in Thailand. Besides, it needs to provide the taxonomic information to Flora of Thailand

which reports into a serial book, including the description of the genus, key to species, description of each species, distribution, and ecology information.

To support the Flora of Thailand project, I intend to revise the genus *Micropera* Lindl. in Thailand based on specimens collected from the field and aboard herbaria in Thailand. Moreover, I will add more DNA sequences of the species which have no report and use them to reconstruct the phylogenetic relationship of this genus, based on morphological and molecular data.

#### 1.1 Research objectives

1. To construct the phylogeny for genus *Micropera* based on molecular and morphological data.

2. To revise the genus *Micropera* Lindl. in Thailand.

#### 1.2 Research hypothesis

1. The genus *Micropera* is a monophyletic group based on morphology and molecular evidence.

2. In Thailand, this genus has 4 species according to Seidenfaden (1988).



## CHAPTER II LITERATURE REVIEW

#### 2.1 Classification of family Orchidaceae

The orchids are the largest family of flowering plants, distributed in 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., ... Cameron, K., 2015). It belongs to Orchidaceae family occurring in almost every habitat nearly worldwide (Chase, M. W., Cameron, K. M., Barrett, R. L., and Freudenstein, J. V., 2003). According to Angiosperm Phylogeny Website (Stevens, P. F., 2020), the orchidaceae is classified into order Asparagales with closed related to Liliales, and Monocotyledons clade. The sister group of the Orchidaceae is the clade comprises Boryaceae, Blandfordiaceae, Lanariaceae, Asteliaceae and Hypoxidaceae. The classification of the Orchidaceae is following as:

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 a persistent outer layer of dead cells, called a velamen. Leaves are entire margin, spirally arranged, usually distichous and often with a basal sheath, rarely reduced to sales. Inflorescences are paniculate or racemose. Flowers are usually twisted through 180 degrees, called resupinate. 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. Pollen 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 phytomelan (adopted from Utteridge and Bramley, 2015).

The revised and updated classification of the flowering plant 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).

Apostasioideae is the most primitive group of this family. They are small or large terrestrial orchids, never being epiphyte. Roots are swollen. Stems are usually erect or ascending, simple or branched. Leaves are spirally arranged and plicate. Leaves texture are herbaceous to papyraceous. Inflorescences are terminal racemose, erect or pendulous. Flowers are more symmetric, spirally arranged, small to mediumsized, white, yellow, or deep yellow. Sepals have 3, not free. Petals have 2, free. Labellum is not differentiated, like sepals and petals. Functional stamens are 2 or 3. Column straight to strongly curved. Ovary is cylindrical or ellipsoid, usually 3 locules. Capsule is thin-walled or fleshy, 3-locular. Seeds are very numerous. Seed shape is ovoid to elliptic (Wu, Z. Y., Raven, P. H., Hong, and D. Y., eds. 2009). They consist of two genera, *Neuwiedia* and *Apostasia*. Both genera are found in Thailand.

Cypripedioideae is terrestrial, lithophytic and epiphytic rhizomatous herbs. Roots are usually succulent and long. Rhizome is short to elongate. Shoots are often unbranched, short to long, erect, and clumpy. Leaves are succulent and coriaceous, plicate or conduplicate. Inflorescence is terminal raceme or one-flowered. The Flowers are showy and colorful. Pedicels are obscure and short. Dorsal sepal is erect to hooded over the labellum. The lateral sepals are forming into a synsepal. Petals are free, inflexed or reflexed. Labellum is usually saccate as a trap for pollinators. Column is short and stalked. Anthers have two. They are bilocular, attaching on short obtuse to acute filaments. Staminode is presented and located at the apex of the column and forming into a shield-like organ. Two fertile anthers are placed on the lateral side of the column. Pollinia is powdery or viscid. Capsule is erect or pendulous, threeribbed, cylindrical to ellipsoidal (cite: flora Wu, Z. Y., Raven, P. H., Hong, and D. Y., eds. 2009). There are five genera namely, *Paphiopedilum, Cypripedium, Mexipedium, Selenipedium* and *Phragmipediumand*. Only the genus *Paphiopedilum* has been recorded in Thailand.

Orchidoideae are terrestrials with fleshy rhizomes or tubers, rarely epiphyte or holomycotrophic. Leaves are simple, spiral and sessile, with one to many. Inflorescence is terminal raceme or baring a single flower. Flowers are small to large, usually resupinate, flat to tubular, often showy, green, white, yellow, pink, purple or red. Pedicels are often short. Dorsal sepal is free or often adnate to the petals to form a hood over the column. Lateral sepals are usually free, sometimes connate and oblique at base to form a spur-like mentum, rarely connate to the base of the column. Petals are often adnate to dorsal sepal. Labellum is entire, three-or five-lobed, with a spur at the base. Column is basifixed, rarely adnate to the other floral segments at the base, erect or decumbent, anther bilocular, longer than or as long as the rostellum. Pollinia has two or four, sectile, attached by short to elongate caudicles to one or two viscidia. Staminodes are two, sessile or stalked, stigma entire or two-lobe, sessile or stalked. Rostellum is usually two- or three-lobed, shorter than or as long as the anther. Ovary is distinct, glabrous or less frequently hairy and glandular (Wu, Z. Y., Raven, P. H., Hong, and D. Y., eds. 2009).

Vanilloideae is terrestrial, perennial, monopodial or sympodial orchid, sometimes achlorophyllus. Roots are elongate, typically fleshy, fibrous or swollen into tubers. Stems are upright, grabrous, sometimes elongated and climbing. Leaves are alternate or whorled, often fleshy or coriaceous. Inflorescences are terminal or axillary raceme, o+r panicle sometime with one flower. Flower is mostly showy, resupinate, and variably fragrant. Sepals are free, glabrous or externally pubescent, mostly fleshy and spreading, yellow, white, green, pink, or brown. Petals are free, glabrous, fleshy or membranaceus, spreading or incurved. Labellum is free or fused with the column, forming a floral tube, simple or more often trilobed. Column is usually slender, glabrous. Stigma is usually emergent. Rostellum is often acute and bent forward. Column apex is frequently hooded. An anther is terminal, hyperincumbent, mobile. Pollen is mostly loose, shed in monads, sometimes tetrad or rarely forming true pollinia without accessory structure. Ovary is unilocular or trilocular. Fruit is a capsule or rarely a fleshy and indehiscent berry (Wu, Z. Y., Raven, P. H., Hong, and D. Y., eds. 2009).

Epidendroideae are the largest group in the family Orchidaceae being epiphyte, lithophyte, terrestrial, rarely myco-heterotrophs. Growth form is monopodial or sympodial. Roots often have velamen. Aerial roots are generally cylindrical or flattened. Rhizome is usually stout, creeping and elongated. Pseudobulbs are usually present with one- to several-noded, swollen or not swollen, usually covered with leaves sheaths. Leaves are one to several distichous or alternate, often fleshy or coriaceous. Inflorescences are lateral, terminal, axillary raceme or panicle. Flowers have one to many, mostly showy, resupinate, variably fragrant. Sepals are free, glabrous or externally pubescent, mostly fleshy and spreading, orange, yellow, white, green, pink, red, or brown, lateral sepals are often obligue. Petals are free, elliptic or oblong to linear-lanceolate, glabrous, usually thinner than sepals, spreading or incurved. Labellum is usually dominant, simple to strongly trilobed, smooth to variously pilose, spur present or lacking. Column is usually curved, glabrous, sometimes with wing-like lateral auricles or wings. Stigma is usually emergent. Column is shorter than its foot. Anther is terminal, hyperincumbent, mobile; pollen is mostly packed, two, four or eight. Ovary is often curved, glabrous, somewhat waxy with 6-grooved. Fruit is a capsule or rarely a fleshy, indehiscent berry (Wu, Z. Y., Raven, P. H., Hong, and D. Y., eds. 2009). ยาลัยเทคโนโลยีสุร

#### 2.2 The genus *Micropera* Lindl.

The generic name of *Micropera*, derived from the Greek words *mikros* meaning little or small and *pera* meaning wallet or pouch, John Linley who first described the genus in 1832 with one species, *Micropera pallida* (Linley, J. 1832). This genus had been accepted for a long time as *Camerotis* Lindl., by Lindley himself changed later in 1833. Until Tang and Wang (1951) and Garay (1972) accepted the generic name, *Micropera*.

This genus can be recognized by being a rather long monopodial epiphyte. Inflorescence is lateral raceme to ca., 15 cm long with few to many flowers. Flowers are usually non-resupinate. The lateral sepals connate for a short distance at base. The petals are linear-obovate or oblong, obtuse, and usually reflexed. The Labellum is porrect, succate, and spurred. The labellum apex is trilobes. The midlobe of the labellum much smaller than sac or spur. Spur is succate providing the longidutinal septum and usually produces a bilobed callus on the front wall. The column is variable, usually extended into a proboscis-like structure. The pollinia has four, as two unequal, attached on linear stripes (Figure 2.1). The classification of the Micropera is following as:

Family: Orchidaceae

Subfamily: Epidendroideae

Tribe: Vandeae

Sub<mark>tribe</mark>: Aeridinae

Genus: Micropera Lindl.



Figure 2.1 General characters of the genus *Micropera* Lindl. drawn by Thotsaporn Chanokkhun.

## 2.3 Taxonomy history of Micropera in Thailand

Seidenfaden et al., 1988 reported the *Micropera* species found in Thailand comprising four species (Table 2.1).

No.	Species	Locations in Thailand		
1.	<i>Micropera pallida</i> (Roxb.) Lindl.	Nakorn Sawan, Kamphengpet, Doi Suthep		
		(Chiang Mai), Phu Khieo (Chayaphum), Nam		
		Prom, Pitsanulok to Lomsak at km 80, Dat		
		Don (Chayaphum, Beteen Nam prom and		
		Tunkamang (Chayaphum), N of Thung		
		Sala <mark>eng</mark> Luang, Namnao, Koh Chang, Khlung,		
		Chan <mark>tabu</mark> ri, Krabin ( Prachinburi) , Khao		
		Soidao Nua, Between Chantaburi and Trat,		
		Makham, Chantaburi, Near Whanka, Saiyok,		
		Sam Roy Yat (Prachuap), Ban Tak Tawan		
		( Pranburi) , Huay Nam Wing, Banrai		
		(Uthaidhani), Bangbao (Surat), Khao Chem		
		Tungsong, Ban Prakap (Songkla), Huay Ai		
		Chuang, W of Bangto, W of an Khao Tong		
	E.	(Phang-nga)		
2.	Micropera thailandica (Seidenf.	Phu Khieo (Chayaphum), Khao Yai, Makham,		
	& Smitinand) Garay	Sriracha, Foothills of Khao Sabab, NW of		
		Phato (Ranong), Eastern foothills of Khao		
		Phra Mi, Thungnui (Satul), Huay Yawt, Khao		
		Den (Patalung), Khao Soidao (Patalung),		
		Khao Kheo Range		
3.	<i>Micropera obtusa</i> (Lindl.) Tang	Khao Bangto, Phang-nga		

 Table 2.1 Micropera species and distribution in Thailand.

& Wang

No.	Species	Locations in Thailand
4.	Micropera rostrata (Roxb.)	Ban Mussoe ( Tak) , Muang Fang, Phu
	Balakrishnan	Krading, Nakorn Nayok

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

### 2.4 Phylogeny of Micropera

According to the molecular phylogenetic analysis by Topik, H., Peter, W., Tomohisa, and Y., Motomi, I. (2005), based on two DNA regions of ITS and *mat*K. The result shows the genus Micropera is related to the genus *Adenoncos* and *Acampe* forming a clade together, *Acampe* alliance. However, they used only 1 taxon of the genus *Micropera*.

The phylogenetic analyses of subtribe Aeridinae by Topik, H., Peter, W., Tomohisa, Y., Motomi, I., and Rod, R. (2012), using two DNA sequences; ITS and *mat*K. The taxa are grouped into 11 subgroups, the result inconsistent with previous analyses (Topik, H., Peter, W., Tomohisa, and Y., Motomi, I., 2005). However, the genus *Micropera* still was placed within the *Acampe* alliance.

The phylogeny analysis of subtribe Aeridinae including 211 species in 74 genera (Figure 2.2), the study using 1 taxon of *Micropera*, that is *M. pallida*. The genus *Micropera* was placed within the *Trichoglottis*, and formed a clade to the genus *Acampe* and *Adenoncos* (Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y., 2015).



Figure 2.2 The Bayesian tree obtained from analysis of the combined dataset of subtribe Aeridinae (Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y., 2015).

# CHAPTER III MATERIALS AND METHODS

#### 3.1 Morphological phylogenetic analysis

#### 3.1.1 Taxon selection

According to the molecular phylogenetic analysis by Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y. (2015), the genus *Micropera* is related to the genus *Acampe* and *Adenoncos* forming a clade together. The well statistics supporting this clade indicated that these three genera have shared common ancestor. In the other word, these three genera are a homology group, sharing the same common ancestor. However, they used only 1 taxa of the genus *Micropera*. I am interested in determining whether *Micropera* is a monophyletic group or not, based on morphology when comparing it with the related genera. Thus, all species of *Micropera, Acampe, Adenoncos*, and *Cottonia* represented in Thailand shall be selected as an ingroup, due to the specimen examined opportunity. For the outgroup, *Trichoglottis triflora* (Guillaumin) Garay & Seidenf., was selected due to being the sister group with the *Acampe-Micropera-Adenoncos* clade.

#### 3.1.2 Morphological Dataset

For building the morphological matrix table between species and discrete characters, 1 compared the morphological character of each species which were selected for ingroup and outgroup. Characters and character states were based on my own observation from the herbarium specimens and the literature (Seidenfaden, 1988). Morphological terminology was followed by Stearn (1989). Data were observed by using a Nikon coolpix P950 stereomicroscope. All 27 morphological characters derived from spirit and herbarium specimens and relevant literatures have been employed for cladistics analysis, 25 binary and 2 multistate characters (Table 3.1), where missing data are represented by "?" and non-applicable data by "-".

Table 3.1 List of morphological characters and character states used for phylogenyanalysis. The letter a, dash and question mark are employed for apolymorphic character state, inapplicable data and missing data,respectively.

No.	Character	Character state	
1.	Stem growth direction	erect (0), more or pendulous (1)	
2.	Stem length	more than 30 cm long (0), less than 20	
		cm long (1)	
3.	Leaves apex	lobed (0), without lobe (1)	
4.	Leaves shape	flatted (0), semiterete (1)	
5.	Leaves sheath	smooth (0), finely wrinkled (1)	
6.	Peduncle length	long, more than 1 cm (0), short less	
		than 1 cm (1)	
7.	Rachis branching	mo <mark>re th</mark> an 3 (0) less than 3 (1)	
8.	Pedicel and ova <mark>ry le</mark> ngth	more than 1.5 times as long as dorsal	
		sepal (0), <mark>s</mark> horter there 1.5 times (1)	
9.	Petal color		
10.	Sepal and petal marking	with reddish brown longitudinal brands	
		(0), concolor (1), variegated (2) botched	
		(3)	
11.	Labellum size: length from base of	more than 2 times longer than dorsal	
	column to apex o <mark>f epichil</mark>	sepal(0), less than 2 times (1)	
12.	Labellum with spur	flatted without spur (0) succate (1) with	
	ั <sup>71</sup> ยาลัยเทคโ	spur (2)	
13.	Adaxial epichil color	with (dark-) reddish brown marking (0),	
		without (1)	
14.	Labellum mid-lobe size	larger than sidelobe (0) smaller than	
		sidelobe (1)	
15.	Labellum sidelobe	present (0), not present (1)	
16.	Labellum hairiness: margin of	hairy (0), glabrous (1)	
	hypochil		

Table 3.1 List of morphological characters and character states used for phylogenyanalysis. The letter a, dash and question mark are employed for apolymorphic character state, inapplicable data and missing data,respectively (Continued).

No.	Character	Character state
17.	Spur septum	without (0) or with (1)
18.	Spur color	pure yellow, whitish or brownish (0),
		yellow tint with dark brown (1), white
		tint with pink (2)
19.	Spur appendage at back wall spur	without (0), tongue-like, thick (1)
20.	Spur appendage at front wall spur	without (0), with (1)
21.	Labellum appendage: <mark>at e</mark> pichil	without appendage at apex of epichil
		(0), with appendage at apex of epichil
		(1)
22.	Labellum callu <mark>s:</mark> at hypochil	with <mark>2 call</mark> i (0) with 1 callus (1)
23.	Column size	short (length/width <1) (0), long
		(length/width>1) (1)
24.	Column twist	no (0), yes (1)
25.	Column with apical appendage	with (0), without (1)
26.	Pollinia number	4 (1), 2 (0)
27.	Rostellum size	equal or shorter than column diameter
	· ·	(0) or longer(1)
	- Oha	

<sup>อุ</sup>กยาลัยเทคโนโลยีสุจ

#### 3.1.3 Morphological phylogenetic analyses

The maximum parsimony (MP) analysis was run in TNT v. 1.1 (Goloboff, Farris, and Nixon, 2008). All characters were equally weighted and unordered (Fitch, W. M., 1971). The data sets were analyzed by the heuristic search with bisection-reconnection (TBR) branch swapping and the MULTREES option on, saving all the most parsimonious trees (MPTs). The bootstrap analysis will be conducted to evaluate the internal support values of clades (Felsenstein, J., 1985) with 10,000 replicates with faststep searching (Watthana et al., 2006).

## 3.2 Molecular Phylogeny

#### 3.2.1 Taxon selection

Table 3.2 Species and DNA regions used in this study, vouchers and GenBankaccession numbers. A hyphen (-) indicates missing data, an asterisk (\*)denotes sequences obtained in this study, and the remaining sequencesare from GenBank.

Species	Voucher	nrITS	matK	
Acampe orchracea	Carlsward 206 (SEL)	DQ091707	DQ091314	
Acampe prarmorsa	SBB-0163	MN517126	JN004343	
Acampe rigida	JK-DEBCR-mat-35	KJ733385	MN523477	
Adenoncos parviflora	KFBG2703	KY966412	AB217703	
Cottonia peduncularis	SBB-0863	JN114477	JN004395	
Micropera obtusa				
Micropera pallida				
Micropera rostrata	สยเทคเนเด		-	
Micropera thailandica	-	-	-	
Trichoglottis triflora	KFBG2682A	KY966964	KY966678	

#### 3.2.2 DNA extraction

DNA was extracted from plants using the Genomic DNA Isolation Kit (Plant), (Bio-Helix, Taiwan), following the manufacturer's protocols.

1) Sample Preparation: 50 mg of fresh plant tissue or 25 mg of dry plant tissue were used. Using a mortar and pestle, sample was ground to a fine powder under liquid nitrogen.

2) Lysis: 500  $\mu$ l of Buffer PL and 0.5  $\mu$ l of RNase A (50 mg/mL) were added before the sample was ground. The sample was transferred to a 1.5 mL microcentrifuge tube. The sample was Incubated for 30 minutes at 75 °C (Every 10 minutes, inverted the tube), The sample was centrifuged at 14,000 x g for 5 minutes, and transferred a new 1.5 mL microcentrifuge tube with the supernatant.

3) DNA Binding: To the clear supernatant from the previous step, isopropanol was added the same volume of and vortex for 5 seconds (e.g., add 350  $\mu$ L Isopropanol to the 350  $\mu$ L supernatant). After that, the column PC was put in a 2 mL Collection Tube, the clear supernatant was transferred the mixture to the column PC, and centrifuged for 30 seconds at 14,000 x g, removed the flow-through and placed the column PC back into the collection tube.

4) Wash: the column PC was added 400  $\mu$ L of the buffer W1, centrifuged at 14,000 x g for 30 seconds. The flow-through was removed and placed the column PC back into the same collection tube, 600  $\mu$ L of the Buffer W2 (Ethanol added) were added into the column PC, centrifuged for 30 seconds at 14,000 x g. The flow through was discarded and placed the column PC back into the same collection Tube. Buffer W2 was removed by centrifuged for 2 minutes at 14,000 x g again.

5) DNA Elution: The supernatant was transferred to 1.5 mL microcentrifuge tube with the dried column PC. The supernatant was added 50-200  $\mu$ L of the Pre-Heated Buffer BE or TE into the center of the column matrix, let aside for 3 minutes at 75°C. Elution of the purified DNA by centrifuged at 14,000 x g for 2 minutes to. Extracted DNA was stored in -20°C.

#### 3.2.3 PCR Amplification and Sequencing

The amplification of the *mat*K region was performed using a primer pair, 390F and 1326R (Cuénoud, P., Savolainen, V., Chatrou, L. W., Powell, M., Grayer, R. J., and Chase, M. W., 2002). The 50  $\mu$ L amplification reaction included 25  $\mu$ L OnePCR Ultra, 2.5  $\mu$ L each primer, see Table 3.3 (5 pmol/ $\mu$ L), 1  $\mu$ l of template DNAs and 19  $\mu$ L of free water. The polymerase chain reaction (PCR) profile consisted of an initial 5 min premelting stage at 95°C, followed by 30 cycles of 30 s at 95°C (denaturation), 1 min at 55°C (annealing), 40 s at 72°C (extension), and a final 7 min extension at 72°C (Kocyan, A., Vogel, E. F., Conti, E., and Gravendeel, B., 2008).

For ITS sequences, amplification was performed using a primer pair, 17SE and 26SE (Sun, Y., Skinner, D. Z., and Liang, G. H., 1994). The 50  $\mu$ L amplification reaction included 25  $\mu$ L OnePCR Ultra, 2.5  $\mu$ L each primer, see Table 3.3 (2 pmol/ $\mu$ L), 1  $\mu$ L of template DNAs, 1  $\mu$ L of DMSO, and 18  $\mu$ L of free water. The polymerase chain reaction (PCR) profile consisted of an initial 5 min premelting stage at 95°C, followed by 30 cycles of 30 s at 95°C (denaturation), 1 min at 55°C (annealing), 40 s at 72°C (extension), and a final 7 min extension at 72°C (Kocyan, A., Vogel, E. F., Conti, E., and Gravendeel, B., 2008).

Loci	Primers	Sequences	References
matK	390F	CGATCTATTCATTCAATATTTC	Cuénoud et al.,
	5.		2002
	1326R	TCTAGCACACGAAAGTCGAAGT	Cuénoud et al.,
		าสยเทคเนเลอง	2002
ITS	17SE	ACGAATTCATGGTCCGGTGAAGTGTTCG	Sun <i>et al.,</i> 1994
	(Forward)		
	26SE	GAATTCCCCGGTTCGCTCGCCGTTAC	Sun <i>et al.</i> ,1994
	(Reverse)		

Table 3.3 The amplification reaction of gene sequences.

#### 3.2.4 Phylogenetic analyses

Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y. (2015) studied the phylogeny of tribe Aeridinae. They included the genus *Micropera* by using only one taxa of *M. pallida* as a representative. In this study, I intended to reveal the phylogeny position of the genus *Micropera* whether it is a monophyletic or not by adding 3 taxa of Thai Micropera for molecular phylogeny analysis. The ingroup in this study included the genus Acampe and Adenoncos which formed a clade with the genus Micropera according Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y. (2015) and Hiyadat et al., 2005, 2013. The genus Cottonia peduncularis a monotypic species is a sister group with the subclade of *Micropera*, *Acampe* and Adenoncos. Thus, I used it as an outgroup. Trichoglottis triflora also was selected as another outgroup based on Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y. (2015). New ITS and *mat*K sequences of Thai Micropera including *M. pallida*, *M.* thailanda, M. obtusa and M. rostrata derived from this study. The other taxa were downloaded from genbank (Table 3.2). Maximum pasomony (MP) was employed with MEGA-XI software version 10.1 to construct the phylogenetic tree. Unordered and equally weighted of all characters were set before analysis (Fitch, W. M., 1971). The data set was analysed by the heristic search method with bisection-reconnection (TBR) branch swapping. Consensus tree with bootstrap value was presented (Felsenstein, J., 1985) with 1,000 replications and fast step searching was used to evaluate clades for internal support. กยาลยเทคโนโลยีสร้

#### 3.3 Taxonomic revision

#### 3.3.1 Materials

All specimen that collected in Thailand, including The Forest Herbarium (BKF), Queen Sirikit Botanic Garden (QBG), Bangkok Herbarium (BK), Kasin Suvatabandhu Herbarium Museum Chulalongkorn University (BCU), Chiang Mai University Herbarium (CMUB), Khon Kaen University Herbarium (KKU), Prince of Songkla University Herbarium (PSU) were studied. The specimen information shall be noted, including dried and spirit specimens. In addition, I studied the specimen pictures from KEW Herbarium (K) and Copenhagen Herbarium (C) taken by Santi Watthana during 2009, as well as type specimens which are available from the website of each relevant Herbaria.

#### 3.3.2 Morphological data collection

I exclusively measured and note morphological characters from dried and spirit specimens by using a stereo microscope or a light microscope to compare the morphological characters covering parts of the stem, leaves and flower of each specimen, collected from Thailand. All vegetative characters were measured in a dry condition. Measurements of the reproductive parts from dried specimens were made after soaked with hot water and a few drops of detergent added for about 15 minutes. The botanical glossary of Stern (1989) will be consulted for the descriptive terminology.

#### 3.3.3 Taxonomic problem solution

All specimens were sorted in separated groups based on similarity and continuous variation of character set. The discrete characters wear detected to define the scope in the species. The principles of species delimitation used in this revision wera relied on traditional, morphological species concept (cf. van Steenis, 1957; Davis and Heywood, 1963, that is, the accepted species were recognized by discrete variation in various characters. Indeed, the taxonomic character shall be the discrete character, which were be used for key identification construction. If there is much similarity among taxa, the taxonomic unit of subspecies or variety was applied instead. Non-overlapping geographical range will be treated as subspecies, whereas overlapping geographical range was treated as varieties. The morphological measurement of some complicated structure is indicated in Figure 3.1.

#### 3.3.4 Nomenclature problem solution

Each defined species will be compared with type specimens of nomenclature. Whenever the type is in the set of the species group, the corrected name of that type shall be applied as the accepted group. In case, if there are more than one type specimen matching with the species group, the accepted name shall be followed by the rule of the International Code of Nomenclature (ICN). All synonyms shall be noted.

#### 3.3.5 Documentation

In this study, I followed the Flora of Thailand format, providing literature, description, ecology, distribution, and vernacular name. Some notes for each species, if there is, were added. Each species was illustrated. Mapping of each species in

Thailand also was provided. For conservation status, I followed the Guidelines for Using the IUCN Red List Categories and Criteria. Version 14 (IUCN Standards and Petitions Committee., 2019).



Figure 3.1 Floral diagram for measuring. A: column length; B: pedicel and ovary length; C: front side length of labellum or labellum height; D: back side length of labellum or labellum length; E: upper side of labellum; F: labellum side lobe length; G: labellum side lobe length.

# CHAPTER IV RESULTS

#### 4.1 General morphology of the genus Micropera

*Micropera* is a monopodial perennial epiphytic herb. Roots arise on the main steam and are typical of vandaceous orchids, thick upto 3 mm in diameter and up to more than 1 m long. The aerial root is terete. Perching roots are slightly flattened on the substrate side. Stems are terete erect or slightly pendulous, covered by leaf sheaths.

Young leaves are conduplicate, spiral arrangement. Leaf blades are dorsoventrally flattened, linear and thick, unequal lobbed, entire. It is no petiole but its base is a sheath covering the stem.

Inflorescence is racemose, which arises near the apical part of the stem. Basal bracts are short tubular, while the upper ones are triangular. Scape is modulate long, more than 5 cm and up to 17 cm. Flowers are medium size

Flowers are non-resupinate, small to medium-sized, (pale-) yellow or light pink. *Sepals* and *petals* are free, similar. *Labellum* is conspicuously spurred or saccate, 3-lobed. The lateral lobes are broad, erect. The mid-lobe is smaller, fleshy. The spur has a longitudinal septum inside, often ornamented at its entrance.

Column is stout to slender, lacking a foot. The rostellum projection is prominent beaked, sometimes twisted. Pollinia has 4, in 2 subequal pairs on a common long stipe. The viscidium is elliptic, very small. Fruit a capsule, teret. Seeds dust-like, numerous.



Figure 4.1 The vegetative and reproductive parts of *Micropera* Lindl. A. Habit of *M. pallida* in nature. B. Showing the monopodial epiphytic herb of *M. thailandica*. C. Inflorescence of *M. pallida*. C. Flower of *M. rostrata*.

#### 4.2 Phylogeny of *Micropera* based on morphological data

Data metrics among ingroup and outgroup taxa were shown in Table 4.1. Figure 4.2 shows a consensus tree based on morphological characters with bootstrap value more than 50. The analysis of the data matrix, comprising 13 terminal taxa and 27 characters, resulted in 76 shortest maximum parsimony with a minimum length of 42 steps, a retention index (RI) of 0.94 and a consistency index (CI) of 0.90.

The analysis showed that the genus *Micropera* and *Adenoncos* formed a monophyletic group with highly bootstrap support 99 and 100, respectively. The *Micropera* has spur septum, thick tongue-like appendage at the back wall of the spur, thick appendage at the front wall of the spur, column without apical appendage, and rostellum size longer than column as synapomorphies evolution characters. *M. thailandica* and *M. obtusa* formed a sister group with a long column (longer than wide) as a synapomorphy, with low bootstrap support. However, the relationship among the 4 species is not clear.

It should be noted that the genus *Adenoncos* has quite different morphological characters among this group which formed a monophyletic group based on molecular phylogeny (Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y., 2015). While, the genus *Acampe* mixed with the genus *Cottonia peduncularis*, a monotypic genus. The low resolution of phylogenetic analysis seems to be high homoplasy of each character state, appearing several times. Although the morphological inference for phylogenetic analysis usually gives vague results, it is still more important for science (Wiens, J. J., 2004).

Species	Character state
Cottonia peduncularis	00000 0-000 00000 00 00000 00
Micropera pallida	00000 01101 12110 11111 0-001 01
Micropera thailandica	00000 01101 12110 11111 0-101 01
Micropera obtusa	00000 01101 12110 11011 0-111 01
Micropera rostrata	00000 01101 12110 11211 0-001 01
Adenoncos sumatrana	11111 11101 111-1 10000 1100- 10
Adenoncos major	11111 11101 11100 10000 11000 10
Adenoncos parviflora	11111 11101 11100 10000 1100- 10
Adenoncos vesiculosa	11111 11101 111-1 10000 1100- 10
Acampe praemosa	00000 11002 12100 10000 0-000 00
Acampe rigida	00000 01002 12100 10000 0-000 00
Acampe ochracea	00000 0-002 12100 10000 0-000 00
Acampe joiceyana	00000 00013 12100 10200 0-000 00

**Table 4.1** The data metric is based on morphological characters. "-"=inapplicable and polymorphic; "?" =missing data (Dogru-Koca, 2016).



Figure 4.2 Tree based on morphological characters of a consensus tree. The number on the branch shows the bootstrap value of more than 50. Each character is mapped on the branches, the black rectangular shows the unique synapomorphic characters, the white rectangular shows the homoplasy characters.
# 4.3 Phylogeny of *Micropera* based on molecular data4.3.1 Polymerase Chain Reaction

The size of PCR products calculated from the standard curve (Appendix A). The bands of *mat*K gene of *Micropera* are shown in Figure 4.3. The product size of *M. pallida* 1,200 bp., *M. thailandica* 1,200 bp., *M. obtusa* 1,600 bp., and *M. rostrata* 1,400 bp.

The length of ITS fragments (Figure 4.4). The product size of *M. pallida* 1,400 bp., *M. thailandica* 1,500 bp., *M. obtusa* 1,400 bp., and *M. rostrata* 1,400 bp. The sequences are shown in the appendix B.



Figure 4.3 Agarose gel electrophoresis for amplified matK gene of Micropera. Bands were fractionated by 1.5 % TBE agarose gel (25 min., 100 V/cm); Lane: M = One Mark B DNA Ladder, 1 = M. pallida, 2 = M. thailandica, 3 = M. obtusa, 4 = M. rostrata, and NC = Negative control.



Figure 4.4 Agarose gel electrophoresis for amplified ITS gene of *Micropera*. Bands were fractionated by 1.5 % TBE agarose gel (25 min., 100 V/cm); Lane: M = One Mark B DNA Ladder, 1 = *M. pallida*, 2 = *M. thailandica*, 3 = *M. obtusa*, 4 = *M. rostrata*, and NC = Negative control.

## 4.3.2 Sequences and alignment

In this study, 4 new sequences and 6 sequences from genbank were included. The aligned sequence lengths are 911 bp for the ITS region, 1,801 bp for *mat*K and 2,715 bp of the alignment of the combined ITS and *mat*K genes. The information of ITS, *mat*K and combined sequences by parsimony analysis are shown in Table 4.2. The ITS and *mat*K sequences from this study are more or less the same length with other studies of the tribe *Aeridinae*, Orchidaceae (i.e. Topik, H., Peter, W., Tomohisa, and Y., Motomi, I., 2005; 2012; Zou, L. H., Huang, J. X., Zhang, G. Q., Liu, Z. J., and Zhuang, X. Y., 2015).

information	ITS	matK	Combined
Aligned sites	911	1,804	2,715
Number of most parsimonious trees	4	5	7
No. parsimony-informative characters	911	1,804	2,715
Tree length	112	44	161
Consistency index (CI)	0.86	0.95	0.86
Retention index (RI)	0.79	0.90	0.76

**Table 4.2** The information derived from the parsimony analysis of the *mat*K, ITS and<br/>combined genes.

## 4.3.3 Phylogenetics analysis

ITS analysis by maximum-parsimony analysis retrieved 4 most parsimonious trees (MPTs). A total length of MPTs is 112. The consistency index is 0.86 and retention index is 0.79 (Table 4.2). One of the original trees is shown in Figure 4.5. The consensus tree (Figure 4.6) shows two major clades which are a clade of *Micropera* and a clade of *Acampe, Cottonia,* and *Adenoncos.* The *Micropera* clade was supported by 99% of bootstrap showing strong support as a monophyletic group. *Cottonia peduncularis* is a sister group with *Acampe* clade with moderately bootstrap support (81%). Topik, H., Peter, W., Tomohisa, and Y., Motomi, I. (2005) showed that the *Acampe alliance* consisting of *A. rigida, A. orchracea, Adenoncos parviflora,* and *Micropera pallida* formed a monophyletic group. This picture is congruent with Topik, H., Peter, W., Tomohisa, and Y., Motomi, I. (2005, 2012).



Figure 4.5 One of the original trees of *Trichoglottis* clade based on the ITS markers.



*mat*K analysis by maximum-parsimony analysis retrieved 5 (MPTs). The total length of the MPTs are 44. The consistency index is 0.95 and retention index is 0.90. One of the original trees is shown in Figure 4.7. The consensus tree (Figure 4.8) shows two major clades which are a clade of *Micropera* (92%) and a clade of *Acampe* and *Adenoncos* (62%). Contrary to the result from ITS, *Cottonia peduncularis* is separated out from the ingroup taxa. This is congruent with Topik, H., Peter, W., Tomohisa, and Y., Motomi, I. (2005, 2012), he showed that *Acampe, Adenoncos* and *Micropera* form a clade as *Acampe* alliance.



Figure 4.7 Original tree of *Trichoglottis* clade based on the *mat*K markers.



The combined ITS and *mat*K by maximum-parsimony analysis retrieved 5 MPTs, consistency index of 0.86 and retention index of 0.76. A total length of MPTs is 161. One of the original trees is shown in Figure 4.9. The Consensus tree (Figure 4.10) indicates two major clades which are *Micropera* and *Acampe*, *Cottonia* and *Adenoncos*. The overall topology is congruent with Topik, H., Peter, W., Tomohisa, and Y., Motomi, I. (2005, 2012) and Zou et al., 2015. Adding more taxa of *Micropera* makes the solution better on the generic level position.

Pridgeon, A. M., Cribb, P. J., Chase, M. W., and Rasmussen, F. N. (2014) pointed out that the genus *Micropera* is not yet stable. From this study, *Micropera clade* is strongly supported by bootstrap (100%) indicating that the genus *Micropera* is a monophyletic group. Thus, it is an accepted genus. The relationship in the genus based on combined ITS and *mat*K revealed that Thai *Micropera* form two groups with weak bootstrap support. The characteristic of column twist may be a taxonomic character to group between the group of *M. rostrata* and *M. obtusa*, with twist column and the group of *M. pallida* and *M. thailandica*, with no twist column. All species of *Micropera* are needed for phylogenetic analysis to reveal the intraspecific evolutionary relationship in this genus.

Beside the objective of this study, focused on the genus *Micropera*, it revealed better solutions of related genera position compared to previous study after adding more taxa of the *Micropera*. On the second clade, it can be seen that the genus *Acampe* is a monophyletic group with rather strong bootstrap support (93%). However, more taxa of *Adenoncos* is needed to include for the analysis. In this study, the position of *Adenoncos* is separated out from *Acampe*.

The morphological phylogenetic analysis showed that *Micropera* and *Adenoncos* are monophyletic groups, except *Acampe*. It is common in the orchidaceae which the morphological data rarely gives the relationship among the closed genera, while the molecular evidence gives better relationship (i.e. *Watthana* et al., 2006).



Figure 4.9 Original tree of Trichoglottis complex based on the combined nuclear

(ITS), and plastid (matK) markers.



Figure 4.10 Bootstrap consensus tree of Trichoglottis complex based on the combined nuclear (ITS), and plastid (*mat*K) markers.

้<sup>3</sup>าวักยาลัยเทคโนโลยีสุรบ

#### 4.4 Taxonomic Treatment

The genus *Micropera* is found in Thailand 4 species, which are *M. pallida* (Roxb.) Lindl., *M. thailandica* Seidenf. & Smitinand, *M. obtusa* (Lindl.) Tang & Wang and *M. rostrata* (Roxb.) Balakrishnan. This enumeration is based on the herbarium specimens from Thai Forest Herbarium (BKF), Copenhagen Herbarium (C), Kew Herbarium (K), and Queen Sirikit Botanic Garden Herbarium (QBG). The taxonomic treatment in this study following Flora of Thailand format. For the geographical area, I followed Burmitt (2011), the world geographical scheme for recording plant distributions.

#### Micropera Lindl.

Lindl., Edwards's Bot. Reg. 18: t. 1522. 1832; Seidenf., Opera. Bot. 95: 120. 1988; J. B. Comber., Orchids Java: 343. 1990; N. Pearce & P. J. Cribb., Fl. Bhutan 3(3): 531. 2002. Pridgeon *et al.*, Gen. Orchid. 6: 210. 2014.— *Camarotis* Lindl., Gen. Sp. Orchid. Pl.: 219. 1833; Holttum, Orch. Malaya 633. 1957; J. J. Smith., und die nachstverwandten Gattungen 72: 79-115. 1912; A. F. G. Kerr., J. Siam Soc. Nat. Suppl. 9, 2: 225-243. 1933.

Monopodial epiphytic perennial herbs, climbing; stems long, terete, with many long roots and leaves. *Leaves* distichous, conduplicate, usually many, dorsi-ventral compress, fleshy, oblong to linear, basal part forming a sheath covering the stem, articulate, apex unequal bilobed. *Inflorescence* lateral racemose sometimes paniculate with many flowers, often arising opposite leaves. *Flowers* non-resupinate, small to medium-sized, (pale-) yellow or light pink. *Sepals* and *petals* free, similar. *Labellum* conspicuously spurred or saccate, 3-lobed; lateral lobes broad, erect; midlobe smaller, fleshy; spur with a longitudinal septum inside, often ornamented at its entrance. *Column* stout to slender, lacking a foot; rostellum projection prominent, beaked, sometimes twist; pollinia 4, in 2 subequal pairs on a common long stipe; viscidium elliptic, very small. Fruit a capsule; seeds dust-like, numerous.

#### Key to Species

1.	Rostellum very long, more than 3 times as column height,		
	flower pinkish, not yellow	M. rostrato	
1.	Rostellum short, less than 2 times as column height, flower		
	yellowish or yellow with pinkish	2	
2.	Rostellum twist	M. obtusc	
2.	Rostellum not twist	3	
3.	Sidelobe conspicuous more or less quadrangular	M. pallida	
3.	Sidelobe inconspicuous	M. thailandica	

Micropera pallida (Roxb.) Lindl., Edwards's Bot. Reg. 18: 1522. 1832; Seidenf., Opera.
 Bot. 95: 120. Fig. 71, 1988; Seidenf. & J. J. Wood., Orchids Penins. Malays. Singap.: 617.
 1992. 1992.— Aerides pallida Roxb., Fl. Ind. 3: 475. 1832. Type: s.coll. (K).

*Camarotis pallida* (Roxb.) auct. non Lindl.: J. J. Smith., und die nachstverwandten Gattungen 72: 79-115. 1912; Kerr., J. Siam Soc. Nat. Hist. Suppl. 9: 238. 1933.

*Camarotis apiculata* Rchb.f., Bonplandia (Hannover) 5: 39. 1857; J. J. Smith., und die nachstverwandten Gattungen 72: 79-115. 1912; Bull. Buitenz. 3. s. 11: 83-160. 1931; Fed. Repert. 32: 129-386. 1933; Carr., Gard. Bull. Sing. 7: 54. 1932; Masamune, Enumeratio Phanerogamarum Bornearum: 137. 1942; Holttum., Orch. Malaya: 633. 1957, Figure 187 c-h; Seidenfaden & Smitinand., A preliminary list. 708. 1965, Fig. 527; Backer & Bakhuizen., Orch. Java. 3: 440. 1968; A. D. Kerr., Nat. Hist. Bull. Siam Soc. 23, 1-2: 185-211. 1969; Seidenf., Bull. Mus. Paris. 112. 1973.— *Micropera apiculata* (Rchb.f.) Garay., Bot. T. Leafl. 23: 186. 1972. - *Sarcanthus apiculatus* (Rchb.f.) J.J.Sm., Orch. Java: 598. 1905.— *Dendrocolla apiculata* (Rchb.f.) Zoll. ex Rchb.f., Bonplandia (Hannover). 5: 39. 1857. Type: not located.

*Sarcochilus cochinchinensis* G.Nicholson, Ill. Dict. Gard. 3: 360. 1886. Type: not located

*Sarcochilus roxburghii* Hook.f., Fl. Brit. India 6: 36. 1894. Type: Bangladesh, Comilla, Bengal, Clarke, C.B. 14202 (K).

*Saccolabium saxicola* Ridl., Trans. Linn. Soc. London, Bot. 3: 374. 1893. Type: not located

Sarcanthus thorelii Guillaumin., Bull. Soc. Bot. France 77: 331. 1930. Type: Vietnam, C. Thorel, 159 (P).

Monopodial epiphytic perennial herb, up to 30 cm long, glabrous, covered by the smooth leaf sheaths; roots arising along the stem. *Leaves* distichous, ca. 2 cm apart, flat, conduplicate, oblong-linear,  $8-15 \times 1-1.5$  cm, apex unequally bilobed with rounded lobes, fleshy. *Inflorescences* hanging, 5-8 cm long, several-flowered; peduncle 5-10 cm long, with 2-3 sheathing sterile bracts 1.5-2 mm long; rachis 5-8 cm or more long, unbranched; bracts broadly triangular, obtuse,  $ca. 2.5 \times 2.5$  mm. Flowers yellow, labellum yellow, sepals yellow sometimes tipped with a purple midline on the back, column white or cream; pedicel and ovary terete with longitudinal grooves, 1-1.2 mm long. *Dorsal sepal* elliptic-ovate, 6-6.5 x 2-2.5 mm, apex broadly rounded; 5-veined; lateral sepal broad elliptic, 6-6.3 x 2.3-2.6 mm, 5veined. *Petals* elliptic-lanceolate, upto  $5-5.5 \times 2-2.5$  mm, acute; 3-veined. *Labellum* fleshy, chin shape, 5-5.2 mm long, 8-9 mm high; lateral lobes conspicuous, quadrangular,  $4-4.5 \times 2-2.2$  mm; mid-lobe triangular,  $2-2.2 \times 1-1.3$  mm; spur 6-6.5 mm long, with a longitudinal septum, with a triangular callus at the base of the midlobe, split at the end; back wall of spur with obscure callus; spur with a longitudinal septum. Operculum ca. 2.3 mm long, with a long beak. Column not twist, 2-3 mm long; rostellum beak c. 2 mm long, not twisted. Capsule terete, 5-6 x 0.5-0.7 cm <sup>ชา</sup>ลยเทคโนโล long.

Ecology: Epiphyte in evergreen forest. Flowering during July-October. Fruiting during October.

Distribution: Assam, India, Myanmar, Thailand, Cambodia, Vietnam, Malaysia, Borneo, Java and Sumatra

Thailand: Nakorn Sawan, Kamphengpet, Doi Suthep (Chiang Mai), Phu Khieo (Chayaphum), Nam Prom, Pitsanulok to Lomsak at km 80, Dat Don (Chayaphum, Beteen Nam prom and Tunkamang (Chayaphum), N of Thung Salaeng Luang, Namnao, Koh

Chang, Khlung, Chantaburi, Krabin (Prachinburi), Khao Soidao Nua, Between Chantaburi and Trat, Makham, Chantaburi, Near Whanka, Saiyok, Sam Roy Yat (Prachuap), Ban Tak Tawan (Pranburi), Huay Nam Wing, Banrai (Uthaidhani), Bangbao (Surat), Khao Chem Tungsong, Ban Prakap (Songkla), Huay Ai Chuang, W of Bangto, W of an Khao Tong (Phang-nga)

Conservation status: This species is considerable as less concern (LC), due to midle distribution.

Specimens examined: A. F. G. Kerr 01000 (C); C. F. van Beusekom & T. Smitinand (BKF); C. Niyomdham (BKF); C. Niyomdham CN 8277 (BKF); David J. Middleton, Chandee Hemrat, Stuart Lindsay, Somran Suddee & Suwat Suwanachat 3345 (BKF); Egerod 373 (C); Geesnik 6909 (C); G. Seidenfaden & T. Smitinand 7614 (C); G. Seidenfaden & T. Smitinand 8796 (C); G. Seidenfaden & T. Smitinand 8822 (C); J. Phelps (BKF); J. F. Naxwell (BKF); Middleton et al. (BKF); Kai Larsen, Supee S, Larsen, I. Nielsen & T. Santisuk (BKF); Kasem 308 (C); Marcan 2700 (C); N. Jacobsen 48 (C); N. Jacobsen 72 (C); N. Jacobsen 77 (C); N. Toolmal & W. Somprasong (BKF); O. Phueakkhlai, P. Tippayasri & N. Anuraktrakoon (BKF); Rabil 125 (C); S. Raksue (BKF); S. Suddee et al. (BKF); Tixier 7 (C); T. Smitinand (BKF); T. Smitinand 7436 (C); Vichian Rattanabunno (BKF); Zolling 1359 (C); Wall 7321 (C).

Note: This species can be distinguished by having the large side lobe of labellum.

ะ รัววักยาลัยเทคโนโลยีสุรุบาว 38



Figure 4.11 *Micropera pallida* (Roxb.) Lindl. a. plant, b. flower, c. petal & sepal d. section through lip, e. pollinarium, f. operculum. Drawn by Thotsaporn Chanokkhun.



Figure 4.12 Micropera pallida (Roxb.) Lindl. Photo by Thotsaporn Chanokkhun.





Figure 4.13 Distribution map of *Micropera pallida* (Roxb.) Lindl. N = Northern, NE = Northeastern, E = Eastern, C = Central, SE = Southeastern, SW = Southwestern and PEN = Peninsular. Photo by Thawatphong Boonma.

2. Micropera thailandica Garay ex Seidenf., Opera Bot. 95: 126. 1988.

*Micropera thailandica* (Seidenf. & Smitinand) Garay, Bot. Mus. Leafl. 23: 187, 1972; Seidenf., Opera Bot. 95: 126, fig. 72, 1988; Vaddhanaphuti, Wild Orch. Thailand, ed. 4, 186, incl. colour photo, 2005. — *Camarotis thailandica* Seidenf. & Smitinand, Orch. Thailand (Prelim. List) 712, fig. 529, 1965. Kurzweil and Lwin, Gard. Bull. Singapore. 67(1): 107–122, 2015. TYPE: Thailand, Satun Province, Feb 1961, Seidenfaden, G. & Smitinand, T. GT 4118 (holotype C).

*Camarotis thailandica* Seidenf. & Smitinand., Orchid. Thail. 4, 2: 712. Figure. 529, 1965 (not validly publ.); P. F. Cumberlege., Nat. Hist. Bull. Siam Soc. 20, 3: 155-174. 1963.

Monopodial epiphytic perennial herb up to 30 cm long, glabrous, covered by the dark, rugose bases of the leaves. Leaves distichous, ca. 2 cm apart, flat, conduplicate, oblong-linear,  $8-15 \times 1-1.5$  cm, apex unequally bilobed with rounded lobes, fleshy. *Inflorescences* more or less upright, 17-23 cm long, several-flowered; peduncle 10-15 cm long, with 2-3 sheathing sterile bracts 3-4 mm long; rachis 8-10 cm long, unbranched; bracts broadly triangular, obtuse,  $2-2.5 \times ca.$  4 mm, fleshy. Flowers yellow or light orange-yellow, lip base sometimes white, sepals sometimes tipped with brown, column white or cream. *Dorsal sepal* elliptic-ovate, 9 × 3.5 mm, apex broadly rounded, concave, fleshy; lateral sepals similar but somewhat shorter and wider, adnate to the column-foot. *Petals* elliptic-lanceolate, upto 8 × 3.3 mm, acute. Labellum fleshy, deeply boat-shaped 6-6.5 mm long, 5.5-6 mm high, obscurely 3-lobed; lateral lobes inconspicuous broadly triangular 5-5.5 x 1.5-1.8 mm, edges minutely serrate; midlobe triangular, ca.  $2 \times 2-2.5$  mm; spur 5-6.5 mm long, with a longitudinal septum; with a triangular tongue callus at the base of the midlobe,. Operculum ca 2 mm long, with a short beak. Column not twist, slender, 5.5-6 mm long; rostellum beak c. 2 mm long, not twisted. *Capsule* not seen.

Ecology: Epiphyte in evergreen forest. Flowering during April. Distribution. Myanmar, Thailand, Cambodia and Vietnam. Thailand: Phu Khieo (Chayaphum), Khao Yai, Makham, Sriracha, Foothills of Khao Sabab, NW of Phato (Ranong), Eastern foothills of Khao Phra Mi, Thungnui (Satul), Huay Yawt, Khao Den (Patalung), Khao Soidao (Patalung), Khao Kheo Range.

Conservation atatus: This species is considered as less concern (LC) and a common species in Thailand.

Specimens examined: A. D. Kerr 3342 (C); A. F. G. Kerr 0528 (C); Cumberlege 1081 (BKF); G. Seidenfaden & T. Smitinand 5773 (C); J. Phelps 7680 (BKF); Middleton et al. (BKF); P. Puudjaa 321 (BKF); P. Puudjaa 1322 (BKF); S. Kaitongsuk 211 (BKF).

Notes: This species is similar to *M. obtusa* but it differs on its rostellum not distinctly twisted.





Figure 4.14 *Micropera thailandica* (Seidenf. & Smitinand) Garay. a. plant, b. flower, c. petal & sepal d. section through lip, e. pollinarium, f. operculum. Drawn by Thotsaporn Chanokkhun.



Figure 4.15 Micropera thailandica (Seidenf. & Smitinand) Garay. Photo by Laksika Rodpothong.



Figure 4.16 Distribution map of *Micropera thailandica* (Seidenf. & Smitinand) Garay.
 N = Northern, NE = Northeastern, E = Eastern, C = Central, SE = Southeastern, SW = Southwestern and PEN = Peninsular. Photo by Thawatphong Boonma.

3. *Micropera obtusa* (Lindl.) Tang & Wang., Acta Phytotax. Sin. 1: 94. 1951; Pradhan., Guide to identification and culture 1. 497. 1979; Seidenfaden., Nord. J. Bot. 2: 193-218. 1982; Christenson., Am. Orch. Soc. Bull. 54. 955-958. 1985;— *Sarcochilus obtusus* (Lindl.) Benth. ex Hook.f., Fl. Brit. India 6: 36. 1894.— *Camarotis obtusa* Lindl., Edwards's Bot. Reg. 30: 73. 1844; Reichenbach f., Bot. Zeit. 22, 39: 881. 1864; Trans. Linn. Soc. 30, 1: 136. 1874; King & Pantling., J. As Soc. Beng. 66, 3: 238. 1898; Smith, J. J., Natuurk. Tijds. Ned Ind. 72: 97. 1912; Bruhl., A guide to the orchids of Sikkim.: 141. 1926; Seidenfaden & Smitinand., A preliminary list. 714. 1965, Figure. 1-9; Hegde., Arunachal Forest News 3, 3: 7. 1980; Forest Dept.: 76. 1984. Type: Loddiges, G., (K).

Monopodial epiphytic perennial herb to 25-40 cm long, glabrous, covered by the smooth leaf sheaths. Leaves about 10, distichous, ca. 2 cm apart, flat, conduplicate, oblong-linear,  $6-9 \times 1-1.5$  cm, apex unequally bilobed with rounded lobes, fleshy. Inflorescences hanging, 4-7.5 cm long, 6-12 flowers; peduncle 2-2.5 cm long, with 2-3 sheathing sterile bracts 3-4 mm long; rachis 3-4 cm long, unbranched; bracts broadly triangular, obtuse,  $3-3.5 \times c.4$  mm. Flowers yellowish or pale purplish, lip yellow and sometimes paler at base, sepals white sometimes tinct with pale purple, column white with purplish apex. *Dorsal sepal* elliptic-ovate, upto 7.5 × 3 mm, apex broadly rounded, 5-veined; lateral sepals obovate-oblong, ca. 6.5 x 3.5 mm, slightly falcate, 5-veined, Petals elliptic-lanceolate, ca. 7.7 x 3 mm, acute, 5veined. Labellum fleshy, deeply boat-shaped, 5-5.5 mm long, 6-6.5 mm high, obscurely 3-lobed; lateral lobes inconspicuous broadly triangular, 4.5-5 mm; midlobe triangular,  $ca. 2 \times 1$  mm; spur 5.8-6 mm long, with a longitudinal septum, with a triangular tongue callus at the base of the midlobe. *Operculum* ca. 1 x 1 mm long with a long beak. Column twist, slender, 2.5-3.5 mm long; rostellum beak ca. 2.5 mm long, strongly twisted. *Capsule* not seen.

Ecology: Epiphyte in evergreen forest. Flowering during May-June. Distribution: Borneo, Nepal, Myanmar, and Thailand. Thailand: Khao Bangto, Phang-nga Conservation status: This species is considered as a rara species in Thailand, due to only locality found.

Specimens examined: Kerr s.n. (C, K).

Note: There are few specimens of this species in Thailand. It is similar to *M. thailandica* but its rostellum is strongly twisted.





Figure 4.17 *Micropera obtusa* (Lindl.) Tang & Wang. a. plant, b. flower, c. petal & sepal d. section through lip, e. pollinarium, f. operculum. Drawn by Thotsaporn Chanokkhun.



**Figure 4.18** Distribution map of *Micropera obtusa* (Lindl.) Tang & Wang. N = Northern, NE = Northeastern, E = Eastern, C = Central, SE = Southeastern, SW = Southwestern and PEN = Peninsular. Photo by Thawatphong Boonma.

Micropera rostrata (Roxb.) Balakrishnan., J. Bombay Nat. Hist. Soc. 67: 66. 1970;
 Seidenfaden., Nord. J. Bot. 2: 217. 1982; Christenson., Am. Orch. Soc. Bull. 54, 8: 956.
 1985.— Camarotis rostrata (Roxb.) Rchb.f. in W.G. Walpers, Ann. Bot. Syst. 6: 881.
 1861; Prain., Repr. p.: 769. 1903; Hunt., Bot. Mag. 177: 537. 1969.— Aerides rostrata
 Roxb., Fl. Ind. 3: 474. 1832. Type: India, Assam, Jainteapur, Jaintea Hills, Prain's 111
 (M).

Sarcochilus purpureus (Lindl.) Benth. ex Hook.f., Fl. Brit. India 6: 37. 1890; Panigrahi & Naik., Bull. Bot. Surv. Ind. 3,3-4: 381. 1962.— *Micropera purpurea* (Lindl.) Pradhan., Indian Orchids: Guide Identif. & Cult. 2: 619. 1979.— *Camarotis purpurea* Lindl., Gen. Sp. Orchid. Pl.: 219. 1833; Lindley., Sertum Orchidaceum.: 19. 1838; Paxt. Mag. Bot. 7: 25. 1840; J. Linn. Soc. 3: 37. 1859; Smith, J. J., Natuurk. Tijds. Ned Ind. 72: 98. 1912; Schlechter., Die Orchideen.: 589. 1915; Die Orchideen.: 580. 1927; Fischer., ec. Bot. Surv. Ind. 12, 2: 136. 1938; Seidenfaden & Smitinand., A preliminary list. 715. 1965, Fig. 522; Rao & Deori., J. Ind. For. 3, 3-4: 258. 1980. Type: Sylhet, Gomez, W. 7329 (K).

Monopodial epiphytic perennial herb 12-20 cm long, glabrous, covered by the glabrous leaf sheaths. *Leaves* about 10, distichous, about 2 cm apart, flat, conduplicate, oblong-linear, 10-12 × 1.5-2 cm, apex unequally bilobed with rounded lobes, fleshy. *Inflorescences* hanging, 10-15 cm long, up to 30 flowers; peduncle 3-5 cm long, with 2-3 sheathing sterile bracts 0.3-0.4 cm long; rachis up to 30 cm long, unbranched; bracts broadly triangular, obtuse, 2-2.5 × c. 2 mm, fleshy. *Flowers* pale to bright purple pink, lip base sometimes white with purple pink margin, column white tinct with purple pink. *Dorsal sepal* elliptic-oblong, 7.5-8.5 × 3-3.5 mm, apex broadly rounded, 5-veined; lateral sepal obovate-oblong, 7.5-8.5 × 3.3-4 mm, 5-veined. *Petals* elliptic-lanceolate, to 8.5 × 3.5 mm, acute, 5-veined. *Labellum* fleshy, deeply boat-shaped, 9-10 mm long, 6-6.5 mm high, obscurely 3-lobed; lateral lobes inconspicuous broadly triangular, 8-8.5 × 1.5-2 mm; midlobe triangular, *ca.* 2 × 1 mm; spur 7.5-9 mm long, with a longitudinal septum, with a triangular tongue callus at the base of the midlobe. *Operculum ca.* 3 × 1 mm long with a long beak. *Column* twit,

stout, 2.5-3 mm long; rostellum beak *ca.* 3.5 mm long, strongly twisted. *Capsule* not seen.

Ecology: Epiphyte in evergreen forest. Flowering during May-June.

Distribution: Assam, Borneo, and Thailand.

Thailand: Ban Mussoe (Tak), Muang Fang, Phu Krading, Nakorn Nayok.

Conservation status: This species is considered as a rare species in Thailand, due to only one locality found.

Specimens examined: *W. Songkakul* 17 (BKF).

Note: This species is easily recognised by having very long and twist rostellum. It is one of the beautiful species. This species have not list in the World Checklist of Selected Plant Family (WCSP), although Seidenfaden reported in 1988.





Figure 4.19 *Micropera rostrata* (Roxb.) Balakrishnan. a. plant, b. flower, c. petal & sepal d. section through lip, e. pollen strip, f. operculum and pollinia. Drawn by Thotsaporn Chanokkhun.



Figure 4.20 Micropera rostrata (Roxb.) Balakrishnan. Photo by Santi Watthana.



Figure 4.21 Distribution map of *Micropera rostrata* (Roxb.) Balakrishnan. N = Northern,
 NE = Northeastern, E = Eastern, C = Central, SE = Southeastern, SW = Southwestern and PEN = Peninsular. Photo by Thawatphong Boonma.

# CHAPTER V CONCLUSION

# 5.1 Morphological and molecular phylogeny

This study supported that the genus *Micropera* is accepted which has answered the doubtful phylogenetic position previously (Pridgeon, A. M., Cribb P.J., Chase M. W., and Rasmussen F. N. 2014). Both morphological and molecular evidence are supported that this genus is a monophyletic group, acceptable for systematics. Although the phylogenetic tree based on morphological evidence gave very poor resolution, the *Micropera* and *Adenoncos* are monophyletic. It is usual that many reversed characters on morphology.

For molecular evidence, ITS and *mat*K and combined genes gave the same pattern of the tree. The consensus tree gave strong bootstrap support for *Micropera* (100%). Adding more taxa of *Micropera* gave a better solution. However, all of *Micropera* should be included to picture the intraspecific evolution relationship for this genus. The genus *Adenoncos* is also one to add more taxa to prove whether this genus is monophyletic or not.

10

# 5.2 Revision of Thai Micropera

The second objective of this study is to revise the genus *Micropera* in Thailand. The generic name was a bit confusing, previously called *Camerotis* in Thailand. Now it is accepted as *Micropera*, as well as supported by phylogenetic analysis from this study. This study accepted Seidenfaden species delimitation (Seidenfaden, G., 1988). There are 4 species in Thailand, which are, *M. pallida* (Roxb.) Lindl., *M. thailandica* Garay ex Seidenf., *Micropera obtusa* (Lindl.) Tang & Wang and *M. rostrata* (Roxb.) Balakrishnan based on specimens from Thai Forest Herbarium (BKF), Copenhagen Herbarium (C), Kew Herbarium (K), and Queen Sirikit Botanic Garden Herbarium (QBG). I provided a key to species in Thailand, description of each species, ecology and distribution. The illustrations of each species are provided. *M. obtusa* and *M. rostrata* are considered as rare species in Thailand.



# REFERENCES

- Brummitt, R. K. (2001). World geographical scheme for recording plant distribution (ed2). Hunt Institute for Botanical Cocumentation Carnegia Mellon University, Pittsburgh.
- Burleigh, J. G., and Mathews, S. (2004). Phylogenetic signal in nucleotide data from seed plants: implications for resolving the seed plant tree of life. *American Journal of Botany*. *91*(10), 1599-1613.
- 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.
- Cash, C. (1991). The Slipper Orchids. *Timber Press*. Portland.
- 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.
- 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*, *atp*B, and *mat*K DNA sequences. *American Journal of Botany*. *89*(1), 132–144.
- 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.
- Dressler, R. (1981). The Orchids Natural History and Classification. *Systematic Botany*. *6*, 201-202.

- Felsenstein, J. (1985). Phylogenies and the Comparative Method. The American Naturalist. *125*, 1-15.
- Fitch, W. M. (1971). Toward Defining the Course of Evolution: Minimum Change for a Specific Tree Topology. *Systematic Zoology*, *20*(4), 406–416.
- Freudenstein, J. V., and Chase M. W. (2015). Phylogenetic relationships in
  Epidendroideae (Orchidaceae), one of the great flowering plant radiations:
  progressive specialization and diversification. *Annals of Botany*. 115(4), 665-681.
- Gitzendanner, M. A., Soltis P. S., Wong G. K., Ruhfel B. R., and Soltis D. E. (2018). Plastid phylogenomic analysis of green plants: A billion years of evolutionary history. *American Journal of Botany*. *105*(3), 291-301.
- 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(1814), 20151553.
- Goloboff, P. A., Farris, J., and Nixon, K. (2008). T.N.T. Tree analysis using new technology. *Cladistics*. *24*, 774–786. doi: 10.1111/j.1096-0031.2008.00217.x
- Judd, W. S., Campbell, C. S., Kellogg E. A., Stevens, P. F., and Donoghue, M. J. (2016). Plant systematics: a phylogenetic approach. *Rhodora*. *118*(976). 418-420.
- Kocyan, A., Vogel, E. F., Conti, E., and Gravendeel, B. (2008). Molecular phylogeny of Aerides (Orchidaceae) based on one nuclear and two plastid markers: a step forward in understanding the evolution of the Aeridinae. *Molecular Phylogenetics Evolution*. 48(2), 422-443.
- Linley, J. (1832). Micropera. Edwards's botanical register. 18, 1522.
- Petruzzello, M. (2018). List of plants in the family Orchidaceae. *Encyclopaedia Britannica*.
- Pridgeon, A. M., Cribb P.J., Chase M. W., and Rasmussen, F. N. (1999). *Genera* Orchidacearum Volume 3: Epidendroideae (Part 2). Oxford University press.
- Pridgeon, A. M., Cribb P.J., Chase M. W., and Rasmussen F. N. (2014). *Genera* Orchidacearum Volume 6: Epidendroideae (Part 3). Oxford University press.

Seidenfaden, G. (1988). Orchid Genera in Thailand. Opera Botanica. 14, 120-126.

Simpson, M. G. (2010). Plant Systematics. Academic press.

- Stevens, P. F. (2020). Angiosperm Phylogeny Website. Angiosperm Phylogeny Website. access on 7 December 2020.
- Sun, Y., Skinner, D. Z., and Liang, G. H. (1994). Phylogenetic analysis of Sorghum and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics.* 89, 26–32.
- The Angiosperm Phylogeny Group, Chase, M. W., Christenhusz, M. J. M., Fay, M. F., Byng, J. W., Judd, W. S., Soltis, D. E., Mabberley, D. J., Sennikov, A. N., Soltis, P. S., and Stevens, P. F. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*. 181(1), 1–20.
- The Angiosperm Phylogeny Group. (1998). An ordinal Classification for the families of flowering plants. *Annals of the Missouri Botanical Garden. 85*(4), 531–553.
- The Angiosperm Phylogeny Group (2009). An update of The Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society. 161(2), 105-121.
- Topik, H., Peter, W., Tomohisa, and Y., Motomi, I. (2005). Molecular phylogenetics of subtribe Aeridinae (Orchidaceae): Insights from plastid *mat*K and nuclear ribosomal ITS sequences. *Journal of plant research*. *118*(4), 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*(1), 87-95.
- Wiens, J. J. (2004). The role of morphological data in phylogeny reconstruction. *Systematic Biology*. *53*(4), 653-661.
- Wu, Z. Y., Raven P. H., Hong, and D. Y., eds. (2009). Flora of China. Vol. 25 (Orchidaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.
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 nuclear and chloroplast regions. *Molecular Phylogenetics and Evolution*. *85*, 247-254.



APPENDICES

#### APPENDIX A

### DNA MARKER STANDARD CURVE



## APPENDIX B DNA SEQUENCES

Table B.1 DNA sequences ITS.

Species	DNA sequences
Acampe orchracea	ACCTGCGGAAGGATCATTGTCGAGACCGAAACTATATCGAGCG
	ATTCGGAGAACCCGTGAAGTAAGCGGCGGCGGCCGTCGCCGCG
	AA <mark>ACG</mark> GCAATCCCCGTCGTCGCTCCCCCCGCCTGGAGGGGGGCC
	CCGACGAGGGACGGCTGAAACCCCAAACCGGCGCAGATTGGCG
	CCAAGGGAGCTATCGAAAAACGCGAGCCCGGCATCGGGTCTTC
	GTGGGGTGGAGCGGTGCTGCGCGCCGCACGTATTGACACGACT
	CTCGACAATGGATATCTCGGCTCTCGCATCGATGAAGAGCGCA
	GCGAAATGCGATACGTGGTGCGAATTGCAGAATCCCGCGAACC
	ATCGAGTCTTTGAACGCAAGTTGCGCCCGAGGCCAATCGGTCG
	AGGGCACGTCCGCCTGGGCGTCAAGCGTCGCGCCGCTCCGTGC
	CGAGTCCCCACCATCCCACCGCAGTGGGGGGGGGCGGGGGGGG
	CCGGACGTGCAGAGTGGCTCGTCGTGCCCGTCGGCGGCGGG
51508	CTGAAGAGCGGGTTATCGTCTCATTGGCCACGAACAACGAGGG
	GTGGATGAAAGCTGCCGCGGGCGAGGCCCGCGTTGTCTCGTGC
	CGGCCCGAGAGAAGATCACACCCTGCGTGCGATCCCTTCCCAT
	GCGCCGCCCCGTGCGGCGGCTTGGAATGCGACCCCAGGATG
	GGCG

Acampe prarmorsa	AACCGAAACTATATCCGAGCGATTCGGGAGAACCTGTGAAGTA
	AGCGGCGGCGGCCGTCGCCGCGAAACGGCAATCCCCGTCGTCG
	CTCCCCCGCCTGGAGGGGGCCCCGACGAGGGACGGCCGAAAC
	CCCAAACCGGCGCAGATTGGCGCCAAGGGAGCTATCGAAAAAC

Species	DNA sequences
	GCGAGCCCGGCATCGGGTCTTCGTGGGGTGGAGCGGTGCTGCG
	CGCCGCACGTATTGACACGACTCTCGACAATGGATATCTCGGCT
	CTCGCATCGATGAAGAGCGCAGCGAAATGCGATACGTGGTGCGA
	ATTGCAGAATCCCGCGAACCATCGAGTCTTTGAACGCAAGTTGC
	GCCCGAG <mark>GCC</mark> AATCGGTCGAGGGCACGTCCGCCTGGGCGTCAA
	GCGTTG <mark>CGCCGC</mark> TCCGTGCCGAGTCCGCACCATCCCACCGCAGT
	GGGGG <mark>TGCCGG</mark> GCGAGGCTCGGACGTGCAGAGTGGCTCGCCGT
	GCCCGTCGGCGCGGGCGGGCTGAAGAGCGGGTTATCGTCTCATT
	GGCCACGGACAACGAGGGGTGGATGAAAGCTGCCGCGGGCGAG
	GCCCGCGTTGTCTCGTGCCGGCCCGAGAGAAGATTACACCCTCC
	GTGCGATCCCTTCCCATGCGCCGCACCCCGTGCGGCGGCTTGG
	AATGCGACCCCAGGACGGGCGAGACCACCCGCCGAGTTTAAGC
Acampe rigida	TCGAGACCGAAACTATATCGAGCGATTCGGAGAACCTGTGAAGT
	AAGCGGCGGCGGCCGTCGCCGCGAAACGGCAATCCCCGTCGTC
	GCTCCCCCGCCTGGAGGGGGGCCCCGACGAGGGACGGCCGAAA
	CCCCAAACCGGCGCAGATTGGCGCCAAGGGAGCTATCGAAAAAC
C.	GCGAGCCCGGCATCGGGTCTTCGTGGGGTGGAGCGGTGCTGCG
52	CGCCGCACGTATTGACACGACTCTCGACAATGGATATCTCGGCT
'Ons	CTCGCATCGATGAAGAGCGCAGCGAAATGCGATACGTGGTGCGA
	ATTGCAGAATCCCGCGAACCATCGAGTCTTTGAACGCAAGTTGC
	GCCCGAGGCCAATCGGTCGAGGGCACGTCCGCCTGGGCGTCAA
	GCGTTGCGCCGCTCCGTGCCGAGTCCCCACCATCCCACCGCAGT
	GGGGGTGCCGGGCGAGGCTCGGACGTGCAGAGTGGCTCGTCGT
	GCCCGTCGGCGCGGGGGGGGGGGGGGGGGGGGGGGGGGG
	GGCCACGAACAACGAGGGGTGGAAGAAAGCTGCCGCGGGCGAG
	GCCCGCGTTGTCTCGTGCCGGCCCGAGAGAAGATTACACCCTCC
	GTGCGATCCCTTCCCATGCGCCGCCCCCGTGCGGCGGCTTGGA

Species	DNA sequences
	AT
Adenoncos parviflora	TCGAGACCGAAACTATATCGAGCGATTCGGAGAACCTGTGAAGT
	AAGCGGCGGCGGCGTCGCCGCGAAACGGCAATCCCCGTCGTC
	GCTCCCCCCGCCTGGAGGGGGCCCCGACGAGGGACGGCCGAAA
	CCCCAAACCGGCGCAGATTGGCGCCAAGGGAGCTATCGAAAAA
	CGCGA <mark>GCCCGG</mark> CATCGGGTCTTCGTGGGGTGGAGCGGTGCTGC
	GCGCC <mark>G</mark> CACGTATTGACACGACTCTCGACAATGGATATCTCGGC
	TCTCGCATCGATGAAGAGCGCAGCGAAATGCGATACGTGGTGC
	GA <mark>ATT</mark> GCAGAATCCCGCGAACCATCGAGTCTTTGAACGCAAGTT
	GCGCCCGAGGCCAATCGGTCGAGGGCACGTCCGCCTGGGCGTC
	AAGCGTTGCGCCGCTCCGTGCCGAGTCCCCACCATCCCACCGCA
	GTGGGGGTGCCGGGCGAGGCTCGGACGTGCAGAGTGGCTCGTC
	GTGCCCGTCGGCGCGGCGGGCTGAAGAGCGGGTTATCGTCTCA
	TTGGCCACGAACAACGAGGGGTGGAAGAAAGCTGCCGCGGGCG
	AGGCCCGCGTTGTCTCGTGCCGGCCCGAGAGAAGATTACACCCT
	CCGTGCGATCCCTTCCCATGCGCCGCCCCCGTGCGGCGGCTT
	GGAAT
6	100
Cottonia peduncularis	CCGTAGGTGAACCTGCGGAAGGATCATTGTCGAGACCGAAACTA
"Onsi	TATCGAGCGATTCGGAGAACCCGTGAAACAAGCGGCGGCGGCC
	GTCGCCGTGAAACGGCCATCCCCGTCGTCGCTCCCCCGTCCG
	GAGGGGGCCCCGACGAGGGACGGCCGAAACCCCAAACCGGCGC
	AGATTGGCGCCAAGGGAGCTATCGAAAAACACGAGCCCGTCATC
	GGGTCTTCGTGGGGTGGAGCGGTGCAGCGCACCGCACGTATTG
	ACACGACTCTCGACAATGGATATCTCGGCTCTCGCATCGATGAA
	GAGCGCAGCGAAATGCGATACGTGGTGCGAATTGCAGAATCCC
	GCGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAGGCCAAT
	CGGTCGAGGGCACGTCCGCCTGGGCGTCAAGCGTCGCGCCGCT

Species	DNA sequences
	CCGTGCCGGGTCCCCACCATCCCACCGCTGTGGGGGTGCCGGG
	CGAGGCTCGGACGTGCAGAGTGGCTCGTCGTGCCCATCGGTGC
	GGCGGGCTGAAGAGCGGGTTATCGTCTCATTGGCCACGAACAAC
	GAGGGGT <mark>GG</mark> ATGAAAGCTGCCGCGGGCGAGGCCCGCGTTGTCT
	CGTGCCG <mark>GCC</mark> CGAGAGAAGATTACGCCCTTCGTGCGATCCCTCC
	CCATGCGCCGCCCCCGTGCGGCGGCTTGGAATGCGACCCCAG
	GACGGGCGAGA
Micropera obtusa	GAGCCACTCTGCACGTCCGCGCCTCGCCCGGCACCCCCACTGCG
	GT <mark>GGG</mark> ATGTGGC <mark>GGG</mark> GACTCGGCACGGAGCGGCGCAACGCTTG
	ACGCCCAGGCGGACGTGCCCTCGACCGATTGGCCTCGGGCGCA
	ACTTGCGTTCAAAGACTCGATGGTTCGCGGGATTCTGCAATTCG
	CGAGATATCCATTGTCGAGAGTCGTGTCAATACGCGCGGCGCGC
	AGCACCGCTCCACCCACGAAGACCCGATGCCGGGCCCGTGTTT
	TTCGATTGCTCCCTTGGCGCCAATCTGCGCCGGTTTTGGGTTCC
	AGACGTCCCTCGCCGCGGCTCCCCGGACGGGGGGGGGGG
	ACGGGGACGGCCGCTTCGCGGCGACAACGGCCGCCACCGCTTA
6	TTTCACGGGTTCTCCGAATCGCTCGATATAATTTCGGTCTCGAC
52	AATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACT
'She	TCTCCTTCCTCTAAATGATAAGGTTCAATGGACTTCTCGCAGAG
	TCGCGGGCGGCGGACCGCCGCGTCGCAGCGATCCGAACACTT
	CACCGGAC

 Micropera pallida
 GTCCGGTGAAGTGTTCGGATCGCTGCGACGCGGGCGGTTCGCC

 GCCCGCGACTCTGCGAGAAGTCCATTGAACCTTATCATTTAGAG
 GAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAA

 GGATCATTGTCGAGACCGAAATTATATCGAGCGATTCGGAGAAC
 CCGTGAAATAAGCGGCGGCGGCCGTCGCCGCGAAGCGGCCGTC

Species	DNA sequences
	CCCGTCGTCGCTCCCCCGTCAGGAGGGGGCCGCGGCGAGCGA
	CGGCCGAAACCCAAAACCGGCGCAGATTGGCGCCAAGGGAGCA
	ATCGAAATACACGGGCCCGGCATCGGGTCTTCGTGGGTGG
	GGTGCTGCGCACCGCGCGTATTGACACGACTCTCGACAATGGAT
	ATCTCGGCTCTCGCATCGATGAAGAGCGCAGCGAAATGCGATAC
	GTGGTGCGAATTGCAGAATCCCGCGAACCATCGAGTCTTTGAAC
	GCAAGTTGCGCCCGAGGCCAATCGGTCGAGGGCACGTCCGCCT
	GGGCGTCAAGCGTTGCGCCGCTCCGTGCCGAGTCCCCACCATC
	CCACCGCAGTGGGGGGGCGGGGGGGGGGGGGGGGGGGGG
	GG <mark>CTC</mark> GTCGTGC <mark>CCA</mark> TCGGTGCGGCGGGCTGAAGACCGGGTTA
	TCGTCTCATTGGCCACGAACAACGAGGGGTGGATGAAAGCTGCC
	GCGGGCGAGGCCCGCGTTGTCTCGTGCCGGCCACAGAGAAGAG
	TGCACCCTTCGTGCGATCCCATCCCATGCGCCGCCCCCGTGCG
	GCGGCTTGGAATGCGACCCCAGGATGGGCGAGACCACCCGCCG
	AGTTTAAGCATATCAATAAGCGGAGGAGAAGAAACTTACGAGGA
	ТТССССТАДТА
Micropera rostrata	CCGGTGAAGTGTTCGGATCGCTGCGACGCGGGCGGTCCGCCGC
	CCGCGACTCTGCGAGAAGTCCATTGAACCTTATCATTTAGAGGA
	AGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG
15	GATCATTGTCGAGACCGAAATTATATCGAGCGATTCGGAGAACC
BUS	TGTGAAATAAGCGGCGGCGGCCGTCGTCGCCGCGAACCGGCCG
	TCCCCGTCGTCGCTCCCCGTCCGGAGGGGGCCGCGGGGGGG
	GACGGCTGAAACCCAAAACCGGCGCAGATTGGCGCCAAGGGAG
	CAATCGAAAAACACGGGGCCCGGCATCGGGTCTTCGTGGGGTG
	GAGCGGTGCTGTGCGCCGCGCGTATTGACACGACTCTCGACAAT
	GGATATCTCGGCTCTCGCATCGATGAAGAGCGCAGCGAAATGCG
	ATACGTGGTGCGAATTGCAGAATCCCGCGAACCATCGAGTCTTT
	GAACGCAAGTTGCGCCCGAGGCCAATCGGTCGAGGGCACGTCC

Table B.1 DN	VA sequences	ITS (Continued).
--------------	--------------	------------------

Species	DNA sequences
	GCCTGGGCGTCAAGCGTTGCGCCGCTCCGTGCCGAGTCCGCGC
	CACATCCCACCGCAGTGGGGGTGCCGGGCGAGGCGCGGACGTG
	CAGAGTGGCTCGTCGTGCCAGCGGTGCGGCGGGCTGAAGAGC
	GGGTTATCGTCTCATTGGCCACGAACAACGAGGGGTGGATGAAA
	GCTGCCG <mark>CGG</mark> GCGAGGCCCGCGTTGTCTCGTGCCGGCCACAGA
	GAAGATTGCACCCTTCGTGCGATCCCATCCCATGCGCCGCCCCC
	CGTGC <mark>GGCGGCT</mark> TGGAATGCGACCCCAGGATGGGCGAGACCAC
	CCGCCGAGTTTAAGCATATCAATAAGCGGAGGAGAAGAAACTTA
	CGAGGATTCCCCTAGTAACGGCGAGCGAACCGGGG
Micropera thailandica	GCGGGCGGTTCGCCGCCGCGACTCTGCGAGAAGTCCATTGAA
	CCTTATCATTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTA
	GGTGAACCTGCGGAAGGATCATTGTCGAGACCGAAATTATATCG
	AGCGATTCGGAGAACCCGTGAAATAAGCGGCGGCGGCCGTCGC
	CGCGACGCGGCCGCCCGTCGTCGCTCCCCCGTCCGGAGGG
	GGCCGCGGCGAGGGACGGCTGAAACCCAAAACCGGCGCAGATT
	GGCGCCAAGGAAGCAATCGAAATACACGGGCCCGGCATCGGGT
	CTTCGTGGGGTGGAGCGGTGCTGCGCACCGCGCGTACTGACAC
	GACTCTCGACAATGGATATCTCGGCTCTCGCATCGATGAAGAGC
	GCAGCGAAATGCGATACGTGGTGCGAATTGCAGAATCCCGCGA
1500	ACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAGGCCAATCGGT
31	CGAGGGCACGTCCGCCTGGGCGTCAAGCGTTGCGCCGCTCCGT
	GCCGAGTCCCCACCATCCCACCGCAGTGGGGGTGCCGGGCGAG
	GCGCGGACGTGCAGAGTGGCTCGTCGTGCCCATCGGTGCGGCG
	GGCTGAAGAGCGGGTTATCGTCTCATTGGCCACGAACAACGAG
	GGGTGGATGAAAGCTGCCGCGGGCGAGGCCCGCGTTGTCTCGT
	GCAGGCCACAGAGAAGATTGCACCCTTCGTGCGATCCCATCCCA
	TGCGCCGCCCACGCGCGGCGGCTTGGAATGCGACCCCAGGAT
	GGGCGAGACCACCCGCCGAGTTTAAGCATATCAATAAGCGGAG

Species	DNA sequences
	GAGAAGAAACTTACGAGGATTCCCCTAGTAACGGCGAGCGA
	GGGG
Trichoglottis triflora	ACAAGAATTCTTTTCTTATCATTTTTATTCTCAAATGGTATCAG
	AAGGTTTT <mark>GG</mark> AGTCATTCTGGAAATTTCATTCTCGTCGCGATTA
	GTATCCTCCCTTGAAGAAAAAAGAATACCAAAATCTCAGAATTT
	ACGATCTATTCATTCAATATTTCCCTTTTTAGAGGATAAATTATC
	ACATTTAAATTATGTGTCGGATCTACTAATACCCTATCCCATCCA
	TCTGG <mark>A</mark> AATCTTGGTTCAAATTCTTCAATGCTGGATCAAAGATG
	ATTTGAATAGTCTCATTACTTCAAAAAAATCCATTTACGTCTTTT
	CAAAAAAAAAGAAAAGATTCTTTTGGTTCCTACATAATTTTTATT
	ATATGAATGCGAATATATATTCCTCTTTCTTCGTAAACAGTCTTC
	TTATTTACGATCAATATCTTCTGGAGTCTTTCTTGAGCGAACACA
	TTTTTATGGAAAAATAGAATATCTTAGAGTCGTGTTTTGTAATTC
	TTTTCAGAGGATCCTATGGTTTCTCAAAGATATTTTCATACATTA
	TGTTCGATATCAAGGAAAAGCAATTTTGGCTTCAAAAGGAACTC
	TTATTCTGATGAATAAATGGAAATTTCATTTTGTGAATTTTTGGC
	AATCTTATITTCACTITTGGTTTCAACCTTATAGGATCCATATAA
	AGCAATTACCCAATTATTCCTTCTCTTTTCTGGGATATTTTTCAA
15	GTGTACTAAAAAACCCTTTGGTAGTAAGAAATCAAATGCTAGAG
BIL	ΑΑΤΤCΑΤΤΤCTAATAAATACTCTGACTAAGAAATTAGATACCATA
	GCTCCAGTTATTTTCTTATTGGATCATTGTCGAAAGCTCAATTT
	TGTACTGTATTAGGTCATCCTATTAGTAAACCGATCTGGACCAA
	TTTATCGGATTCTGATATTCTTGATCGATTTTGTCGGATATGTAG
	AAATCTTTGTCGTTATCACAACGGATCCTCAAAGAAACAGGTTT
	TGTATCGTATAAAGTATATACTTCGACTTTCGTGTGCTAGAACT
	TTGGCTCGTAAACATAAAAGTACAGTACGCACTTTTATGCGAAG
	ATTAGGTTCGGGATTCTTAGAAGAATTTT

Table B.1 DNA sequences ITS (Continued).

Species	DNA sequences
Acampe orchracea	TTCAATAGTTTGATTTTTTATGAACCTGTGGAAATTATCGGTTAT
	GACAATAAATCTAGTTTGGTACTTGTGAAACGTTTAATTACTCGA
	ATGTATCAACAGAAATATTTGATTTCTTCGGTGAATGATTCTAAC
	CAAAATGG <mark>AT</mark> TTTCGCTGCACAAGAATTCTTTTCTTATCATTTT
	TATTCTCAAATGGTATCAGAAGGTTTTGGAGTCATTCTGGAAATT
	TCATTCTCGTCGCGATTAGTATCCTCCCTTGAAGAAAAAAGAATA
	CCAAAATCTCAGAATTTACGATCTATTCATTCAATATTTCCCTTT
	TTAGA <mark>G</mark> GATAAATTATCACATTTAAATTATGTGTCGGATCTACTA
	ATACCCTATCCCATCCATCTGGAAATCTTGGTTCAAATCCTTCAA
	TGCTGGATCAAAGATGTTCCTTCTTTGCATTTCTTGCGATTGATT
	TCCATTTACGTCTTTTCAAAAAAAAAAAAAAAAAAAAAA
	СТАСАТААТТТТТАТGТАТАТGААТGCGAATATATATTCCTCTTT
	CTTCGTAAACAGTCTTCTTATTTACGATCAATATCTTCTGGAGTC
	тттсттдадсдаасасатттттатддааааатадаататсттада
	GTTGTTTCTTGTAATTCTTTTCAGAGGATCCTATGGTTCCTCAAA
	GATATTTTCATACATTATGTTCGATATCAAGGAAAAGCAATTTTG
	GCTTCAAAAGGAACTCTTATTCTGATGAATAAATGGAAATTTCAT
C I	TTTGTGAATTTTTGGCAATCTTATTTTCACTTTTGGTTTCAACCT
7.5	татаддатссататааадсааттасссааттаттссттстсттт
BUN	CTGGGATATTTTTCAAGTGTACTAAAAAACCCTTTGGTAGTAAGA
	AATCAAATGCTAGAGAATTCATTTCTAATAAATACTCTGACTAAG
	AAATTAGATACCATAGCTCCAGTTCTTTTCTTATTGGATCATTG
	TCGAAAGCTCAATTTTGTACTGTATTAGGTCATCCTATTAGTAAA
	CCGATCTGGACCAATTTATCGGATTCTGATATTCTTGATCGATTT
	TGTCGGATATGTAGAAATCTTTGTCGTTATCACAGCGGATCCTCA
	AAGAAACAGGTTTTGTATCGTATAAAGTATATACTTCGACTTTCG
	TGTGCTAGAACTTTGGCTCGTAAACATAAAAGTACAGTACGCA

Species	DNA sequences
	TTTATGCGAAGATTAGGTTCGGGATTCTTAGAAGAATTTTTTTG
	GAAGAAGAACAATTTCTTTCCTTAATCTTTCTCCAAA
Acampe prarmorsa	GCTGGATC <mark>AA</mark> AGATGTTCCTTCTTTGCATTTCTTGCGATTGATTT
	TCCACGAATATCATAATTTGAATAGTCTCATTACTTCAAAAAAAT
	СТАТТТАССТСТТТТСАААААААААААААААААТТСТТТТССТ
	TACATAATTTTTATGTATATGAATGCGAATATATATTCCTCTTTC
	TTCGTAAACAGTCTTCTTATTTACGATCAATATCTTCTGGAGTCT
	TTCTTGAGCGAACACATTTTTATGGAAAAATAGAATATCTTAGAG
	TCGTGTCTTGTAATTCTTTTCAGAGGATCCTATGGTTCCTCAAAG
	ATATTTTCATACATTATGTTCGATATCAAGGAAAAGCAATTTTGG
	CTTCAAAAGGAACTCTTATTCTGATGAATAAATGGAAATTTCATT
	TTGTGAATTTTTGGCAATCTTATTTTCACTTTTGGTTTCAACCTT
	ATAGGATCCATATAAAGCAATTACCCAATTATTCCTTCTCTTTC
	TGGGATATTTTTCAAGTGTACTAAAAAACCCTTTGGTAGTAAGAA
	ATCAAATGCTAGAGAATTCATTTCTAATAAATACTCTGACTAATA
	AATTAGATACCATAGCTCCAGTTCTTTTTCTTATTGGATCATTGT
	CGAAAGCTCAATITTGTACTGTATTAGGTCATCCTATTAGTAAAC
	CGATCTGGACCAATTTATCGGATTCTGATATTCTTGATCGATTTT
7.5	GTCGGATATGTAGAAATCTTTGTCGTTATCACAGCGGATCCTCAA
BUN	AGAAACAGGTTTTGTA
Acampe rigida	ACAAGATGTTCCTTCTTTGCATTTCTTGCGATTGATTTTCCACGA
	ΑΤΑΤCΑΤΑΑΤΤΤGAATAGTCTCATTACTTCAAAAAAATCCATTTA
	CGTCTTTTCAAAAAAAAAAGAAAAGATTCTTTTGGTTCCTACATAA
	TTTTTATGTATATGAATGCGAATATATATTCCTCTTTCTT
	ACAGTCTTCTTATTTACGATCAATATCTTCTGGAGTCTTTCTT
	GCGAACACATTTTTATGGAAAAATAGAATATCTTAGAGTCGTGTC

Species	DNA sequences
	TTGTAATTCTTTTCAGAGGATCCTATGGTTCCTCAAAGATATTTT
	CATACATTATGTTCGATATCAAGGAAAAGCAATTTTGGCTTCAAA
	AGGAACTCTTATTCTGATGAATAAATGGAAATTTCATTTTGTGAA
	TTTTTGGC <mark>AAT</mark> CTTATTTTCACTTTTGGTTTCAACCTTATAGGAT
	CCATATAAAGCAATTACCCAATTATTCCTTCTCTTTCTGGGATA
	TTTTTCAAGTGTACTAAAAAACCCTTTGGTAGTAAGAAATCAAAT
	GCTAGAGAATTCATTTCTAATAAATACTCTGACTAAGAAATTAAA
	TACCATAGCTCCAGTTCTTTTTCTTATTGGATCATTGTCGAAAGC
	TCAATTTTGTACTGTATTAGGTCATCCTATTAGTAAACCGATCTG
	GACCAATTTATCGGATTCTGATATTCTTGATCGATTTTGTCGGAT
	ATGTAGAAATCTTTGTCGTTATCACAGCGGATCCTCAAAGAAACA
	GGTTTTGTATCGTATAAAGTATATACTTCGACTTTCGTGTGCTAG
	AACTTTGGCTCGTAAACACAAAAGTACTGTA
Adenoncos parviflora	CATATTGCACTATGTATCATTTCAGGACACAAGACATGCCTCTCT
	ATTTTGGTTATAGTAGAAATGTATTTAAATAGCAGAATTACAAGG
	ATATTTAGATTGAAAAAAGAGAGATTTTGGCAACAAAACTTCCTA
	TATCCGCTACTCCTTCAGGAGTATATTTACTCACTTGCTCATTAT
	CATAGCTTCAATAGTTTGATTTTTTATGAACCTGTGGAAATTATC
15	GGTTATGACAATAAATCTAGTTTGGTACTTGTGAAACGTTTAATT
BUS	ACTCGAATGTATCAACAGAAATATTTGATTTCTTCGGTGAATGAT
	TCTAACCAAAATGGATTTTCGCTGCACAAGAATTCTTTTCTTAT
	CATTTTTATTCTCAAATGGTATCAGAAGGTTTTGGAGTCATTCTG
	GAAATTTCATTCTCGTCGCGATTAGTATCCTCCCTTGAAGACATA
	TTGCACTATGTATCATTTCAGGACACAAGACATGCCTCTCTATTT
	TGGTTATAGTAGAAATGTATTTAAATAGCAGAATTACAAGGATAT
	TTAGATTGAAAAAAGAGAGATTTTGGCAACAAAACTTCCTATATC
	CGCTACTCCTTCAGGAGTATATTTACTCACTTGCTCATTATCATA

Species	DNA sequences
	GCTTCAATAGTTTGATTTTTTATGAACCTGTGGAAATTATCGGTT
	ATGACAATAAATCTAGTTTGGTACTTGTGAAACGTTTAATTACTC
	GAATGTATCAACAGAAATATTTGATTTCTTCGGTGAATGATTCTA
	ACCAAAAT <mark>GGA</mark> TTTTCGCTGCACAAGAATTCTTT <b>TCTTATCATT</b> T
	TTATTCTCAAATGGTATCAGAAGGTTTTGGAGTCATTCTGGAAAT
	TTCATTCTCGTCGCGATTAGTATCCTCCCTTGAAGATGCATACCT
	СТТТТСТСТТТБАТТБАТАТАТ
Cottonia peduncularis	TGTTCCTTCTTTGCATTTCTTGCGATTGATTTTCCACGAATATCAT
	AAT <mark>TTG</mark> AATAGTCTCATTACTTCAAAAAAATCCATTTACGTCTTTT
	CAAAAAAAAAGAAAAGATTCTTTTGGTTCCTACATAATTTTTATG
	TATATGAATGCGAATATATATTCCTCTTTCTTCGTAAACAGTCTTC
	TTATTTACGATCAATATCTTCTGGAGTCTTTCTTGAGCGAACACA
	TTTTTATGGAAAAATAGAGTATCTTAGAGTCGTGTCTTGTAATTC
	TGTTCGATATCAAGGAAAAGCAATTTTGACTTCAAAAGGAACTCT
	TATTCTGATGAATAAATGGAAATTTCATTTTGTGAATTTTTGGCA
	ATCTTATTTTCACTTTTGGTTTCAACCTTATAGAATCCATATAAAG
57508	CAATTACCCAATTATTCCTTCTCTTTTCTGGGATATTTTTCAAGTG
	тасталалассстттддтадтадалатсалатдстададаатт
	CATTTCTAATAAATACTCTGACTAAGAAATTAGATACCATAGCTC
	CAGTTATTTTTCTTATTGGATCATTGTCGAAAGCTCAATTTTGTAC
	TGTATTAGGTCATCCTATTAGTAAACCGATCTGGACCAATTTATC
	GGATTCTGATATTCTTGA
Micropera obtusa	CATTTAAATTATGTGTCGGATCTACTAATACCCTATCCCATCCAT
	CTGGAAATCTTGGTTCAAATCCTTCAATGCTGGATCAAAGATGTT
	CCTTCTTTGCATTTATTGCGATTGATTTTCCACGAATATCATAATT
	ΤGΑΑΤΑGTCTCATTACTTCAAAAAAATCCATTTACGTCTTTTCAAA

Species	DNA sequences
	AAAAAAGAAAAGATTCTTTTGGTTCCTACATAATTTTTATGTATAT
	GAATGCGAATATATATTCCTCTTTCTTCGTAAACAGTCTTCTTATT
	TACGATCAATATCTTCTGGAGTCTTTCTTGAGCGAACACATTTTT
	ATGGAAAAATAGAATATCTTAGAGTCGTGTCTTGTCATTCTTTTC
	AGAGGATCCTATGGTTATTCAAAGATATTTTCATACATTATGTTC
	GATATCAAGGAAAAGCTATTTTGGCTTCAAAAGGAACTCTT
Micropera pallida	CCTGTTCTAGCACACGAAAGTCGAAGTATATACTTTATACGATAC
	AAAACCTGTTTCTTTGAAGATCCGCTGTGATAACGACAAAGATTT
	CTACATATCCGACAAAATCGATCAAGAATATCAGAATCCGATAAA
	TTGGTCCAGATCGGTTTACTAACAGGATGACCTAATACAGTACAA
	AATTGAGCTTTCGACAATGATCCAATAAGAAAAATAACTGGAGCT
	ATGGTATCTAATTTCTTAGTCAGAGTCTTTATTAGAAATGAATTCT
	CTAGCATTTGATTTCTTACTACCAAAGGATTTTTTAGTACACTTG
	AAAAATATCCCAGAAAAGAGAAGGAATAATTGGGTAATTGCTTTA
	TATGGATCCTATAAGGTTGAAACCAAAAGTGAAAATAAGATTGCC
	AAAAATTCACAAAATGAAATTTCCATTTATTCATCAGAATAAGAG
	TTCCTTTTGAAGCCAAAATCGCTTTTCCTTGATATCGAACATAAT
6	GTATGAA 10
77-	
Micropera rostrata	GACAAAGATTTCTACATATCCGACAAAATCGATCAAGAATATCAG
-0	AATCCGATAAATTGGTCCAGATCGGTTTACTAACAGGATGACCTA
	ATACAGTACAAAATTGAGCTTTCGACAATGATCCAATAAGAAGAA
	TAACTGGAGCTATGGTATCTAATTTCTTAGTCAGAGTCTTTATTA
	GAAATGAATTCTCTAGCATTTGATTTCTTACTACCAAATTGTTTTT
	TAGTACACTTGAAAAATATCCCAGAAAAGAGAAGGAATAATTGGG
	TAATTGCTTTATATGGATCCTATAAGGTTGAAACCAAAAGTGAAA
	ΑΤΑΑGATTGCCAAAAATTCACAAAATGAAATTTCCATTTATTCAT

CAGAATAAGAGTTCCTTTTGAAGCCAAAATTGCTTTTCCTTGATA

Species	DNA sequences
	TCGAACATAATGTATGAAAATATCTTTGAATAACCATAGGATCCT
	CTGAAAAGAATTACAAGACACGACTCTAAGATATTCTATTTTCC
	ATAAAAATGTGTTCGCTCAAGAAAGACTCCAGAAGATATTGATCG
	TAAATAAG <mark>AAG</mark> ACTGTTTACGAAGAAGAGGGAATATATATTCGCA
	TTCATATAC <mark>AT</mark> AAAGATTATGTAGGAACCAAAAGAATCTTTCTT
	TTTTTTG <mark>AAA</mark> AGACGTAAATGGATTTTTTGAAGTAATGAGACT
	ATTCAA <mark>ATT</mark> ATGATATTCGTGGAAAATCAATCGCAATAAATGTAA
	AGAAGGAACATCTTTGATCCAGCATTGAAGGATTTGAACCAAGAT
	TTCCAGATGGATGGGATAGGGTATTAGTAGATCCAACACATAATT
	TAAATGTGATAATTTATCCTCTAAAAAGGGAAATAT
Micropera thailandica	CACGAAAGTCGAAGTATATACTTTATACGATACAAAACCTGTTTC
	TITGAGGATCCGCTGTAATAACGACAAAGATTTCTACATATCCGA
	CAAAATTGATCAAGAATATCAGAATCCGATAAATTGGTCCAGATC
	GGTTTACTAACAGGATGACCTAATACAGTACAAAATTGAGCTTTT
	GACAATGATCCAATAAGAAAAAGAACTGGAGCTATGGTATCTAAT
	TTCTTAGTCAGAGTCTTTATTAGAAATGAATTCTCTAGCATTTGAT
	TTCTTACTACCAAAGGGTTTTTTAGTACACTTGAAAAATATCCCA
5	GAAAAGAGAAGGAATAATTGGGTAATTGCTTTATATGGATCCTAT
475NE	AAGGTTGAAACCAAAAGTGAAAATAAGATTGCCAAAAATTCACAA
	AATGAAATTTCCATTTATTCATCAGAATAAGAGTTCCTTTTGAAG
	CCAAAATTGCTTTTCCTTGATATCGAACATAATGTATGAAAATAT
	CTTTGAATAACCATAGGATCCTCTGAAAAGAATTACAAGACACGA
	СТСТААБАТАТТСТАТТТТССАТАААААТGTGTTCGCTCAAGAA
	AGACTCCAGAAGATATTGATCGTAAATAAGAAGACTGTTTACGAA
	GAAAGAGGAATATATATTCGCATTCATATACATAAAAATTATGTA
	GGAACCAAAAGAATCTTTTCTTTTTTTTGAAAAGACGTAAATGG
	ATTTTTTGAAGTAATGAGACTATTCAAATTATGATATTCGTGGA
	AAATAAATCGCAATAAATGCAAAGAAGGAACATCTTTGATCCAGC

Species	DNA sequences
	ATTGAAGGATTTGAACCAAGATTTCCAGATGGATGGGATAGGGTA
	TTAGTAGATCC
Trichoglottis triflora	TCGAGACCGAAGTTATATCGAGCGATTCGGAGAACCTGTGAAACA
	AGCGGCGG <mark>CG</mark> GCCGTCGCGGCGAAACAGCCGCCACCCCGTCGC
	TCCCCCCGTCTGGAGGGGGCCGCGGCGAGGGACGGCTGAAACCC
	CAAACC <mark>GGC</mark> GCAGATTGGCGCCAAGGGAGCTATCGAAAAACACG
	AGCCCG <mark>G</mark> CATCG <mark>G</mark> GTCTTCGTGGGTTGGAGCGGTGCTGCGCGCC
	GCGCGTATTGACACGACTCTCGACAATGGATATCTCGGCTCTCGC
	ATC <mark>GAT</mark> GAAGAGCGCAGCGAAATGCGATACGTGGTGCGAATTGC
	AGAATCCCGCGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGA
	GGCCAATCGGTCGAGGGCACGTCCGCCTGGGCGTCAAGCGTTGC
	GCCGCTCCGTGCCGAGTCCCCACCATCCCGCCGCAGTGGGGGTG
	CCGGGCGAGGCTCGGACGTGCAGAGTGGCTCGTCGTGCCCATCG
	GTGCGGCGGGCTGAAGAGCGGGTTATCGTCTCATTGGGGCCACG
	AACAACGAGGGGTGGATGAAAGCTGCCGCGGGCGAGGCCCGCGT
	TGTCTCGTGCCGGCCCGAGAGAAGATCACACCCTTCGTGCGATC
	CCTTCCCATGCGCCGCCCCCGGGCGGCGGCTTGGAATGTCTCG
	TGCCGGCCCGAGAGAAGATCACACCCTTCGTGCGATCCCTTCCCA
5.	TGCGCCGCCCCGGGCGGCGGCTTGGAA
100	

# <sup>อุ</sup>กยาลัยเทคโนโลยีสุจ

#### APPENDIX C

#### PARSIMONOUS TREES FROM THIS ANALYSIS



Figure C.1 All shortest tree from maximum parsimony analysis, based on the ITS





Figure C.1 All shortest tree from maximum parsimony analysis, based on the ITS gene (Continued).



Figure C.2 All shortest tree from maximum parsimony analysis, based on the *mat*K gene.



**Figure C.2** All shortest tree from maximum parsimony analysis, based on the *mat*K gene (Continued).



Figure C.2 All shortest tree from maximum parsimony analysis, based on the *mat*K gene (Continued).





**Figure C.3** All shortest tree from maximum parsimony analysis, based on the combined nuclear (ITS) and plastid (*mat*K) gene.



**Figure C.3** All shortest tree from maximum parsimony analysis, based on the combined nuclear (ITS) and plastid (*mat*K) gene (Continued).

### CURRICULUM VITAE

Name	Mr. Tammanoon Jitpromma
E-mail	tammanoo <mark>n_</mark> bio@hotmail.com
Phone	092 139 68 <mark>5</mark> 4
Date of Birth	12 December 1994
Place of Birth	Roi ET, Thailand
Education	Bachelor of Science (Biology), School of biology, Institute
	of S <mark>cien</mark> ce, Suran <mark>aree</mark> University of Technology, Thailand
	in 2017

#### Conferences

2017. Jitpromma T. and Krubprachaya P. Comparison of heterologous expression of  $\alpha$ amylase engineering in *Escherichia coli* with the wide type  $\alpha$ -amylase from *Bacillus amyloliquefaciences*. Undergraduate student research presentations, school of biology, Suranaree University of Technology, Thailand (Poster Presentation).

Grants and Fellowships Suranaree University of Technology

<sup>3</sup>่าวักยาลัยเทคโนโลยีสุรบ