TAXONOMIC REVISION OF FERN *Microsorum punctatum* (L.) Copel. COMPLEX (POLYPODIACEAE)

Mr. Sahanat Petchsri

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Biological Science
Faculty of Science
Chulalongkorn University
Academic Year 2007

TAXONOMIC REVISION OF FERN *Microsorum punctatum* (L.) Copel. COMPLEX (POLYPODIACEAE)

Mr. Sahanat Petchsri

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Biological Science
Faculty of Science
Chulalongkorn University
Academic year 2007

ทบทวนอนุกรมวิธานของเฟิร์นชนิดเชิงซ้อน *Microsorum punctatum* (L.) Copel. (POLYPODIACEAE)

นายสหณัฐ เพชรศรี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิทยาศาสตร์ชีวภาพ
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2550

Thesis Title	TAXONOMIC REVISION OF FERN *Microsorum punctatum* (L.) Copel. COMPLEX (POLYPODIACEAE)
By	Mr. Sahanat Petchsri
Field of Study	Biological Science
Thesis Advisor	Professor Thaweesakdi Boonkerd, Ph.D.
Thesis Co-advisor	Bernard R. Baum, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

S. Hannongbua .....Dean of the Faculty of Science

(Professor Supot Hannongbua, Ph.D.)

THESIS COMMITTEE

.....Chairman

(Associate Professor Preeda Boon-Long, Ph.D.)

Thaweesakdi Boonkerd ..... Thesis Advisor

(Professor Thaweesakdi Boonkerd, Ph.D.)

B. Baum ..... Thesis Co-advisor

(Bernard R. Baum, Ph.D.)

Wina Meckvichai ..... Member

(Associate Professor Wina Meckvichai)

Manit Kidyoo .....Member

(Manit Kidyoo, Ph.D.)

..... Member

(Associate Professor Kitichate Sridith, Ph.D.)

สหณัฐ เพชรศรี : ทบทวนอนุกรมวิธานของเฟิร์นชนิดเชิงซ้อน *Microsorum punctatum* (L.) Copel. (Taxonomic revision of fern *Microsorum punctatum* (L.) Copel. complex (POLYPODIACEAE)) อ. ที่ปรึกษา: ศ.ดร.ทวีศักดิ์ บุญเกิด, อ. ที่ปรึกษาร่วม: ดร. Bernard R. Baum, 114 หน้า.

"กระปรอกหางสิงห์ (*Microsorum punctatum* (L.) Copel.)" เป็นเฟิร์นที่เจริญตามธรรมชาติตั้งแต่ระดับน้ำทะเลในป่าชายเลนไปจนถึงบริเวณภูเขาสูงกว่า 2,800 เมตร กระจายพันธุ์อยู่ในเขตร้อนและเขตกึ่งร้อน มีสถานภาพทางอนุกรมวิธานไม่เสถียรและมีความแปรผันสูงในลักษณะทางสัณฐานวิทยาซึ่งไม่สอดคล้องกับระบบการจัดจำแนกที่มีมาก่อน จึงจำเป็นต้องมีการศึกษาทบทวนเพิ่มเติม จากการศึกษาลักษณะสัณฐานวิทยาและกายวิภาคของตัวอย่างพันธุ์ไม้แห้งของเฟิร์น *M. punctatum* (L.) Copel. และชนิดใกล้เคียงจำนวน มากกว่า 1,500 ตัวอย่างพบว่าสามารถจัดจำแนกเฟิร์นกลุ่มนี้ออกได้เป็น 8 หน่วยอนุกรมวิธาน ซึ่งสอดคล้องกับผลการศึกษาตัวอย่างพันธุ์ไม้แห้งจำนวน 707 ตัวอย่างที่เก็บรักษาไว้ในพิพิธภัณฑ์พืชต่างๆ ทั้งในประเทศและต่างประเทศด้วยเทคนิคทางอนุกรมวิธานเชิงตัวเลขในลักษณะเชิงปริมาณและเชิงคุณภาพรวมทั้งสิ้น 58 ลักษณะ โดยพบว่าการวิเคราะห์การจัดกลุ่ม (Cluster analysis) นั้นสามารถแบ่งตัวอย่างพันธุ์ไม้ทั้งหมดออกเป็น 8 กลุ่มเช่นกัน ในขณะที่การวิเคราะห์การจัดจำแนก (Discriminant analysis) นั้นพบว่าแต่ละกลุ่มจาก 8 กลุ่มตามข้างต้นนี้ได้แยกออกจากกันอย่างชัดเจน โดยสามารถสรุปได้ว่ามีพรรณไม้ที่ควรจัดเป็นชนิดอิสระจากชนิดอื่นๆ ตามผลการวิเคราะห์ในครั้งนี้คือ *M. siamense M. thailandicum* และ *M. membranaceum, M. musifolium, M. glossophyllum* และ *M. punctatum* ส่วนที่ควรจัดจำแนกให้เป็นระดับพันธุ์คือ *M. steerei* var. *steerei* and *M. steerei* var. *whiteheadii* โดยมีลักษณะสำคัญที่ใช้ในการจำแนก 6 ลักษณะคือ ความยาวของก้านใบ ความยาวของเกล็ดบริเวณไรโซม ความกว้างของ primary areole จำนวนแถวของกลุ่มอับสปอร์ เส้นผ่านศูนย์กลางของกลุ่มอับสปอร์ และความหนาแน่นของกลุ่มอับสปอร์บนแผ่นใบ จากนั้นจะนำทั้งลักษณะเชิงปริมาณและลักษณะเชิงคุณภาพมาใช้ในการสร้างรูปวิธานแยกเฟิร์นกลุ่มนี้ต่อไป อีกทั้งจากผลการศึกษาความแปรผันทางพันธุกรรมด้วยเทคนิค PCR-RAPD ยังพบว่าสมควรให้คงการจัดจำแนกในระดับพันธุ์ปลูกเอาไว้ 2 พันธุ์ด้วยคือ *M. punctatum* cv. *serratum* และ *M. punctatum* cv. *grandiceps* ส่วนผลการวิเคราะห์สายวิวัฒนาการนั้นพบว่าสอดคล้องกับผลการศึกษาต่างๆ ข้างต้นในการแยก *M. glossophyllum* และ *M. musifolium* ออกจาก *M. punctatum* โดยมีสายวิวัฒนาการ *M. membranaceum* และ *M. musifolium* เป็นสายวิวัฒนาการสายแรกที่แยกออกมา ส่วนชนิดอื่นๆที่เหลือต่อมาจึงแยกย่อยและมีสายวิวัฒนาการใหญ่ๆ 4 สาย

สาขาวิชา.....วิทยาศาสตร์ชีวภาพ.....ลายมือชื่อนิสิต.....สหณัฐ เพชรศรี.....

ปีการศึกษา.....2550.....ลายมือชื่ออาจารย์ที่ปรึกษา.....ทวีศักดิ์ บุญเกิด.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....B. Baum.....

4673833123 : MAJOR BIOLOGICAL SCIENCE
KEY WORD : SYSTEMATIC/NUMERICAL TAXONOMY/MORPHOMETRIC ANALYSIS/*Microsorum punctatum*
SAHANAT PETCHSRI : TAXONOMIC REVISION OF FERN *Microsorum punctatum* (L.) Copel. COMPLEX (POLYPODIACEAE). THESIS ADVISOR : PROF. THAWEESAKDI BOONKERD, Ph.D., THESIS COADVISOR : BERNARD R. BAUM, Ph.D. 114 pp.

Microsorum punctatum (L.) Copel. is a widespread and variable species of fern. It is found from sea level to about 2,800 m altitude and has its distribution in the palaeotropics and subtropics. At present, the taxonomic status and boundary of this species is still uncertain due to the great variation in many characters. This variation does not match with previous systematic treatments. So, a number of species were grouped under a proposed species complex worth investigating. In order to investigate this species complex, more than 1,500 specimens were studied. Based on morphological and anatomical characters, *M. punctatum* complex can be divided into 8 taxa. This result rather corresponded with the results of cluster analysis and canonical discriminant analysis performed on 707 herbarium specimens deposited at BCU, BKF, BM, K, B, L and P. Twenty three quantitative and 35 qualitative characters were employed. Cluster analysis revealed the separation of the taxa in the species complex into eight groups. The eight-cluster grouping is discussed. From a canonical discriminant analysis using the eight-cluster grouping as priori groups, it can be concluded that *M. siamense*, *M. thailandicum*, *M. membranaceum*, *M. glossophyllum* and *M. musifolium* are distinct taxa, while specimens of *M. steerei* and *M. whiteheadii* were mixed together and should be proposed as one and the same species. Likewise, the other 10 synonyms of *M. punctatum* are not distinct from *M. punctatum* and treated here as members of *M. punctatum.* The six most important characters that separate the eight recognized species are stipe length, number of sori row between the adjacent secondary vein, sori diameter, sori density, primary-areole width, and spore width. These quantitative characters, together with some qualitative characters, were useful in constructing an identification key to these species. The differentiation between the studied taxa is discussed. According to RAPD data, *M. punctatum* cv. *serratum* and *M. punctatum* cv. *grandiceps*, were still recognized. The result of phylogenic study agreed with morphological and morphometric studies to separate *M. glossophyllum* and *M. musifolium* from *M. punctatum*. *M. membranaceum* and *M. musifolium* were most basal position of monophyletic clade of the complex. The larger clade was divided into four recognizable subclades.

Field of study.....Biological Science.....Student's signature.....Sahanat Petchsri.....
Academic year.....2007.....Advisor's signature.....Thaweesakdi Boonkerd.....
Co-advisor's signature.....B. Baum.....

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude and sincere appreciation to my thesis advisors, Professor Dr. Thaweesakdi Boonkerd, for his assistance in the thesis's problem, guidance, encouragement and many valuable suggestions, which has a great benefit throughout my study.

I also would like to express my sincere thanks to my thesis co-advisor, Dr. Bernard R. Baum, for his helpful advice and criticism, especially in molecular and numerical techniques, throughout the course of this study.

I wish to express my sincere thanks to the thesis committee, Associate Professor Dr. Preeda Boon-Long, Associate Professor Wina Meckvichai, Associate Professor Dr. Kitichate Sridith, Dr. Manit Kidyue and Assistant Professor Dr. Tosak Seelanan for their valuable suggestions and advice.

I also wish to thank Mr. Yotsawate Sirichamorn, Miss Maliwan Nakkuntod, Miss Thanikarn Udomchalothorn, Miss Paweena Traiperm, Miss Oratai Neamsuvan, and Miss Apirada Sthapattayanon for their friendship and their helps during my field observations and my laboratory. Thanks are also due to all staff, the directors and curators of B, BCU, BKF, BM, K, L and P herbarium for their generous help and making their collections available for this study.

Thanks to Department of Botany, Faculty of Science, Chulalongkorn University, the Plant of Thailand Research Unit, and Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada for providing laboratory facilities for this thesis.

Special appreciation goes to my family for their supports and encouragement throughout the study period.

This project was supported by the Thai government budget 2005, under the Research Program on Conservation and Utilization of Biodiversity and the Center of Excellence in Biodiversity, Faculty of Science, Chulalongkorn University; Chulalongkorn University graduate scholarship to commemorate the 72nd anniversary of His Majesty King Bhumibol Adulyadej and the TRF/ BIOTEC Special Program for Biodiversity Research and Training program (grant No. T_145007).

CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATION

A, T, C, G	=	Nucleotide containing the base Adenie, Thymine, Cytosine, and Guanine, respectively
AC	=	costal areole
ALA	=	Angle of frond apex
ALB	=	Angle of frond base
APS	=	Angle between primary and secondary vein
BCU	=	Professor Kasin Suvatabhundbu Herbarium, Department of Botany, Faculty of Science, Chulalongkorn University
BKF	=	National Park, Wildlife and Plant Conservation Department, Bangkok, Thailand
B	=	The Botanical Museum Berlin-Dahlem, Berlin, Germany
BM	=	The Natural History Museum, London, Enhland
°C	=	Degree Celsius
cm	=	Centimeter
cv.	=	*cultivarietas*, cultivar variety
comb. nov.	=	*combinatio*, new combination of name end epithet
CTAB	=	Cetyl Trimethyl Ammonium Bromide
DBSV	=	Distance between closest secondary vein in mm
diam	=	in diameter
DSL	=	Density of sori per cm2
DSO	=	Diameter of sori in mm
DSR	=	Diameter of sporangium in mm
e.g.	=	*example gratia*, byway of example, for example
mm	=	Millimeter
g	=	gram
K	=	Kew Herbarium, Royal Botanic Gardens, England
kb	=	kilobase pair
L	=	The Rijkherbarium, Leiden, Nederland
LMW	=	Lamina width in mm
LML	=	Lamina length in mm
M	=	Molar

m	=	meter
mg	=	Milligram
$MgCl_2$	=	Magnesium Chloride
mM	=	Millimolar
ml	=	milliliter
NAC	=	Number of annulus cell
NaCl	=	Sodium chloride
ng	=	Nanogram
NSR	=	Number of sori rows between closest secondary vein
n.v.	=	*non visus*, not seen
OTU	=	Operational Taxonomic Unit
P	=	Muséum National d'Historie Naturelle, Paris, France
PAW	=	Primary areole width in mm
PCR	=	Polymerase chain reaction
PDL	=	Phyllopodia length in mm
PHS	=	Distance between highest sori and frond apex in mm
PLS	=	Distance between lowest sori and frond base in mm
RAPD	=	Random Amplification of Polymorphic DNA
RDL	=	Distance between closest phyllopodia in mm
RHD	=	Rhizome diameter in mm
rpm	=	Revolution per minute
SCL	=	Scale length in mm
SCW	=	Scale width in mm
SEM	=	Scanning electron microscope
s. l.	=	sensu lato
s.n.	=	*sine numero*, without a number, unnumbered
SPL	=	Spore length in mm
SPW	=	Spore width in mm
s. str.	=	sensu stricto
stat. nov.	=	*status novus*, new rank
STL	=	Stipe length in mm
STD	=	Stipe diameter in mm
subg.	=	Subgenus

subsect.	=	Subsection
ssp.	=	Subspecies
Tris	=	Tris (hydroxyl methyl) aminomethane
UPGMA	=	Unweighted Pair Group Method with Arithmetic mean
µg	=	Microgram
µl	=	Microlitre
µm	=	Micrometer
V	=	Volt
var.	=	Variety

CHAPTER 1

INTRODUCTION

Microsorum punctatum (L.) Copel. is a common species, having a widespread distribution in various forest types of temperate and tropical parts of the Old World, extending from the Pacific Islands to Northeast Australia, Malaysia, Southeast Asia, Southern China, the Indian subcontinent extending to Madagascar and Tropical Africa, whereas the highest species diversity occurs in East Asia (Bosman, 1991; Roux, 2005). *M. punctatum* (L.) Copel. and its related species are terrestrial, lithophyte or epiphyte in evergreen forests, mostly in deep shade. These species can be found from mangrove or sandy beach forest at sea level up to high elevation of Montane forest. At present, *Microsorum* includes about 50 species. Many of them are widely cultivated either as house or garden plants.

Microsorum punctatum and its related species have a great variation in frond forms, sizes, and venation patterns and were treated differently by different authors. For example, *M. musifolium* Copel. and *M. glossophyllum* Copel. were treated as synonyms of *M. punctatum* while they were recognized as distinct species by some workers (Bosman, 1991). Nooteboom (1998) noted that *M. glossophyllum* is a form differing in blackish narrow rhizome scales, but this character is variable and intermediates occur with the more brownish and wider scale. *M. musifolium* is a form with wider fronds and more connecting veins and is connected with *M. punctatum* by many intermediates. Moreover, *Polypodium punctatum* ssp. *subirideum* H. Christ and *P. punctatum* ssp. *subdrynariaceum* H. Christ were first proposed in 1906 and were transferred into *M. subirideum* by Copeland, who proposed name *M. punctatum*, in 1947 and were treated as synonym of *M. punctatum* by some worker (Bosman, 1991; Bosman et al., 1998; Nooteboom, 1997). *Polypodium polycarpon* Cav. was considered as a basionym of *M. polycarpon* Tardieu., this species have rather different morphological character of frond-form from *M. punctatum*. It was also considered as a synonym of *M. punctatum* by some pteridologists. Because these variations do not match with previous recognized taxa, Nooteboom placed these collective taxa as a species complex and suggested that they are worth investigating (personal communication, 9 September 2005).

These examples show that *M. punctatum* and related species are not clearly circumscribed and delimited. Moreover, these taxa, especially cultivated plants, exhibit variations in frond forms (e.g. irregularly lobed). Some of these forms have been described as cultivars, i.e. *M. punctatum* (L.) Copel. cv. *serratum*. These variants are not included in previous recognized systematic treatments. It can be seen that the members of this species complex have a history of circumscriptional uncertainty, suggesting the need for further taxonomic evaluation.

Aim of the thesis

1. To investigate morphological, anatomical and molecular variation of *Microsorum punctatum* (L.) Copel. and its related taxa. With these objectives in mind, both cluster analysis (CA) and discriminant analysis (DA) were performed based on both qualitative and quantitative characters (i.e. 56 characters) examined from 707 herbarium specimens.

2. To infer phylogenetic relationship of *Microsorum punctatum* (L.) Copel. and its related taxa based on morphological data.

3. To determine the taxonomic status of taxa in the *Microsorum punctatum* (L.) Copel. complex.

CHAPTER 2

LITERATRE REVIEW

2.1 Taxonomic History of the "*Microsorum punctatum* (L.) Copel. complex"

"*Microsorum punctatum* complex" occurs in various forest types of temperate and tropical parts of the Old World, extending from the Pacific Islands to Northeast Australia, Sumatra, Malaysia, Southeast Asia, Southern China, the Indian subcontinent extending to Madagascar and Tropical Africa, whereas the highest species diversity occurs in East (Bosman, 1991; Roux, 2005). It grows as terrestrial, lithophytes or epiphytes in evergreen forests, mostly in semi- or deep shade. (Fig. 2.1)

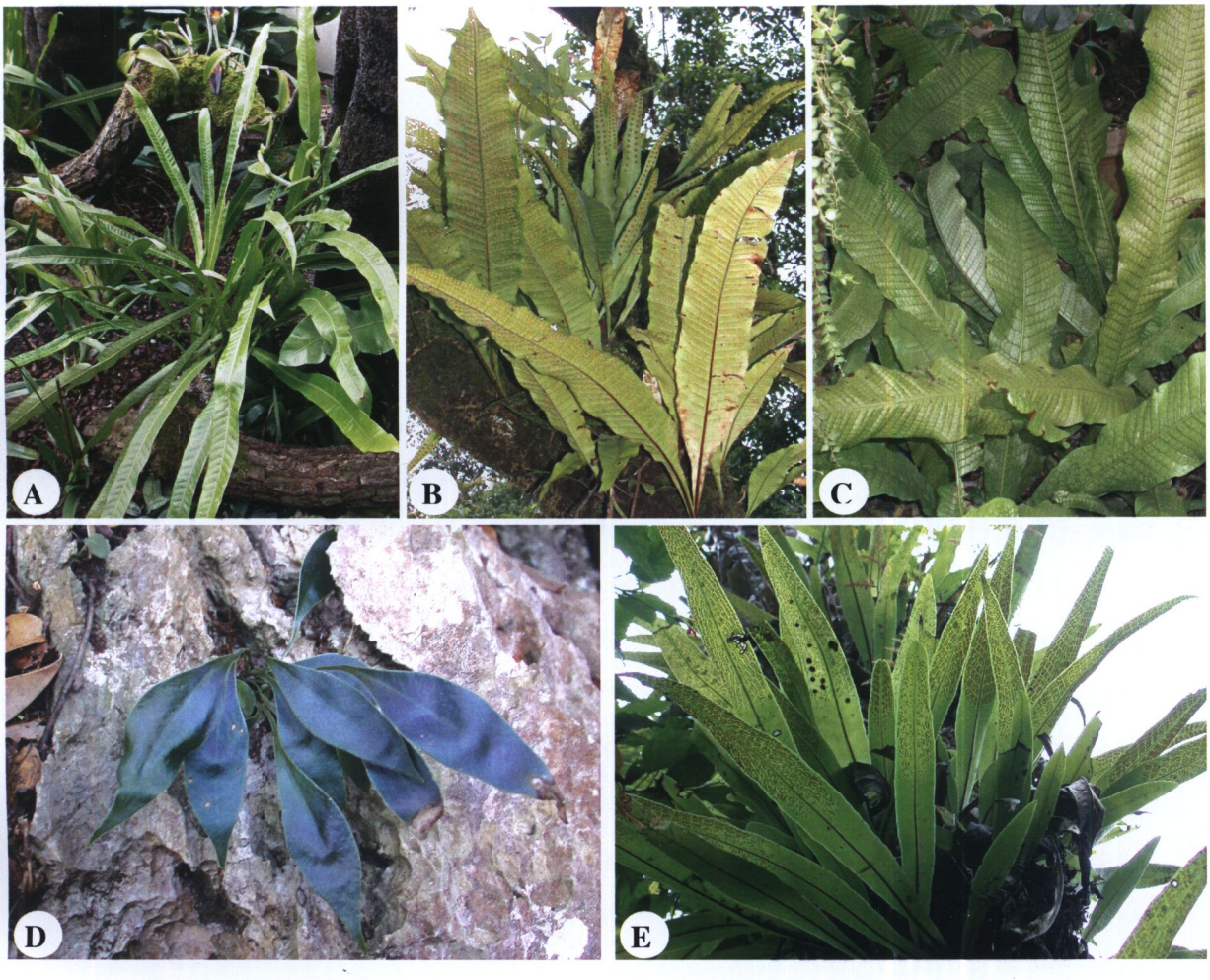

Figure 2.1 Habitats of "*M. punctatum* complex".—A. and E. *M. punctatum* (L.) Copel., Lithophyte and Epiphyte; B. *M. membranaceum* (D. Don) Ching., Epiphyte; C. *M. musifolium* (Blume) Copel., Terrestrial; D. *M. siamense* Boonkerd, Calciphyte

The specific epithet "*punctatum*" refers to the minutely pitted dotted nature of the lamina. This species was proposed in 1763 by Linnaeus. He had already described this plant under *Acrostichum punctatum* L. which became the basionym of *M. punctatum* (L.) Copel (Bosman, 1991). Link (1833) is the first who established the genus *Microsorum*, but described only one species, i.e. *M. irregulare* Link using another type specimen. Up till now, this name was regarded as a synonym of *M. punctatum* (L.) Copel (Nooteboom, 1997).

After the genus *Microsorum* was established, many species of *Polypodium* were transferred to *Microsorum*. Particularly many of the *Polypodium* species described by Blume (1829) are still accepted as basionyms of some microsoroid ferns, e.g. *P. heterocarpum*, *P. musifolium*, *P. insigne*, *P. pteropus*, *P. superficiale* and *P. zippelii*. Likewise, *P. validum* Copel. was transferred into *Microsorum* under *M. validum* by Ching in 1933 and was treated as synonyms of *M. punctatum* (L.) Copel. by Hovenkamp (1998).

The most up-to-date treatment of *Microsorum* was in Flora Malesiana where many species were treated as synonyms of this species (Bosman et al., 1998), for example *M. musifolium* Copel., and *M. glossophyllum* Copel. were previously recognized as distinct species by Bosman (1991). Nooteboom (1997) noted that *M. glossophyllum* is a form of *M. punctatum* differing only in the blackish narrow rhizome scales, however this character is rather variable with intermediate forms occurring with more brownish and wider scales. He also noted that *M. musifolium* is a form of *M. punctatum* having wider fronds and more connecting veins and is connected with *M. punctatum* by many intermediates. Previously, Holttum (1954) pointed out that dried herbarium specimens of *M. musifolium* are very similar to the narrow-frond specimens of *M. punctatum* and it is not easy to distinguish them; but living specimens of *M. musifolium* have distinctly raised main veins and the dark color of most veins appears to be very distinct from *M. punctatum*. It seems also that the scales of *M. punctatum* differ in having thin small cells on the edges, but this character needs further verification.

Polypodium punctatum L. ssp. *subiridum* H. Christ and *P. punctatum* ssp. *subdrynariaceum* H. Christ were firstly proposed in 1906 and were transferred to *Microsorum subirideum* by Copeland, who proposed the name *M. punctatum* in 1947. These taxa were treated as synonyms of *M. punctatum* by Nooteboom (1997). Another

related species, *Polypodium polycarpon* Cav. was previously considered as the basionym of *Microsorum polycarpon* Tardieu, due to having different morphological character of their fronds from *M. punctatum*; but currently was considered as a synonym of *M. punctatum* by some pteridologists. It can be seen that the taxonomic status of *M. punctatum* and its related taxa is still unclear due to great variations in frond form, sizes, and venation patterns. Because these variations do not match with previous recognized taxa, Nooteboom placed these collective taxa as a species complex and suggested that they are worth investigating (personal communication, 9 September 2005). He also pointed out that *M. membranaceum* and *M. steerei* are very close to *M. punctatum* and that they might be conspecific (Nooteboom, 1997).

Recently, *Microsorum whiteheadii* A.R. Sm. & Hoshiz. was discovered from a limestone cliff in Sumatra. It is related to widespread polymorphic forms of *M. punctatum*, but differs from that species by the shorter-creeping rhizomes, more succulent, thicker, very dark green, oblanceolate lamina, prominent hydathodes on upper surface of fronds, and less visible venation (Smith & Hoshizaki, 2000). Boonkerd and Nooteboom (2001) described *Microsorum thailandicum* Boon. & Noot., from a limestone hill in peninsular Thailand. They noted that *M. thailandicum* resembles *M. punctatum* and *M. steerei*, but differs from both species in the iridescent bluish-green fronds and in having more annulus cells. Another iridescent blue *Microsorum*, also found from limestone outcrops in southern Thailand, was named as *Microsorum siamense* (Boonkerd, 2006). This species also has scales and sporangia characters in common with *M. punctatum* and *M. thailandicum* but differs in always having conspicuous hydathodes on the upper frond surface.

These examples show that *M. punctatum* and related species are not clearly circumscribed and delimited. Moreover, these taxa, especially cultivated plants, exhibit variations in frond forms (e.g. irregularly lobed). Some of these forms have been described as cultivars, i.e. *M. punctatum* (L.) Copel. cv. *serratum*. These variants are not included in previous recognized systematic treatments. It can be seen that the members of this species complex have a history of circumscriptional uncertainty, suggesting the need for further taxonomic evaluation.

2.2 Methodological review

Cryptic species complex is a group of species which satisfy the biological definition of species, that is, they are reproductively isolated from each other, but they are not morphologically distinguishable (or at least are not readily or reliably distinguishable on a morphological basis). Prior to about 1988, cryptic species were thought to be uncommon due to they were not easy to recognize. But the increasingly widespread application of molecular genetic methods to studies of population structure and systematics has revealed that many long-established "species" are really composed of two or more fully isolated and ecologically differentiated forms. The following are examples of methods currently used to clarify the taxonomic status of pteridophytes and flowering plants.

Morphological and anatomical feature (including wood anatomy and pollen morphology) were studied in order to investigate taxonomic status of *Pagameopsis* (Rubiaceae) by Piesschaert et al. in 2001. They showed that *Pagameopsis* is definitely not a member of the Psychotrieae because of significant differences in wood anatomy and gynoecial and fruit structure. Starr and Ford (2001) tried to revise taxonomy and phylogenetics of *Carex* section *Phyllostachys* using both macro- and micro morphological and anatomical characters. They concluded that the anatomical characters strongly supported the recognition of three species within the *Carex willdenowii* complex, namely *C. willdenowii* s.s., *C. basiantha*, *C. superata* as well as in the two close species pair of *C. backii* and *C. saximontana*, and *C. latebracteata* and *C. juniperorum*.

Speer and Hilu (1998), and Thomson (2000) used both morphometric analysis and DNA fingerprinting to study taxonomy and relationships in all species of the cosmopolitan bracken-fern, *Pteridium* Gled. ex Scop. (Dennstaedtiaceae). The results supported a taxonomic treatment at the varietals level. Furthermore, Thomson et al. (2005) studied two African morphotypes of bracken fern occur in sub-Saharan Africa. The results showed that this African form is separable morphometrically and genomically from the European subspecies, *P. aquilinum* ssp. *aquilinum*, and restored its earlier name *P. aquilinum* ssp. *capense*. The second African bracken fern, with a more localized tropical distribution mainly in the drainage basins of the Congo and Zambezi River systems, is confirmed for its taxonomic status as *P. aquilinum* ssp. *centrali-africanum*. Moreover, thirty four species and 3 varieties considered within *Poa* sect. *Dioicopoa* were

examined using numerical techniques by Giussani (2000). Two species complex were established, *Poa bonariensis* complex and *Poa resinulosa* complex, as well as new species and new variety, *Poa lanigera* and *Poa pilcomayensis* var. *pilcomayensis*.

Ward (1993) used phenetic analysis to clarify taxonomic status of the genus *Raoulia* in New Zealand. One hundred and seventeen individuals representing all known taxa of *Raoulia* were scored for 84 morphological characters. Numerical phenetic analysis showed that boundaries of most species are clear-cut except *R. mammillaris* and *R. bryoides*. Of the six varieties currently recognized viz. *R. hookeri* var. *apicinigra*, *R. hookeri* var. *laxa*, *R. hookeri* var. *albosericea*, *R. tenuicaulis* var. *pusilla*, *R. tenuicaulis* var. *dimorpha*, and *R. hectorii* var. *mollis* should be restored. The three main subdivisions of the genus are *Raoulia* subg. *Raoulia*, *Raoulia* subg. *Psychrophyton*, and *R. cinerea*. subg. *Mistura* and the sections of *Raoulia* should not be maintained.

Multivariate and descriptive analyses of morphological data were performed in order to clarify taxonomic status of the *Isoetes karstenii* complex by Small and Hickey (2001). Five species are recognized including the previously described *I. karstenii* and *I. palmeri*. One variety was transferred from *I. lechleri* to *I. karstenii* (*I. karstenii* var. *anomala*). Three of the recognized species are newly described, i.e. *I. fuliginosa*, *I. hemivelata* and *I. preccoia*. UPGMA cluster analysis to determine taxonomically definable limits and to estimate the phenetic relationships among four *Typha* species from Korea and Far East Russia using 25 quantitative characters. The result showed that individuals of those plants form discrete clusters corresponding to four species namely *T. latifolia*, *T. angustifolia*, *T. orientalis* and *T. laxmanni* (Kim et al., 2003). Furthermore, morphometric analyses was also carried out in 33 populations belonging to all nine described and one putative taxa of *Chamaecrista* subsect. *Baseophyllum*. The data from morphometric analyses supported the recognition of eight species in the subsection viz. *C. blanchetii*, *C. brachystachya*, *C. confertiformis*, *C. coriacea*, *C. cytisoides*, *C. decora*, *C. depauperata*, and *C. unijuga*. Regarding to *C. cytisoides* var. *micrantha*, it should be proposed as synonym of *C. brachystachya* (Conceição et al., 2008).

Casiva et al. (2002) studied 4 species of *Acacia* using morphometric and RAPD techniques. The RAPD phenogram shows similar result with morphological data. The

RAPD analysis showed 34 loci which can be used to differentiate the species, with an exception in *A. aroma* and *A. macracantha*, the two most closely related species. Morphometric characters showed highly significant difference among the species. Though *A. aroma* and *A. macracantha* are different only by thorn length. The discovery of another *Zieria* from, *Z.* sp. aff. *smithii*, raised questions in taxonomic status of *Z. prostrata* and its relationship between *Z. prostrata* and the *Z. smithii* species complex. Hogbin and Crisp (2003) try to solved this problem by using RAPD markers and morphometric analysis. It was found that *Z. prostrate* formed a distinct group in phenetic space based upon the morphometric data but with an incomplete discontinuity between it and nearby populations of *Z. smithiii* based upon the genetic data, implying that *Z. prostrata* may be considered as a distinct but incipient species. Meanwhile morphometric data set suggested that *Z.* aff. *smithii* may be a subspecific taxon.

A phenetic study of *Cassia* s. l. (Leguminosae) in Thailand was carried out based on floral morphology. The result from a canonical discriminant analysis can be concluded that *Cassia* s. str., *Senna*, and *Chamaecrista* are really distinct taxa. The three most important characters that separate the three genera are filament length, fruit length, and ovary stalk length. It was also found that *Senna* is rather a heterogeneous taxon (Boonkerd et al. 2005). Kidyue et al. (2005) studied taxonomic status of nine forms of the *Hoya parasitica* complex in Thailand based on 35 quantitative and 14 qualitative characters. It was proposed that the *H. parasitica* complex in Thailand should be treated as 3 species: i.e. *H. rigida*, *Hoya* sp. nov., and *H. parasitica*. Pangua et al. (2006) studied the *Asplenium seelosii* complex including strictly rupicolous plants that live on limestone cliffs mainly in the mountains of south-west Europe and several mountain ranges of the eastern Iberian Peninsula. The previous systematic treatments have been used to distinguish the two species and up to four subspecies using the disjunction distribution of its populations and several morphological characters, i.e. leaf indumentum and the structure of the perispore. The present analysis of all of these characters together with differentiated two groups correspond to the established species, *A. seelosii* and *A. celtibericum*, but still not enough differences were found to separate the subspecies.

It was found that RAPD is a reliable procedure for distinguishing among all nineteen sweet cherry (*Prunus avium* L.) cultivars commonly cultivated in Poland and other *Prunus* species, such as the peach, the plum and the almond (Warburton and Bliss,

1996; Ortiz et al., 1997; Bartolozzi et al., 1998; Dirlewanger et al., 1998; Shimada et al., 1999; Lisek et al., 2006). Moreover, analysis of the RAPD data suggested that natural hybridization has occurred among *Cibotium* species as was originally hypothesized based on morphological examination (Motley and Morden, 2001).

Randomly amplified polymorphic DNAs (RAPD) were used to distinguish among isolates of *Gaeumannomyces graminis* var. *tritici*, *G. graminis* var. *graminis*, *G. graminis* var. *avenae*, *G. incrustans*, and *G. cylindrosporous*. The unweighted pair group method with arithmetical averages (UPGMA) indicated that *G. graminis* var. *tritici* isolates were more closely related to *G. graminis* var. *avenae* than to *G. graminis* var. *graminis* isolates. The results of this study showed that RAPD markers can be used to confirm the identification of *Gaeumannomyces* species and varieties (Hanafy et al., 1996)

Analyses of RAPD profiles from 17 populations of the *Hippocrepis balearica* complex revealed a highly structured geographic pattern, not only among continental-insular areas but also within the eastern Balearic Islands. In marked contrast to previous morphometric results, a clear separation between continental and insular samples was found, and intermediates between *H. balearica* and *H. valentina* samples were not detected. Molecular data indicated that western and eastern Balearic populations of the complex (*H. grosii* and *H. balearica*) were more closely related to each other than to continental populations (*H. valentina*). Multivariate analyses of the RAPD data clearly indicated that the similarities between continental and eastern Balearic samples of the *H. balearica* complex recovered by morphometric methods are due either to parallel evolution or retention of plesiomorphic features (Rossello etal., 2002). Seeprasert et al. (2006) study on DNA polymorphism in 4 species and 2 varieties of Polygonum collected from northeasthern Thailand was examined using random amplified polymorphic DNA (RAPD) technique using twenty primers. The genetic similarities were estimated from banding pattern using UPGMA. Cluster analysis divided the samples into 3 groups.

From the aforementioned researches it can be seen that macro-micromorphological, anatomical, and RAPD techniques were commonly applied to solve classification problems in pteridophytes as well as flowering plants, especially in determining the status of species complex and usually carry out in conjunction with

numerical analysis. There fore, these procedures can be effectively applied to elucidate the taxonomic problem within the *Microsorum punctatum* complex.

CHAPTER 3

MORPHOLOGICAL AND ANATOMICAL STUDY

3.1 Introduction

This work deals with the revision of *Microsorum punctatum* (L.) Copel. complex (Polypodiaceae). Especially the generic boundaries and the systematic position of these plants appeared to be problematic. Studies in morphological and anatomical have widely been utilized in classification of species complex. For example, In *Carex*, vegetative anatomy and fruit epidermal silica bodies have been used to delimit species and sections. Anatomical and silica body characters strongly support the recognition of three species within the *Carex willdenowii* Willdenow complex (Starr and Ford, 2001). Tsukaya (2002) recognized two species and hybrids of *Dendranthema yoshinaganthum* (Makino ex Kitam.) species complex based on variation in leaf anatomy. Three known populations of *Isoetes tennesseensis* were examined to document and analyze their morphological and anatomical characters such as leaf form and size, sporangial wall cells, and ligule morphology. Three types of morphological patterns were found. The discovery of dimorphism between mega- and microsporophylls is of particular interest (Budke et al., 2005).

Separation of phenotypic plasticity from genetically fixed morphological differences is very important for bryophyte taxonomy. Biometric studies together with genetic methods have given an opportunity to find new reliable anatomical and morphological features for some critical species, e.g. *Calypogeia* (Buczkowska, 2004), or even for species previously regarded as cryptic, like *Conocephalum conicum* and *C. salebrosum* (Szweykowski et al., 2005).

In this work, *Microsorum punctatum* (L.) Copel. complex is represented by 21 taxa according previous classification. The taxonomic status of this species is still dubious due to its great variations in frond form, sizes, and venation patterns. These variations do not match and not included in previous recognized systematic treatments. It is reasonable to propose the hypothesis that *M. punctatum* and its allied should be placed as a species complex. Because of this, the morphological, anatomical, together with the

geographical distribution data need to investigated in order to clarify the taxonomic status of this circumscription.

3.2 Materials and Methods

3.2.1 Survey and collection of specimens

Microsorum punctatum (L.) Copel. and its allied will be collected from various localities throughout Thailand and will be raised in the greenhouse of the Department of Botany, Chulalongkorn University. These transplanted plants preserve for anatomical, morphological and molecular studies.

3.2.2 Specimens determinations

All collected specimens were determined to appropriate taxa using previous taxonomic key (Bosman, 1991; Tagawa & Iwasuki, 1989; Hovenkamp et al., 1998; Smith & Hoshizaki, 2000; Boonkerd, 2001).

3.2.3 Morphological study

Each specimen was examined for both vegetative and reproductive characters by stereoscopic microscope. These characters in each specimen include habits, rhizome, shape and texture of fronds, venation pattern, position and distribution of sori on lamina, scale margin, etc. Spore morphology was observed using scanning electron microscope (SEM). Descriptions of spore morphology were focused on shape, size, color, and wall ornamentation.

3.2.4 Anatomical study

Cross section of rhizome, stipe and frond were cut using Automatic MT-3 microtome (Toyozumi Dengenkiki Co., Ltd.) at 8-12 mm thick without embedding in paraffin. For dried sample of herbarium specimens, before cutting, small pieces of rhizome or stipe was boiled in water for several minutes, until they sank, then they were transferred to cold water. Then the section were stained by safranin O for temporary slides and observed using a light microscope. Cross sections of rhizome were made at the internodes and cross sections of stipe within 1 cm from phyllopodia. Preferably at least two specimens per species were selected.

For epidermal character, the preparation were made by scraping pieces of *softened frond with a safety razor blade and then the section were stained by safranin O* for temporary slides. The observation was done using a light microscope.

3.2.5 Key construction

For constructing a key to species, qualitative characters of the segregated taxa were tabulated. Subsequently, the best characters for separating the segregate genera as suggested by DELTA (Dallwitz et al., 1993) were used.

Table 3.1 List of the 21 taxa of the *Microsorum punctatum* (L.) Copel. complex in this present study.

No.	Taxon	Taxon according to Nooteboom (1997), Boonkerd and Nooteboom (2001, and Boonkerd (2006)
1.	*Microsorum whiteheadii* A.R. Sm. & Hoshiz.	*M. whiteheadii* A.R. Sm. & Hoshiz.
2.	*M. siamense* Boon.	*M. siamense* Boon.
3.	*M. thailandicum* Boon. & Noot.	*M. thailandicum* Boon. & Noot.
4.	*M. membranaceum* (D. Don) Ching	*M. membranaceum* (D. Don) Ching
5.	*M. glossophyllum* (Copel.) Copel.	*M. punctatum* (L.) Copel.
6.	*Pleopeltis megalosoides* Alderw.	ditto
7.	*M. musifolium* (Blume) Copel.	ditto
8.	*M. steerei* (Harr.) Ching	*M. steerei* (Harr.) Ching
9.	*P. tonkinense* Baker	ditto
10.	*P. playfairii* Baker	ditto
11.	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
12.	*M. punctatum* ssp. *subirideum* H. Christ	ditto
13.	*M. punctatum* ssp. *subdrynariaceum* H. Christ	ditto
14.	*Polypodium irioides* Poiret	ditto
15.	*M. validum* (Copel.) Ching	ditto
16.	*P. glabrum* Wall.	ditto
17.	*P. millisorum* Baker	ditto
18.	*M. sessile* Kze.	ditto
19.	*P. polycarpon* Cav.	ditto
20.	*M. punctatum* (L.) Copel. cv. *serratum*	ditto
21.	*M. neoquineense* Copel.	ditto

Figure 3.1 Herbarium Specimen of "*M. punctatum* complex". — A. *M. sessile*; B. *M. glossophyllum*; C. *M. punctatum* ssp. *subdrynariaceum*; D. *Polypodium tonkinens*;, E. *Pleopeltis megalosoides*; F. *P. irioides*; G. *P. ambiguum*; H. *M. punctatum* cv. *grandicep*; I. *M. punctatum* cv. *serratum*.

3.3 Result

3.3.1 General morphology and anatomy

Rhizome

All species in the *Microsorum punctatum* complex have long creeping rhizomes that are often enveloped in a felt of roots. Dorso-ventrally flattened or approximately cylindrical rhizome is more or less densely set with scales. Scales occurring on the rhizome are mostly latticed. The surface is smooth to finely wrinkle after drying. Some species is covered by a thin, probably waxy layer (Fig. 3.2A) e.g. in *M. glossophyllum*, *M. punctatum*, *M. steerei*. Phyllopodia vary in distinctness and are usually placed in two alternating rows. Phyllopodia length is 3.5 mm long or shorter; but in *M. punctatum*, *M. glossophyllum*, *M. steerei, and M. musifolium,* they may be over 4.5 mm long.

Rhizome anatomy is rather uniform in cross-section (Fig 3.2C). The epidermis is indistinct. Outer wall of epidermal cells usually thickened with thick cuticle at the outer surface. The stellar structure is highly perforated dictyostele (Schmid, 1982). The strands are round or oval in cross section and are always surrounded by sclerenchyma sheaths (Fig. 3.2E). The perforations in this type of stele are discontinuities more than leaf, branch, or root gaps. Sclerenchymatic tissue is present in all species. It may form scattering strands in the ground tissue (Fig. 3.2F).

Scale

In the *M. punctatum* complex scales are found on the rhizome (including the phyllopodia). Most species also have scales on the fronds (including the stipe) except *M. steerei*. But they usually found in a small number and simpler in structure than those on the rhizome.

The density of the scales is relatively highest on the young parts, i.e., on the rhizome apex, lateral buds, and on the main veins of mature fronds. The general outline of scale is triangular or ovate or narrowly ovate with entire (Fig. 3.2G) to dentate (Fig. 3.2I) margin and acute or acuminate to slightly caudate apex. Scales of some species have dark black and glabrous on central region, or bearing multiseptate hairs at least when young (e.g. *M. siamense*, *M. membranaceum*, and *M. whiteheadii*).

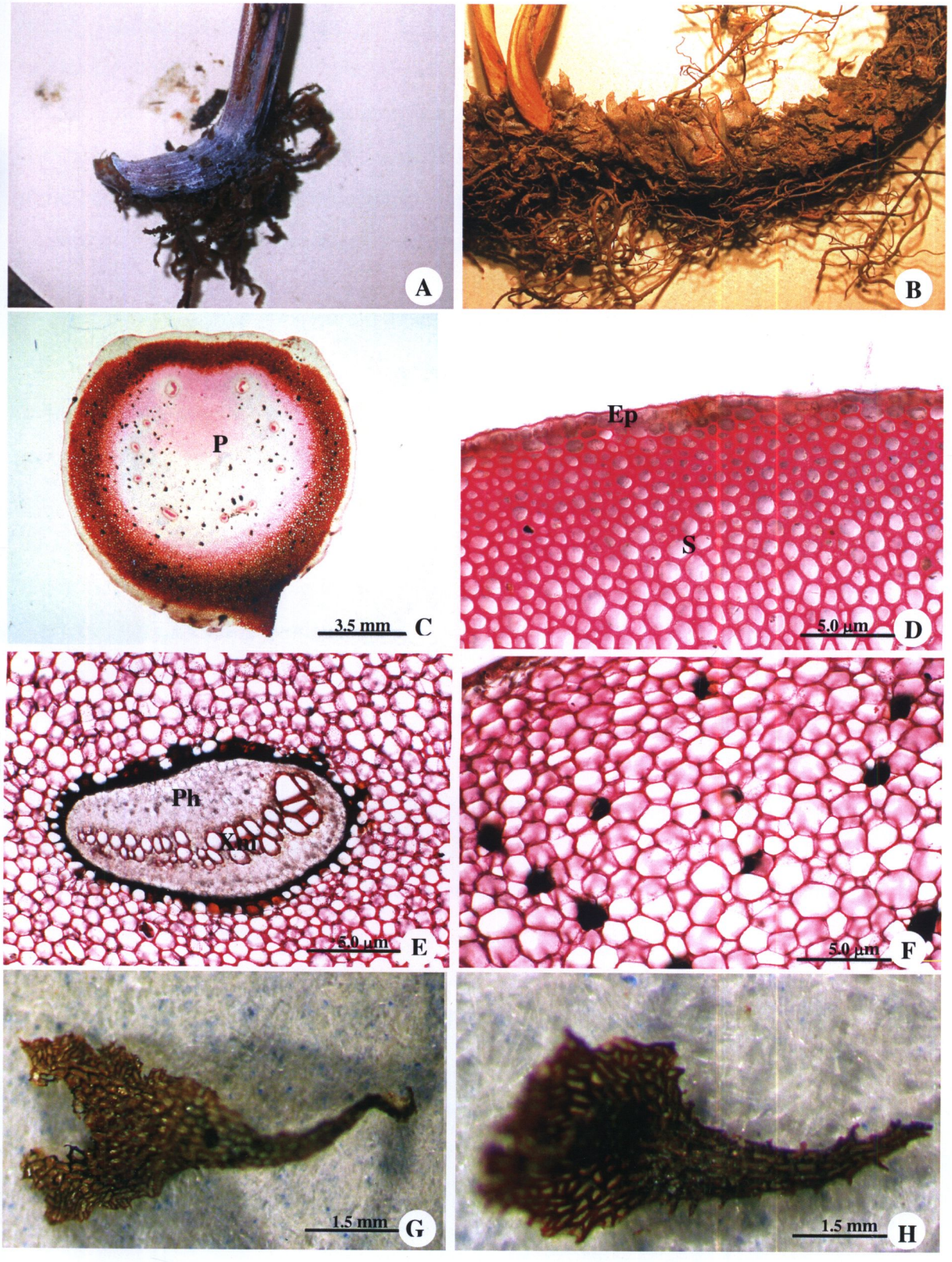

Figure 3.2 Rhizome and scale of "*Microsorum punctatum* complex".— A. waxy rhizome; B. rhizome covered by scales; C. rhizome in cross-section; D. collenchyma tissue; E. vascular bundle; F. sclerenchyma strand; G. entire marginal scale; H. dentate marginal scale. (Ep=epidermis, P=pith, Ph=phloem, S=sclerenchyma, Xm=xylem)

Scales of all species in the *Microsorum punctatum* complex (except those of *M. glossophyllum*) have one cell layer thick, except for the central region around the stalk. The color varies from light to dark brown. Some scales are partly clathrate. It was found that hyaline marginal region was found only in *M. membranaceum* and *M. musifolium*. In a few species all superficial walls are more or less opaque, while the anticlinal walls are thickened; these scales are called subclathrate such as in *M. glossophyllum* and *M. punctatum* (Bosman, 1991).

Fronds

The fronds of all species in the *Microsorum punctatum* complex are monomorphic, sessile or stipitate and articulated to rhizome. The lamina (leaf blade) is simple, usually varies in size and shape. Midrib is usually raised on lower surface, but grooved on upper surface as in *M. thailandicum*. It is likely that frond length is usually correlated with the rhizome diameter. In *M. punctatum*, plant grows more solitary with the fronds clustered, forming irregular nets.

Frond is persistent in most species, except *M. membranaceum* usually shed all fronds during dry season. Frond color when living varies from light to dark green. Iridescent blue-green in living frond is found in *M. thailandicum* and *M. siamense*. This character is still present in the transplanted plants growth in the greenhouse. The often glaucous (or, when dry, grayish or reddish) color of the fronds and midrib is diagnostic character for *M. glossophyllum*.

Stipe is generally distinct with the exception of *M. musifolium* and *M. whiteheadii* these two species have very short stipe or absent. It is found that the shape of transverse sections varies from approximately round to oval (Fig. 3.3A and 3.3B). In some species stipe may be winged for considerable part or wingless in *M. siamense*, *M. thailandicum* and *M. whiteheadii*.

Anatomical characters of stipe were rather uniform. Several layers of collenchyma are found below the epidermis. In *M. glossophyllum* the collenchyma is abaxially darker than adaxially (Fig. 3.3A).

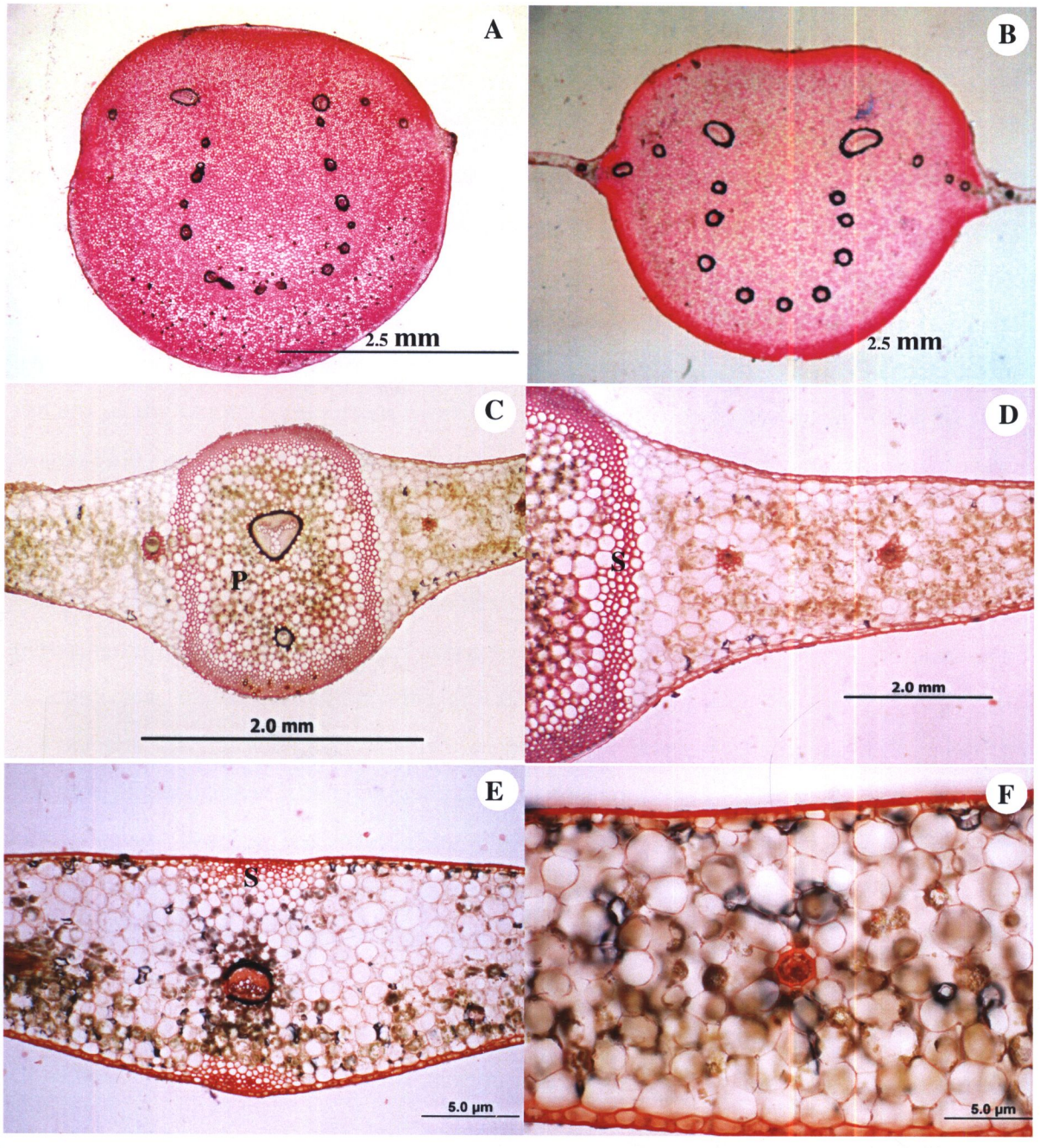

Figure 3.3 Stipe and midrib of "*M. punctatum* complex". — A-B. stipe in cross-section of *M. glossophyllum* and *M. punctatum*, respectively; C-D. midrib in cross-section of *M. punctatum*; E-F. midrib in cross-section of *M. siamense*. (P=pith, S=scerenchyma)

In the ground tissue vascular strands are arranged in an arc, with two relatively large strands on both adaxial ends. The strands are round or oval in cross section, and are always surrounded by sclerenchyma sheaths. The number of strands is usually highest at the base of the stipe but increase in the first centimeter above

phyllopodium. Moreover, the number of strands is correlated with the diameter of the stipe.

Lamina: All species in the *M. punctatum* complex the lamina is flat (except *M. musifolium* between the veins it is slightly raised towards the adaxial side) and simple. The base of the simple lamina is attenuate or narrowly angustate and often slightly unequal (e.g. *M. punctatum*, *M. glossophyllum*, *M. musifolium*, and *M. steerei*) and narrowly cuneate in *M. membranaceum*. In *M. musifolium* and *M. punctatum* it may vary from obtuse to truncate. The margin is usually an entire, but in some species, especially of the *M. siamense*, undulated entire margin is diagnostic character. The texture of the lamina varies from membranaceous lamina in *M. membranaceum* to firm herbaceous in *M. musifolium*, whereas e.g. *M. siamense*, *M. thailandicum*, *M. glossophyllum*, *M. punctatum*, *M. whiteheadii*, and *M. steerei* are coriacecous.

For the general anatomy of lamina in 21 taxa of the *M. punctatum* complex, the mesophyll in this complex is composed of 8 to 10 layer of spongy parenchyma (Fig. 3.3D and 3.3F). Collenchyma is only found in the midrib or costa as a subepidermis sheath surrounding the ground tissue in *M. punctatum* (Fig. 3.3C) or as adaxial and adaxial subepidermis layer in *M. musifolium* and *M. siamense* (Fig. 3.3E). Palisade parenchyma and calcium scale could not be confirmed in this study.

We can see many chloroplasts in the epidermal cell. These cells have sinuate anticlinal walls in surface view (Fig. 3.4E-G) except for the cell overlying the vein, which are more angular with straight walls. The fronds of the *M. punctatum* complex ferns are hypostomatic frond because stomata are only found on abaxial surface. The guard cells are placed at the same level as the epidermis cell (Fig. 3.4D). All plants show both polocytic and copolocytic stomata (Fig. 3.4H-J).

Venation patterns: In the taxonomy of the microsoroid ferns venation patterns have received relatively much attention. In comparing fronds of different sizes it has to be taken into account that the venation pattern in narrow and small fronds is generally a simplified version of that in larger fronds of the same species. Within the *M. punctatum* complex the general venation pattern, formed by the secondary and tertiary veins, is catadromous and can be roughly divide into two types.

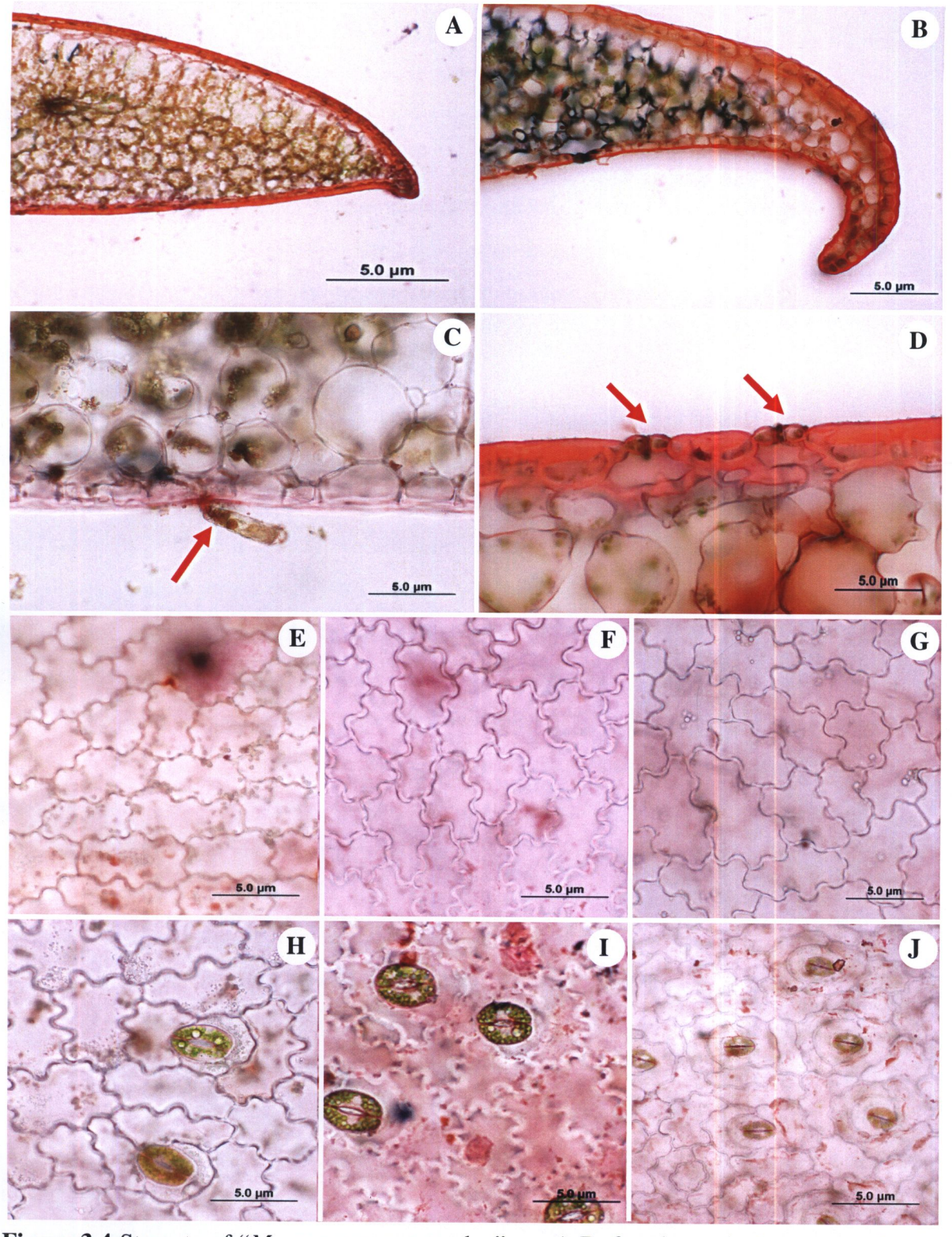

Figure 3.4 Stomata of "*M. punctatum* complex". — A-B. frond margin in cross-section of *M. thailandicum* and *M. siamense*, respectively; C. uniseriate hair with a glandular top-cell; D. guard cells are placed at the same level as the epidermal cell; E-G. epidermal cell; H-I. stomata.

Venation type 1 (Fig. 4.1) -- connecting veins forming a row of about equally sized areoles between two adjacent veins and no prominent veinlet situated parallel to the veins. This type is the most common in the *M. punctatum* complex. It is found in *M. siamense*, *M. membranaceum*, *M. glossophyllum*, *M. whiteheadii*, *M. steerei*, *M. musifolium*, and *M. punctatum*.

Venation type 2 (Fig. 4.1) -- the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger, areoles in a row between two veins. This type is exclusive for the species of *M. thailandicum*.

Sori

The sori in the *M. punctatum* complex usually are numerous, small, round, irregularly scattered, and are not covered by an indusium (covering membrane) (Fig. 3.5). Elongate sori also occur alongside the round one in some species. The same range of sizes and shape is found in the *M. punctatum* complex. Only *M. glossophyllum* and *M. membranaceum* forms an exception, being have relatively large sori rather than the other species. The uniseriate paraphyses are present in *M. glossophyllum*, *M. musifolium* and *M. punctatum*.

In all species of the *M. punctatum* complex the sori are superficial or slightly immersed, in very coriacecous fronds, in the undersurface of the lamina. The sori are distributed over the whole undersurface of the lamina or absent from the basal part. The sori in each species are irregularly scattered or arranged in two to several rows parallel to each secondary vein and may situated on tertiary or smaller veins. The taxonomic value of sori distribution character seems limited due to its variable.

The density of sori on the lamina can be estimated by counting the number of sori per square cm in fully fertile areas. This density varies from 5 to about 74 sori per square cm. In addition, this density also proves to be correlated to sorus size, shape, position, and size of lamina. Species with relatively large sori (up to 2.5 to 3.0 mm) as, e.g. *M. membranaceum* and *M. glossophyllum* show lower densities (up to 22 and 30, respectively). Other species with similar size and distribution sori, *M. thailandicum*, *M. siamense*, and *M. whiteheadii*, still show lower densities than do species with scattered sori and not more than 1.5 mm in averaged (e.g. *M. musifolium*, *M. steerei*, *M. punctatum*, and its synonym) have higher densities (up to 74 sori per cm^2).

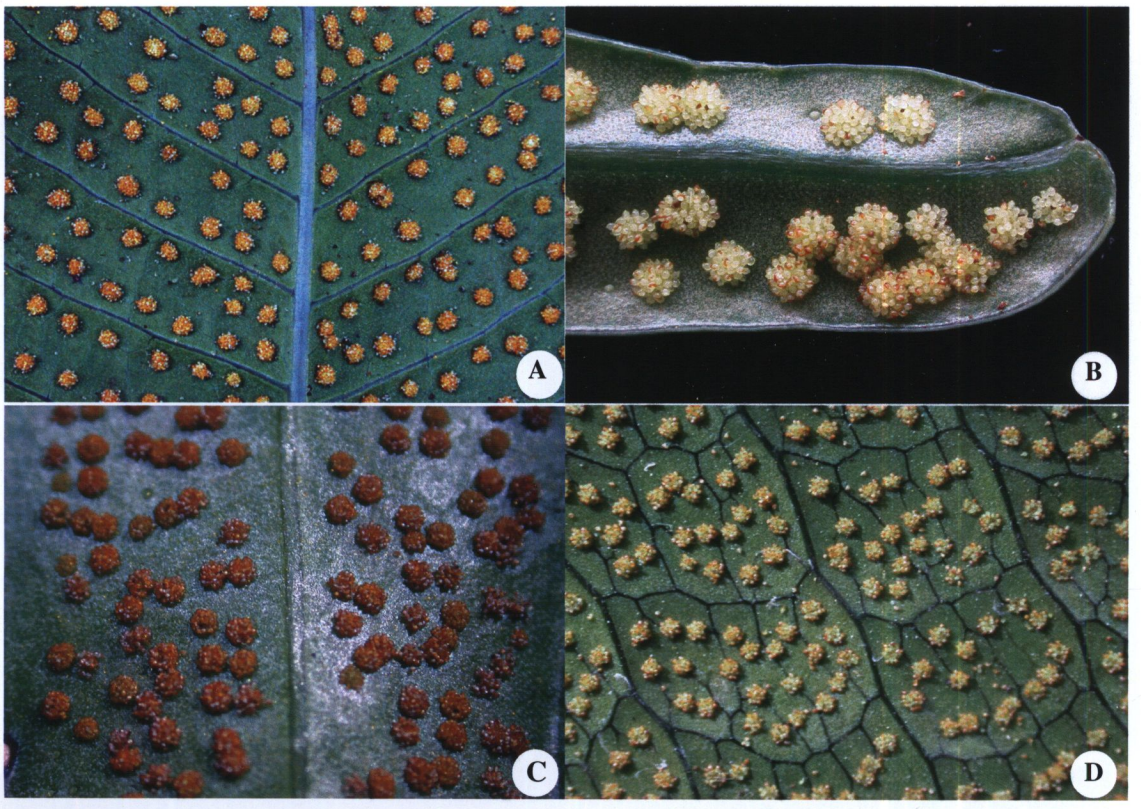

Figure 3.5 Sori distribution patterns of *M. punctatum* complex.— A. *M. membranaceum*; B. *M. thailandicum*; C. *M. punctatum*; D. *M. musifolium*.

Spore

Spore of all species in the *M. punctatum* complex measure in lateral view 37.50-82.50 by 22.50–47.50 μm. Larger spores are found in *M. glossophyllum*. Spore of the *M. punctatum* complex are monolete, bilateral and concavo-convex or plano-convex shape in lateral view (Fig. 3.6 and 3.7). The color of the spores usually varies from hyaline in *M. glossophyllum*, *M. musifolium*, and *M. steerei* to distinctly yellow in *M. siamense*, *M. membranaceum*, *M. whiteheadii*.

The surface of the spores was studies using SEM. Most species showed globules scattered over the surface. This globules varies seems not a very useful diagnostic character. *M. musifolium* is unique in having distinctly larger size. The spores of *M. membranaceum* are very different from all other taxa in this complex. They may best be described as verrucalted spore (Fig. 3.7D).

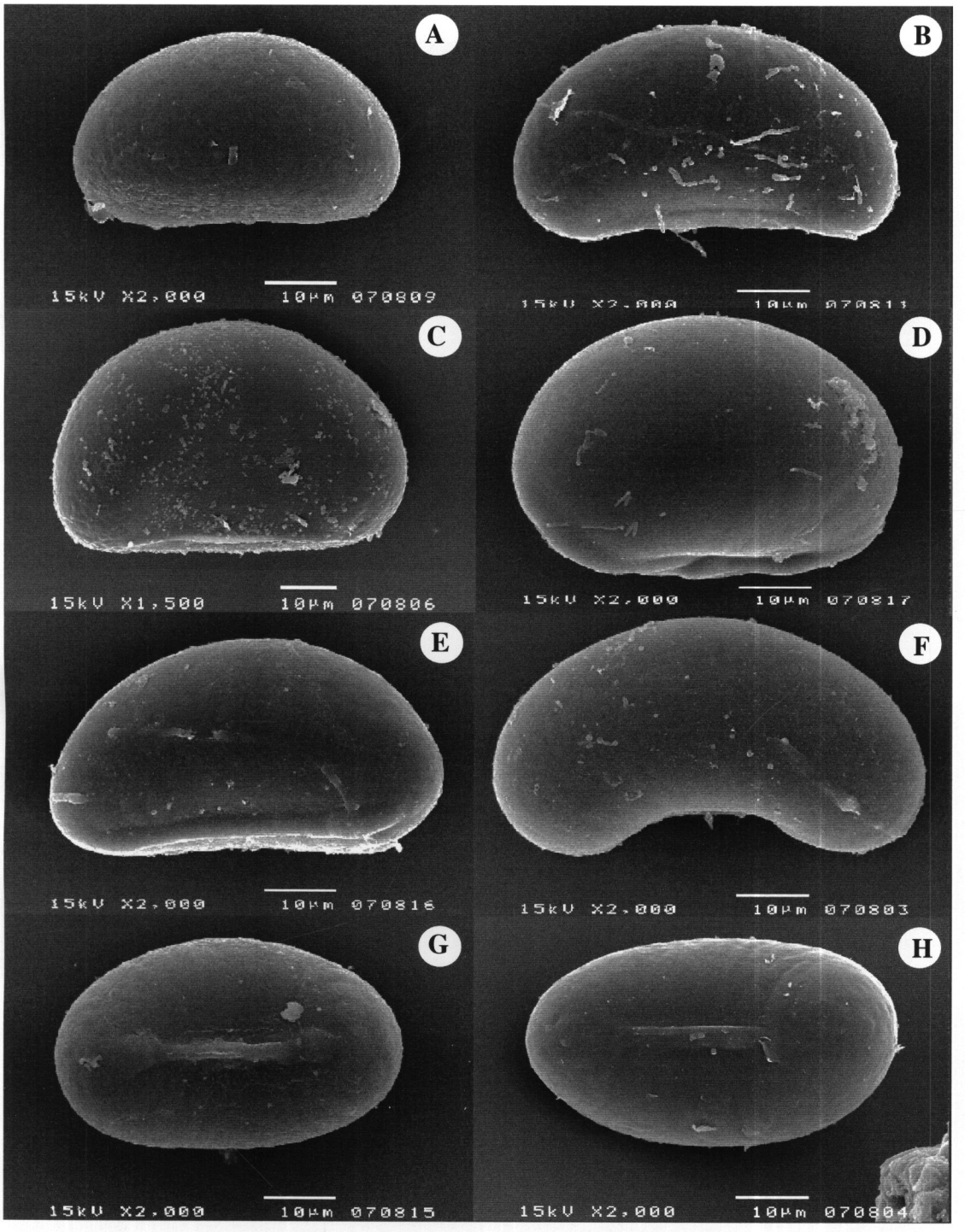

Figure 3.6 Spore of "the *M. punctatum* complex". — A. *Polypodium polycarpon*; B. *P. glabum*; C. *M. glossophyllum*; D. *P. irioides*; E. and G. *M. punctatum* ssp. *subirideum*; F. and H. *M. validum*.

3.3.2 Comparative morphology and anatomy

From the result of general morphological and anatomical study mentioned above, it can be summarized the morphological and anatomical difference among those eight species of the *M. punctatum* complex as show in Table 3.2 below.

Table 3.2 Comparison of 37 qualitative characters of the eight tentatively taxa. (1. *Microsorum siamense*; 2. *M. thailandicum*; 3. *M. membranaceum*; 4. *M. glossophyllum*; 5. *M. whiteheadii*; 6. *M. steerei*; 7. *M. musifolium*, and 8. *M. punctatum*)

No.	Character	Taxa							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1.	Shape of rhizome transverse section: (1) approximately cylindrical, (2) dorso-ventrally slightly flattened or flattened	1	1	1,2	1,2	1,2	1,2	1,2	1,2
2.	Appearance of rhizome surface: (1) not waxy, (2) at least sometimes waxy under the scales or often waxy	1	1	1	2	1	2	1	2
3.	Differentiation of vascular bundle sheaths: (1) vascular bundle sheaths be parenchymatous, (2) vascular bundle sheaths be collenchymatous or occasionally party sclerenchymatous tissue	1	1	1	2	1	1	2	1
4.	Type of attachment of scales: (1) pseudopeltate, (2) peltate	2	1	1	1	1	1	2	1
5.	Density of scales: (1) densely set, (2) apically densely set or moderately densely set	1	1	2	1	2	2	2	2
6.	Spreading of scales: (1) distinctly or slightly spreading, (2) appressed	1	1	1	1	1	1	2	1
7.	Scales shape: (1) narrowly ovate to ovate, (2) triangular	1,2	1,2	1,2	1	1	1,2	1	1,2
8.	Scales margin: (1) entire, (2) dentate to denticulate	2	2	1	2	2	2	1	2
9.	Scales apex: (1) acute (2) acuminate to slightly caudate	2	2	1,2	2	2	1,2	2	2
10.	Presence of hyaline marginal region on rhizome scales: (1) absent, (2) present	1	1	2	1	1	1	2	1
11.	Number of cell layer of rhizome scale: (1) one cell layer thick, (2) more than two cell layer thick	1	1	1	2	1	1	1	1
12.	Indumenta type of central region of scales: (1) dark black on central region or glabrous, (2) bearing multiseptate hairs at least when young	2	1	2	1	2	1	1	1
13.	Scales translucence: (1) opaque and blackish, (2) translucent and brownish	2	2	1	1	2	2	2	2
14.	Phyllopodia distinctness: (1) distinct, (2) obscure	2	2	1	1	2	1	2	2
15.	Lamina texture: (1) coriaceous, (2) subcoriaceous, (3) herbaceous, (4) membranaceous	2	2	4	3	1	2	3	1-3
16.	Frond color when living: (1) light to dark green, (2) iridescent blue-green, (3) glaucous (or grayish to reddish when dry)	2	2	1	3	1	1	1	1
17.	Lamina shape: (1) linear, (2) (narrowly) elliptic, (3) (narrowly) ovate, (4) narrowly obovate, (5) broad to narrowly oblanceolate	2	1	1-3	4	4	5	4	1-4
18.	Lamina base: (1) attenuate, (2) narrowly angustate, (3) narrowly cuneate, (4) truncate to obtuse, (5) cordate	2	1	2	3	1	2	4	2,5

Table 3.2 (continued)

No.	Character	Taxa							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
19.	Stipe character: (1) the stipe winged for considerable part, (2) narrowly winged	2	2	1	1	2	1	1	1
20.	Margins of lamina: (1) margin entire, (2) margin entire, undulate	2	1	1	1	1	1	1	1,2
21.	Lamina apex: (1) acute, (2) acuminate to long acuminate, (3) rotundate	1,2	2	2	1,2	1,2	2	1,2	1,2,3
22.	Presence of indumenta: (1) with only scales, (2) with a few scales and short glandular hairs, (3) with only short glandular hairs	3	3	3	1	3	3	2	2
23.	Presence of stipe: (1) present, (2) absent or obscure	1	1	1	1	2	1	2	1
24.	Midrib character: (1) slightly raised or raised on both surface, (2) raised on lower surface, grooved on upper surface	1	2	1	1	1	1	1	1
25.	Character of collenchyma tissue in midrib: (1) as a subepidermis sheath surrounding the ground tissue, (2) as adaxial and adaxial subepidermis layer	2	1	1	1	1	1	2	1
26.	Venation general pattern: (1) type 1: connecting veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, (2) type 2: the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger, areoles in a row between two veins	1	2	1	1	1	1	1	1
27.	Visibility of veins: (1) all veins distinct, (2) all veins indistinct or secondary and smaller veins more or less immersed and vague (at least in living specimen)	2	2	1	1	2	2	1	2
28.	Branching of included free veinlet venation: (1) free veinlet simple and once-forked, (2) free veinlet simple, once or twice forked,	1	2	2	1	1	2	1	1
29.	Sori distribution pattern: (1) mostly irregularly scattered on simple free or on 2 or 3 connecting veins, (2) forming into 2-4 irregular rows parallel to each pair of secondary veins, (3) forming more than 3-10 (-15) irregular rows parallel to each pair of secondary veins	1	1	2	3	3	3	3	3
30.	The presence of distinct hydathodes: (1) present, (2) absent	1	2	2	2	1	2	2	2
31.	Sori position on lamina surface: (1) superficial, (2) slightly immersed	1	2	1,2	1	1	1,2	1	1
32.	Sori distribution: (1) on the whole surface of the lamina, (2) usually occupying the upper half portion of the lamina, (3) absent from the basal parts for 1/5-4/5 of total length of lamina	2	2	1,2	3	1	2	1,2	1
33.	Presence of sori in the costal areoles or on their bordering veins: (1) absent in costal areoles, (2) present in costal areoles	2	2	2	2	1	1	2	1
34.	Presence of uniseriate paraphyses in sori: (1) absent, (2) present	1	1	2	2	1	2	2	2
35.	Spores shape: (1) plano-convex, (2) concavo-convex	2	2	2	1,2	2	2	2	1
36.	Spores color: (1) hyaline, (2) yellowish hyaline, (3) yellow	3	2	3	1	3	1	1	1,2
37.	Spore surface: (1) plain to slightly verrucate, (2) irregularly rugate	1	1	2	1	1	1	1	1

All species in the *M. punctatum* complex can be described as a wart fern species have long creeping dorso-ventrally flattened or approximately cylindrical rhizomes that are often enveloped in a felt of roots and more or less densely set with scales. It might be covered by a thin waxy layer or not. Scales is relatively highest on young parts. The fronds of all species in the *M. punctatum* complex can be described as a sessile or stalked and articulated to short and vary in distinctness phyllopodia which are variably spaced and usually placed in two alternating rows along the rhizome. The lamina can either be simple and has reticulate venation. The frond length is usually correlated with the rhizome diameter. Fertile and sterile fronds are not different in size and shape.

The sori in the *M. punctatum* complex usually are numerous, small, round, irregularly scattered, and are not covered by an indusium the abaxial surface. The density of sori proves to be correlated to sorus size, shape, position, and size of lamina. However, the taxonomic value of sori distribution character seems limited due to its variable. Spore of all species in the *M. punctatum* complex measure in lateral view is 37.50-82.50 by 22.50–47.50 μm. The spore is monolete, bilateral spores and concavo-convex or plano-convex shape in lateral view.

Finally, based on the investigation of morphological and anatomical variation, the M. punctatum complex is composed of 8 distinct taxa i.e. *Microsorum siamense* (I), *M. thailandicum* (II), *M. membranaceum* (III), *M. glossophyllum* (IV), *M. whiteheadii* (V), *M. steerei* (VI), *M. musifolium* (VII), and *M. punctatum* (VIII). A key to taxa and description are presented below.

Key to taxa

1a. Living fronds iridescent blue-green 2

1b. Living fronds light to dark green 3

2a. Midrib slightly raised on both surfaces; rhizome scales peltate; frond elliptic, base narrowly angustate; hydathodes distinct ..... **1. *M. siamense***

2b. Midrib grooved on the upper surface; rhizome scales pseudopeltate; frond narrowly elliptic to linear, base attenuate; hydathodes indistinct ..... **2. *M. thailandicum***

3a. Scale margins entire, clathrate with hyaline margins; rhizome not white waxy 4

3b. Scale margins denticulate or dentate, clathrate throughout or opaque; rhizome often waxy, at least sometimes waxy under the scales 5

4a. Stipe present, up to 10 cm long; lamina membranaceous, base narrowly angustate; scales distinctly or slightly spreading, pseudopeltate, central region with long hair; sori forming into 2-4 irregular rows ..... **3. *M. membranaceum***

4b. Stipe absent or indistinct; lamina firm-herbaceous or subcoriaceous, base truncate to obtuse; scales appressed, peltate, central region glabrous; sori forming more than 4 irregular rows ..... **7. *M. musifolium***

5a. Lamina usually shorter than 40 cm long, with only short glandular hairs6

5b. Lamina up to 100 cm long, with only scales, or with a few scales and short glandular hairs 7

6a. Rhizome white waxy, phyllopodia obscure; stipe distinct, less than 2 mm diam., lamina base narrowly angustate ..... **6. *M. steerei***

6b. rhizome not white waxy, phyllopodia distinct; stipe absent or obscure, up to 5 mm diam., lamina base attenuate ..... **5. *M. whiteheadii***

7a. Scales opaque and blackish, more than two cell layers thick; stipe less than 2 cm long; lamina with only scales; all veins distinct; sorus present in costal areoles .... **4. *M. glossophyllum***

7b. Scales translucent and brownish, one cell layer thick; stipe up to 12 cm long; lamina with a few scales and short glandular hairs; all veins more or less immersed and vague (at least in living specimen); sorus absent in costal areoles ..... **8. *M. punctatum***

1. *Microsorum siamense* Boonkerd

Rhizome creeping, 3.3–4.1 mm diam., approximately cylindrical, not white waxy, bundle sheaths not differentiated, vascular bundles in cylinder 9–10, sclerenchyma strands 60–240, roots densely set; scales peltate, densely set, widest near the base, slightly spreading, ovate to triangular, up to 1.3 by 3.1 mm, margin denticulate, apex acuminate to slightly caudate, clathrate throughout, dark black on central region. *Phyllopodia* more or less distinct, 2.8–3.9 mm apart; stipe present, 35.3–44.3 mm long, 2.4–2.6 mm diam., raised on lower surface, slightly raised on upper surface. *Fronds* monomorphous, subcoriaceous, iridescent blue-green in color when living; lamina elliptic, up to 13.6 by 4.2 cm, index 3.1–3.8, widest about or above the middle of leaf length, base narrowly angustate, the stipe more or less winged, margin entire undulate, apex acute to long acuminate, with short glandular hairs, scales and acicular hairs absent. *Venation pattern*:

connecting veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, all veins or secondary and smaller veins more or less immersed and vague, free veinlet simple and once- forked, angle between primary and secondary vein 28–32 -degree. *Sori* mostly irregularly scattered on simple free or on 2 or 3 connective veins; round, superficial, usually occupying the upper (apical) half portion of the lamina, 10–13 cm^2, 1.3–1.4 mm diam., absent in the marginal areoles, occasionally present in costal areoles; paraphyses present, sporangium annulus 20–22 –celled; indurated cells 11–14. *Spores* concavo-convex, yellow, 32.5–35 by 50–52.5 μm.

Distribution. — Endemic, Thailand (Yala).

Ecology. — In rock crevices on moist rock of limestone hill in semi-shade, 100 m altitude.

Specimens examined. — **Thailand**: P.V. fern 1 (BCU, L); S.P. 20, 26, 39, 60, 62 (BCU).

2. *Microsorum thailandicum* Boonkerd & Noot.

Rhizome 4.9–5.2 mm diam., approximately cylindrical, not white waxy; bundle sheaths not differentiated, vascular bundles in cylinder 8–12, sclerenchyma strands 40–245, roots densely set. Scales pseudopeltate, sometimes some peltate, densely set, widest near the base, slightly spreading, ovate, or triangular, 2.6–3.3 mm long, 1–1.2 mm wide, margin denticulate, apex acuminate to slightly caudate, clathrate throughout, dark black on central region. *Phyllopodia* more or less distinct, 3–3.9 mm apart; stipe present, 10.8–13.2 mm long, 2.7–3.2 mm diam., raised on lower surface, grooved on upper surface. *Fronds* monomorphous, subcoriaceous, iridescent blue-green in color when living; lamina narrowly elliptic, 21.3–39.6 cm long, 1.2–1.9 cm wide, index 12.8–25.2, the widest indistinct, base attenuate, the stipe more or less winged, margin entire, apex long acuminate, with short glandular hairs, scales and acicular hairs absent. *Venation pattern*: the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger areoles in a row between two veins like in type 1, all veins or secondary and smaller veins more or less immersed and vague (at least in living specimen), free veinlet simple or once- or twice- forked, angle between primary and secondary vein 47–52 –degree. *Sori* mostly irregularly scattered on simple free or on 2 or 3 connective veins; round or slightly elongate, slightly immersed, usually occupying the

upper (apical) half portion of the lamina, 15–23 cm^2, 1.2–1.4 mm diam., absent in the marginal areoles, occasionally present in costal areoles; paraphyses present; sporangium annulus 20–31 -celled; indurated cells 15–20. *Spores* concavo-convex, yellowish hyaline, 27.5–37.5 by 42.5–47.5 μm.

Distribution. — Endemic, Thailand (Chumphon).

Ecology. — In rock crevices on rather dry rock-ceiling of limestone hill in semi-shade, 250–300 m altitude. Its blue leaf iridescence is still retained when was introduced to a home garden where it is not really in deep shade as was in natural habit.

Specimens examined. — Thailand: Boonkerd 1442 (BCU, L); S.P. 2, 6, 49, 64, 71, 88, 120, 121 (BCU).

3. *Microsorum membranaceum* (D. Don) Ching

Rhizome 2.3–9.8 mm diam., approximately cylindrical or dorso-ventrally flattened; not white waxy; bundle sheaths not differentiated, vascular bundles in cylinder 15–20; sclerenchyma strands 54–97, roots densely set; *scales* pseudopeltate, apically densely set, otherwise more or less sparsely set, slightly spreading, ovate, or triangular, up to 8 by 2.5 mm, margin entire, apex acute, clathrate except the hyaline marginal region, central region bearing multiseptate hairs at least when young. *Phyllopodia* more or less distinct, 0.8–5.3 mm apart; stipe present, 0.6–4.4 mm long, 2.6–7.2 mm diam., raised on lower surface, slightly raised on upper surface. *Fronds* monomorphous or slightly dimorphous; membranaceous, light to dark green in color; lamina narrowly elliptic to narrowly ovate; 18.9–94.9 by 1.4–13.8 cm, index 4–13.6, widest below or about the middle of leaf length; base narrowly angustate, the stipe winged for considerable part, margin entire, apex acuminate, with short glandular hairs, scales and acicular hairs absent. *Venation pattern*: connecting veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, or the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger areoles in a row between two veins like in type 1; all veins distinct, free veinlet simple or once to twice- forked, angle between primary and secondary vein 13–31 -degree. *Sori* forming into 2–4 irregular rows parallel to each pair of secondary veins, round or slightly elongate; superficial or slightly immersed on the whole surface of the lamina, or usually occupying the upper (apical) half portion of the lamina, 5–22 cm^2, 1.1–3.2 mm diam., absent in the marginal areoles, generally present in costal areoles; paraphyses absent;

sporangium annulus 16–23 -celled; indurated cells 10–13. *Spores* concavo-convex, yellow, 27.5–47.5 by 47.5–82.5 μm.

Distribution. — Nepal; Sikkim; Bhutan; India; Srilanka; Burma; S China; Taiwan; N Thailand; N Laos; N Vietnam; Philippine.

Ecology. — Epiphyte, epilithic, or terrestrial in evergreen or deciduous forest, 600–2,600 or up to 4,000 m altitude.

Specimens examined. — Burma: Lace 4894 (K); Topping 4200 (K) — Ceylon: Abeysiri 55 (K); Beddome 339 (K); Gardner 1298 (K); Hooker 1145 (K), 1298 (K); Skinner 4828 (K); Sledge 543 (K), 832 (K); Walker 25 (K), 1834 (K) — Himalaya: Barnerji et al. 1313 (K), 2604 (K), 26957 (K); Henry 339 (K); Jalconer 68 (K); Konar 56 (K); Stachey & Winterbottom 1 (K); Stewart 21169 (K); Treutler 661 (K); Trotter 246 (K), 730 (K); Watt 101087 (K) — India: Jati 10 (K); Beddome 67 (K), 101 (K), 159 (K), 177 (K); Clarke 21388 (K), 27186 (K), 33720 (K); Gamble 14409 (K), 14870 (K); Haines 5379 (K); Jacquemont 11600 (K); Madhusoodanan CU29683 (K); Manickam 31442 (K); Mooney 128 (K); Nair 51452 (K); Narasimtan 165111 (K); Ramamoorthy 256 (K); Saldanha 421 (K), 641 (K), 717 (K), 820 (K), 14457 (K), 14800 (K), 15068 (K), 17959 (K) — Indo-China: Matthew 1967 (K) — Nepal: Bliss 41 (K), 51 (K), 189 (K); Gamble 1925 (K), 4847 (K); J.J. 6061 (K); Keke 902 (K); Khasya 1867 (K); Khwaunju 1259 (K); Maddine 1867 (K), Mense 343 (K) — Philippines: Elmer 5873 (K), 8367 (K); Merrill 11691 (K) — Sikkim: Balker 339 (K), Gamble 884 (K), 4000 (K), 6366 (K), 6367 (K), 9699 (K); Sinchal 339 (K); Treutler 661 (K) — Thailand: Garrett 59 (K), H.B.G. Garrett 59b (K), 591 (K); Larsen et al. 2314 (K); Iwatsuki 9600 (K), 15642 (K); Shimizu 10102 (K); Smith 1187 (K) — Vietnam: Balansa 1990 (K).

4. *Microsorum glossophyllum* (Copel.) Copel.

Rhizome 4.1–11.7 mm diam., approximately cylindrical, often waxy, at least sometimes waxy under the scales; bundle sheaths collenchymatous, vascular bundles in cylinder 10–20, sclerenchyma strands 53–94, roots very densely set (forming a thick mat); *scales* pseudopeltate, densely set, widest near the base, slightly spreading, ovate, 2.3–5.9 mm long, 0.8–1.7 mm wide, margin denticulate, apex acuminate, opaque, central region glabrous. *Phyllopodia* more or less distinct, 2.6–10.2(–25.8) mm apart; stipe present, 1.1–14.6 mm long, 2.1–7.9 mm diam., raised on lower surface, slightly raised on upper surface. *Fronds* monomorphous, firm-herbaceous; *lamina* narrowly obovate to

broad oblanceolate, 49.3–146.2 cm long, 3.2–14.8 cm wide, index 6.8–16.9, widest about or above the middle of leaf length, base narrowly cuneate, the stipe winged for considerable part, margin entire, apex acute to acuminate, with a few scales, short glandular hairs and acicular hairs absent. *Venation pattern*: connecting veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, all veins distinct, free veinlet simple and once- forked, angle between primary and secondary vein 21–42 -degree. *Sori* mostly irregularly scattered on simple free or forming irregular rows parallel to each pair of secondary veins; round, superficial, absent from the basal parts for 1/5–4/5 of total length of lamina; 10–30 cm^2, 1.2–2.8 mm diam., absent in the marginal areoles, occasionally present in costal areoles; paraphyses absent; sporangium annulus 18–23 -celled; indurated cells 13–16. *Spores* plano-convex to concavo-convex, hyaline, 25–42.5 by 45–70 μm.

Distribution. — Papua New Guinea (Type); Irian Jaya; Solomon Is.

Ecology. — Epiphyte (low) or terrestrial, rarely epilithic on limestone, volcanic rocks, brown loam and clay. Often nest-forming, 80–2,800 m altitude.

Specimens examined. —Irian Jaya: Johns 7995 (K); Leeuwenberg 9853 (K); McDonald 3829 (K); Widjaja 4293 (K) — Papua New: Blackwood 188 (K); Braithwaite 4721 (K), 4866 (K, P); Brass 11319 (K), 23055 (K), 24483 (K), 29549 (K), 29786 (K), 30498 (K), 31569 (K), 32403 (K); Bulmer 103837 (K); Carr 7660 (B), 13015 (B), 13340 (K); Clemens 7133 (B); Conn & Kairo 152 (K); C. King 388 (P); Croft 151 (K), 203 (L), 451 (L), 533 (L), 568 (K), 1728 (K), 65719 (L); Leland et al. 65641 (L); Edelfelt 220 (P); Flenley 2084 (K); Vinas 5974 (K), 60249 (K); Gay 1086 (K); Hoogland et al. 6877 (K); van Mettenius 276 (B); Nakaike 408 (K); Parris 7751 (K), 9251 (K), 9479 (K); Sand 1780 (K); Veldkamp & Stevens 5911 (K), 6793 (L); Verdcourt 5113 (K); Vink 16534 (L), 17568 (L); Wakefield 1437 (K); Walker 548 (K); Whitmore 1045 (K); Womersley 6820 (K), 11092 (K) . Papua New: Lam 1365 (L).

5. *Microsorum whiteheadii* A.R. Sm. & Hoshiz.

Rhizome 5.2–6 mm diam., approximately cylindrical or dorso-ventrally slightly flattened; not white waxy; bundle sheaths not differentiated, vascular bundles in cylinder 50–80; sclerenchyma strands 98–113, roots very densely set (forming a thick mat); *scales* pseudopeltate, moderately densely set, brown; slightly spreading, ovate; 3.1–5 mm long, 1.5–3.2 mm wide, margin erose-denticulate to dentate, apex attenuate, clathrate

throughout, central region glabrous, or central region bearing multiseptate hairs at least when young. Phyllopodia obscure, 4.2–5.2 mm apart; stipe absent or indistinct, 0.2–1 mm long, 5.4–6.2 mm diam., not or slightly raised on both surfaces. *Fronds* monomorphous, coriaceous, light to dark green in color; lamina broad to narrowly oblanceolate; 27.6–35.5 cm long, 5.5–7.9 cm wide, index 3.1–5.8, widest about or above the middle of leaf length, base attenuate, the stipe more or less winged; margin entire, apex round to acute, or acuminate, with short glandular hairs, scales and acicular hairs absent. *Venation pattern*: the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger areoles in a row between two veins like in type 1; all veins or secondary and smaller veins more or less immersed and vague (at least in living specimen); free veinlet simple and once- forked, angle between primary and secondary vein 38–45 -degree. *Sori* mostly irregularly scattered on simple free on the whole surface of the lamina or forming irregular rows parallel to each pair of secondary veins; round, superficial, 14–19 cm^2, 1.2–1.8 mm diam., absent in the marginal areoles, generally absent in costal areoles; paraphyses present; sporangium annulus 19–28 -celled; indurated cells 18–20. *Spores* concavo-convex, yellow, 27.5–37.5 by 42.5–47.5 μm.

Distribution. — Western Sumatra (type).

Ecology. — Growing on limestone outcrops, 900 m altitude.

Specimens examined. — Sumatra: Whitehead s.n. (L), Kampu 1, 2, 3, 4, 5 (BCU).

6. *Microsorum steerei* (Harr.) Ching

Rhizome 2.5–7.1 mm diam., approximately cylindrical, often waxy, at least sometimes waxy under the scales; bundle sheaths not differentiated, vascular bundles in cylinder 10–15; sclerenchyma strands 50–100, roots densely set; *scales* pseudopeltate, apically densely set, otherwise more or less sparsely set; distinctly spreading, ovate, or triangular; 2.1–5.9(–23.7) mm long, 0.7–1.4 mm wide, margin denticulate, apex acuminate, clathrate throughout, central region glabrous. Phyllopodia distinct, 1.7–7.9 mm apart; stipe present, 0.6–22.6 mm long, 0.5–2.7 mm diam., sharply raised on upper and lower surface. *Fronds* monomorphous or slightly dimorphous, subcoriaceous, light to dark green in color; lamina narrowly elliptic to narrowly obovate to linear; 16.2–56.1 cm long, 1.9–6.5 cm wide, index 4–19.4, widest about or above the middle of leaf length or indistinct, base narrowly angustate, the stipe winged for considerable part, margin entire, apex acuminate, with short glandular hairs, scales and acicular hairs absent. *Venation pattern*: connecting

veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, all veins or secondary and smaller veins more or less immersed and vague (at least in living specimen), free veinlet simple or once- or twice- forked, angle between primary and secondary vein 34–61 -degree. *Sori* mostly irregularly scattered on simple free or forming irregular rows parallel to each pair of secondary veins; round, superficial or slightly immersed, on the whole surface of the lamina or usually occupying the upper (apical) half portion of the lamina, 10–60 cm^2, 1.1–1.6 mm diam., absent in the marginal and costal areoles; paraphyses absent; sporangium annulus 18–24 -celled; indurated cells 10–17. *Spores* concavo-convex, hyaline, 27.5–40 by 42.5–55 μm.

Distribution. — Taiwan; China; Vietnam.

Ecology. — By slope, under wood on limestone, 100–200 m altitude.

Specimens examined. —China: Christensen 1339 (BM), 3418 (BM); Henry 1895 (K), 1951 (K); Kew H1905/85 (K); Suruhoe 82 (K) — Formosa: B20 0091734 (B) — Hanoi, Vietnam: d' Alleizette herb s.n. (P)— Kew, England: Edward 38 (K), 2411 (K); Kew A.D. 19 (K) — Philippines: Loher 867 (K); Vidal 4041 (K) — Tokin, Vietnam: Balansa 45 (P), 70 (P), 107 (P), 148 (P); Bon 1274 (P), Cadière 30 (P), 99 (P); Christ 1940 (P); Giesenhagen 1910 (P); Pételot (Colani) 1339 (P), 1789 (BM), 4871 (P), 4911 (P) — Taiwan: Balansa 198 (K), Playfair 383 (K), J.B. Steere (s.n.) (P).

7. *Microsorum musifolium* Copel.

Rhizome 3.8–10.8 mm diam., approximately cylindrical or dorso-ventrally slightly flattened, not white waxy; bundle sheaths collenchymatous, vascular bundles in cylinder 19–20, sclerenchyma strands 45–87, roots very densely set (forming a thick mat); *scales* peltate, apically densely set, otherwise more or less sparsely set, appressed, ovate, 2.1–4.5 mm long, 1–2.1 mm wide, margin entire, apex acuminate, with hyaline marginal region, central region glabrous. Phyllopodia distinct; 2.4–8.8 mm apart; stipe absent or indistinct, 0.8–12.7 mm long, 2.8–9.1 mm diam., raised on lower surface, slightly raised on upper surface. *Fronds* monomorphous, (firm-)herbaceous, light to dark green in color; lamina narrowly obovate to broad oblanceolate, 43.4–138.1 cm long, 5.3–13.6 cm wide, index 5.9–16.4, widest about or above the middle of leaf length, base truncate to obtuse, the stipe more or less winged, margin entire, apex acute to acuminate, with a few scales and short glandular hairs, acicular hairs absent. *Venation pattern*: connecting veins forming a

row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, all veins distinct, free veinlet simple and once- forked, angle between primary and secondary vein 21–44 -degree. *Sori* mostly irregularly scattered on simple free on the whole surface of the lamina or usually occupying the upper (apical) half portion of the lamina, or forming irregular rows parallel to each pair of secondary veins; round, superficial, 15–62 cm^2, 1.1–1.8 mm diam., absent in the marginal areoles, occasionally present in costal areoles; paraphyses absent; sporangium annulus 17–20 -celled; indurated cells 13–15. *Spores* concavo-convex, hyaline, 27.5–42.5 by 37.5–57.5 μm.

Distribution. — Southern Burma; Peninsular Malaysia; Singapore; Sumatra; Java; Borneo; Philippines: Luzon, Catanduanes and Mindanao; Papua New Guinea.

Ecology. — Primary rain forest, in stream beds or near streams. Low epiphyte or epilithic, 100–900 m altitude. Often cultivated in botanical gardens.

Specimens examined. — Borneo: Elmer 20871 (P); Enders 4022 (L); Hose 1827 (K), 1894 (K); Inder 4022 (K); Iwatsuki et al. 3252 (K); Jaman 4036 (K); Kato et. al B3252 (L) — Java: Bernardi 234 (B); Blume s.n. (L); Zollinger 3005 (P) — Malay Peninsula: Henderson 19708 (BM) — Malaysia: Beddome 1911 (K); Henderson 19704 (K); King 192 (K); Littke 469 (L); Matthew 1928 (K); Yapp 575 (K); Turneau 836, 905 (K); Unesco Limestone Exp. 635 (K) — Papua New: Lauterbach 567 (P); Ledermann 7695 (B), 8549 (B), 8743 (B); Schlechter 2764 (B) — Philippines: Copeland 1537 (B); EBL 1537 (P); Elmer 10500 (K, L); Vanoverberg 3678 (P) — Sarawak: Elmer 20871 (K); Gay 132 (K); Hancook 342 (K); Hose 1827 (BM); J.Smith 1859 (BM) — Singapore: Corner 30247 (K); Haniff 21028 (K) — Sumatra: Brooks 357 (BM); Hancook 1892 (K); Lau 1796 (K); de Wide & de Wide 12385 (L).

8. *Microsorum punctatum* (L.) Copel.

Rhizome 2.2–11.1 mm diam., approximately cylindrical, often waxy, at least sometimes waxy under the scales; bundle sheaths not differentiated, vascular bundles in cylinder 15–20, sclerenchyma strands 50–100, roots very densely set (forming a thick mat); *scales* pseudopeltate, sometimes peltate, apically densely set or otherwise more or less sparsely set, slightly spreading, narrowly ovate to ovate or triangular, 1.5–5.6 mm long, 0.7–2.6 mm wide, margin dentate to denticulate, apex acuminate, clathrate throughout to subclathrate, central region glabrous. Phyllopodia more or less distinct,

1.6–13.7 mm apart. *Fronds* monomorphous or slightly dimorphous, firm-herbaceous to subcoriaceous or coriaceous, light to dark green in color; lamina narrowly elliptic to narrowly ovate to narrowly obovate to broad to narrowly oblanceolate to linear; 27.9–171.9 cm long, 2.6–19.2 cm wide, index 5.6–28.8, widest about or above the middle of leaf length, base cordate to narrowly angustate, the stipe winged for considerable part, margin entire, or entire undulate, or sinuate (in cultivar variety), apex rotundate to acuminate, with a few scales and short glandular hairs, acicular hairs absent; stipe present, 0.7–9.9 mm long, 2.5–7.9 mm diam., sharply raised on upper and lower surface. *Venation pattern*: connecting veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, or the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger areoles in a row between two veins like in type 1; all veins or secondary and smaller veins more or less immersed and vague (at least in living specimen), free veinlet simple and once- forked, angle between primary and secondary vein 20–56 -degree. *Sori* mostly irregularly scattered on simple free veins on the whole surface of the lamina, or forming irregular rows parallel to each pair of secondary veins, round, superficial, 14–74 cm^2, 0.9–2.3 mm diam., absent in the marginal areoles, generally absent in costal areoles; paraphyses absent; sporangium annulus 10–26 -celled; indurated cells (10–)12–18(–20). *Spores* plano-convex, hyaline, or yellowish hyaline, 22.5–37.5 by 40–65 μm.

Distribution. — Paleotropics and subtropics.

Ecology. — Epiphyte, but also epilithic or terrestrial in various types of forest, sometimes in savannah but also in wet places in streambeds, up to 2,800 m altitude.

Specimens examined. —Annobon Is.: Melville 2023 (K); Skinn 283 (K) — Australia: Coveny & Hind 6900; Heward 183 (K); Hooker 1820 (K); Melville et al. 3669 (K); Wall 11232 (K) — Bali: Holstvoogd 772 (L), 844 (L) — Borneo: Ashton 19060 (K); Combes 4097; Korthals 113 (B), 973 (B); Endert 2358 (L); Ismail 2744 (K) — Burma: Dickason 7637 (L); Wallace 191 (K) — Bhutan: Nuttall 1867 (K) — Cameroon: Fris et al 7120 (K); Hepper 8682 (K); Mildbraed 4426 (B); Preuss 2 (B), 309 (B); Sermolli 5232 (K), 7219 (K), 7244 (K); Tchinaye 89 (K) — Ceylon: Beckett 648 (B); Hooker 3799 (K) — China: Cavalerie (& Fortunat) 4012 (P); Esquirol 3601 (P); Fung 20053 (K); Henry 10899 (B, K); Lungchow 83 (K); Matthew 1907 (K); Rochers 2634 (P); Rosenstock 99678 (B); Tutcher 10771; Ying 1657 (K) — Christmas Is.: Andrews 108 (K); Allen 7 (K), 173 (K); Mitchell 132 (K), 154 (K); du Puy 7 (K); Wace 4 (K),

42 (K) — Comoro Is.: Benson 106 (BM), 1293 (BM) — Congo: Anton Cupffert 337 (K); Ben 438; Gutzwiller 1305 (K); Leonard 1618 (K); Louis 1417(K), 1932 (K); de Néré 332 (P), 1412 (P); Thollon 1304 (P); Wide et al. 3734 (B) — Ethiopia: Meyer 7997 (K) — Fiji: Brownlie 1304 (L), 8454 (L), 16074 (L); Seemann 728 (L) — Gabon: Jeffrey 208 (K); Leeuwenberg 13492 (K); Louis et al. 950 (P) — Guinea: Caruallo 2279 (K), 4235 (K); Jacques-Fe'lix 864 (P) — India: Abraham 666 (K); B 200099652 (B); Beddome 48/341 (K); Bhargava et al. 6356 (K), 2836 (L); Cusclah 17347 (K); I.S. Gamble 16350 (K); Gough 3243 (K), 6055, 8289, 16350; Jarrett 766 (K), 784(K); J.D.H. 750 (K), 2223 (K); Manickam 31220 (K); Miller 1364 (K); Mooney 2383 (K); Paush 1931 (K); Piggott 2103 (K); Saldaha 12517 (K), 16392 (K); Sunanda 9557 (K); Tessier 19067 (K); Wallich 281 (BM, K), 1837 (P) — Irian Jaya: Nooteboom 5915 (K); Sands 6730 (K) — Ivory Coast: Chevalier 21088 (P); Leeuwenberg 1785 (K), 2542 (K); Viane 16 (K), 828 (K) — Java: Bakhuizen van den Brink 5739 (L); van Balgooy 4628 (K); Botavae 74 (K); Endert 15062 (L); Hooker 1867 (K); Inaeteay 173 (K); Jati 875 (L); Korthals 148 (L), 527 (L), 684 (L); R.B. le Lunde 156 (P); Matthew 1928 (K); Mousset 20; Pleyte 57, 265; Rosenstock 20 (K); Slanse 6314 (L); de Vriese & Teijsmann 26 (K), 32 (K), 325 (K); Zollinger 935 (B), 1028 (K); Mousset 166 (P) — Kenya: Lucas 230 (K); Verdcourt 3919 (K) — Kl. Soenda Eil.: Elbert 913 (L); Schmitz 5169A (L), F7 (L) — Liberia: Linder 759 (K); Deighton 6056 (P); Wide et al. 3734 (K, P), 3876 (K) — Madagascar: Barrett & Dorr 201 (P); M.R. Decary 17754 (P); Humblot 666 (L); Perrier 6149 (P); Perrier 1747 (P) — Malaccan: Lam 3717 (L) — Malay Peninsular: Castel 1961 (K), 15193 (K); Chusan 1847 (K); Ernst 11045 (K); Hooker 526 (K), 1803 (K); Hose 4823 (K); G. King's collector (= Kunstler) 5069 (K); Maitban 281 (K); Matthew 2 (K), 4 (K); Parrell 11385 (K); Piggott 2973 (K); Rodin 177 (K), 245 (K), 569 (K); Ridley 1917 (K); 6554 (K); Turneau 904 (K); Yapp 296 (K) — Malaysia: Littke 394 (L) — Mayetta: Pascal 923 (P) — Mauritius Is.: M. Boivin 891 (P), 1014 (P); Sieber (Syn. Fil.) 31 (B, BM, K) — McLucas: van Borssom Waalkes 3053 (K), 3288 (K), 3238 (K), 3228 (K); Buwalda 4159, (K) 6052 (K, L); Eyma 3254 (K); Moseley 3412 (K); Parris 11051 (K), 11720 (K); Robinson 1954 (K) — Mozambique: Rehmann 8674 (P) Cameroon: Bos 4106 (K); Leeuwenberg 5032 (P), 6651 (B, K, P); Thorold 28 (BM), 87 (K) — Micronesia: Bryan 1114 (K), 1167 (K); Hutchison 1139 (K) — New Caledonia: Deplanche 198 (L); Germain 40 (L); Franc 11448 (P); Moore 30 (L); Noumea 29 (L), 199 (L); Pancher 186 (P), 506 (P) — New Hebrides: Braithwaite 2306 (L), 2570 (L); Bourdy 306 (L); Savi 340 (L) — Nigeria: Chapman 3132 (K); J.M. Baker 84 (K); Jones 16952

(K); Wright 4 (K) — Nongowa: Bakshi 207 (K) — Nyasaland: Chapeua 581 (BM) — Reunion: Cadet 3824 (P) — Papua New Guinea: Avon 370 (L); Brass 24220 (BM), 25458 (K, L), 29373 (L); Carr 12148 (K); Croft 199 (K), 1129 (K), 61266 (K), 61160 (K), 61578 (L), 65453 (K); Darbyshire 624 (K); Floyd 5682 (K); Forster 10852 (K); Gay 405 (K), 1031 (K); Hartley 11536 (L); Holttum 15702 (K, L); Hoogland 10588 (K); *Køie 1149 (K); Kulong 11582 (L); Jermy (& Rankin) 8220 (K); Lam 1108 (K);* Leeuwenberg 10647 (L); Moseley 1874 (BM); van Royen 3474 (K); Sand 2118(K), 2695 (K); Schultze 104 (B); Schlechter 16304 (P); Schodde 3026 (L); Stevens 58710 (K); Thomas 11536 (K); Wakefield 1435 (BM); Walker 7884 (L) — Philippines: Castro 5910 (L); Copeland 275 (BM), 1535 (B), 1776 (P), 15356 (B); Elmer 5884 (K, P), 7854 (P, L), 7991 (K), 8263 (L), 9946 (BM, K, L), 10920 (K), 13813 (L), 13598 (B, BM, K, P), 16863 (K), Elmer 22330 (K); Foxworthy 42135 (B); Gutierrez 117367 (K); Hatierg 171 (K); Mathew 1928 (K); Merrill 7331 (P); Ramos 973 (P), 14862 (P), 31419 (P), 14779 (K); Ridsdale 5567 (K); USC 288 (L); Wenzel 1216 (BM), 2611 (B) — Polynesian: B20 0099749 (B) — Sarawak: Christensen 529 (BM); Cooks 1909 (K); Kandau 62458 (K); Parris 6900 (K); Paul 64665 (K) — Sierraleone: Deighton 6056 (K) — Singapore: Ridley 8935 (P) — Solomon Is.: Braithwaite 43701 (L); Brass 2756 (P); Beer 7768 (L); Jarrett 68 (L); Whitmor 4321 (L) — South AF: B20 0099 607 (B), Chase 5220 (BM); Melsetter 46915 (BM); Mitchell 378 (BM); Rudatis 1369 (P); Schelpe 5032 (BM, P), 5225 (BM) — Sulawesi: Bünnemeijer 12427 (L); de Joncheere 1325 (L); T.G. Walker T12316 (L) — Sumatra: Buwalda 6978 (K); Coode 6251 (L); Darnaedi 71 (K), 2107 (K); Hennipman 5112 (L), 5462, 5981 (L); Jacobson 10 (L); Lütjeharms 4750 (L), 4990 (K), 5151 (K), 5159 (K); Surbeck 1082 (L) — Tahiti: Braithwaite 4136 (P), 2570 (P); Forsberg 14149 (K); Hooker 1867 (K); Maire 10775 (K); Moseley 6447 (K); Savatier 987 (P); Sloover P195 (P); Vesco 1847 (P); Vieillard 10775 (K) — Taiwan: Tagawa 1853 (K) — Tanganyika: Glover 263 (K) — Tanzania: Balslev 342 (K); Bidgood 4775 (K); Balslev 342 (K) — Thailand: van Beusekom et al. 258 (B, BM); Bloembergen 18 (K); Bunk 384 (K); Christensen 529; Floto 7237 (K); H.B.G. Garrett 288 (K, P); Hennipman 3065 (BM); Iwatsuki et al. 10900 (K); Larsen 2597 (K), 3096 (K), 5078 (K); Murata et. al. 16387 (K), 17674 (K); Phengkai 683 (B); Tem 11209(K) — Timor: Bloembergen 3424 (K) — Uganda: Dawkins 389 (K); Dümmer 472 (K); Faden 69-946 (K); Glokudler 288 (K), 1458 (K), 1488 (K); den Hoed 909 (K); Kostermans 59 (K); Hafashimane 26 (K), 357 (K); Jackson 123 (K); Katendo 1187 (K); Lecerber 2042 (K), 2617 (K); Sangster 630 (K); Tweedie 2432 (K); Thomas 145 (K), 1369 (K) — Vietnam: Braker 4136 (P); Cadière

98 (K); Phustouve 34 (K); Pételot (Colani) 2898 (P), 4101 (B); M. Semesle 580 (P); Tsiang 29192 (K), 36090 (K); Vieillard 459 (P).

2.4 Discussion and Conclusion

Morphological point of view, it can be seen that 8 distinct species, viz. *Microsorum siamense* (I), *M. thailandicum* (II), *M. membranaceum* (III), *M. glossophyllum* (IV), *M. whiteheadii* (V), *M. steerei* (VI), *M. musifolium* (VII), and *M. punctatum* (VIII) are revealed from the study of *Microsorum punctatum* complex. The first two species can be easily distinguished from the others by obtaining iridescent blue-green fronds color when living. *M. siamense*, an endemic species from Yala, Thailand, has distinct hydathodes as diagnostic character; while *M. thailandicum*, from Chumphon, Thailand, has a unique character of general venation pattern as type 2 (the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger, areoles in a row between two veins). Both of them can use these characters to separate itself from the other microsoroid fern in the *M. punctatum* complex.

The result of this work corresponded with Smith and Hoshizaki (2000) who purposed a new species, *M. whiteheadii*, from Indonesia to the genus *Microsorum*. Although *M. whiteheadii* appears related to *M. steerei* and *M. punctatum* but differs from these two species by the generally shorter creeping rhizomes, more succulent, thicker, very dark green, oblanceolate blades, prominent hydathodes on adaxial lamina surface, and less visible venation.

For the character of presence or absence of paraphyses, the term paraphyses can be applied to all sterile structures in a sorus, such as sporangiasters (aborted sporangia), sterile filaments, peltate scales. It is likely that this character cannot be easily assessed and is unusable. However, the structures are under "uniseriate paraphyses" (Bosman, 1991) and can be best useful for construction an identification key. By her definition, "uniseriate paraphyses", the paraphyses with a glandular top-cell, are present in *M. membranaceum*, *M. glossophyllum*, *M. whiteheadii*, *M. steerei*, *M. musifolium*, and *M. punctatum*.

This study also agrees with Bosman (1991) who pointed out that *M. glossophyllum* can be easily distinguished from *M. punctatum* using characters of rhizome scale. The scales of typical *M. glossophyllum* are opaque, blackish and narrow, whereas the scales of *M. punctatum* are translucent, brownish and broadly ovate. This indeed easily observed character seems to be useful and potentially important character in identification of these two very close and often confused taxa. Moreover, *M. glossophyllum* differs from *M. punctatum* in many characters i.e. frond color, presence of indumenta on fronds, visibility of veins and presence of sori in the costal areoles or on their bordering veins (Table 3.2). Therefore, this study disagrees with Nooteboom (1997) who recognized *M. glossophyllum* as synonym of *M. punctatum.*

Nooteboom (1997) also included *M. musifolium* as a synonym of *M. punctatum* due to these two taxa has many intermediates. The present result revealed that *M. musifolium* can be separated from *M. punctatum* in many characters such as appearance of rhizome surface, type of attachment of scales, spreading of scales and presence of hyaline marginal region on rhizome scales (Table 3.2). In pteridophyte, character of rhizome scale has highly taxonomic value, especially in species level classification (Barrington, 1985; Sano et al., 2000; Salgado, 2004). Rhizome scale of *M. musifolium* is entire marginal scale with out hyaline region whereas hyaline marginal region present on denticulate rhizome scales of *M. punctatum*. This study also agrees with Bosman (1991) who noted that characteristic for *M. musifolium* are the large fronds with truncate to obtuse base and the very regular and distinct venation. Because of this, it is reasonable to conclude that *M. musifolium* should be recognized as a clearly distinct species from *M. punctatum*.

The characters of spore morphology such as shape, number of spores per sporangium and perispore ornamentation, specially fine details revealed by scanning electron microscopy, were the most valuable characters in the recognition of taxa in pteridophyte species complex classification (Regalado and Sânchez, 2001). Spore surface of *M. membranaceum* are irregularly rugate while the other taxa in the complex are plain to slightly verrucate. Although most taxa in the complex have yellowish hyaline or hyaline spore, the yellow spore occurs in *M. membranaceum*, *M. siamense* and *M. whiteheadii*.

Regarding the anatomical characters, though most of species in the *M. punctatum* complex are quite uniform, some of these characters are useful to distinguish taxa in the complex. For example, all species in the *M. punctatum* complex have one cell layer thick of rhizome scale, except for the central region around the stalk. Only those of *M. glossophyllum* are not having this character. The numbers of cell layer in rhizome scale of *M. glossophyllum* are up to two or three cell layers thick. The mesophyll of plants in the complex is consisted of 8-10 layers of spongy parenchyma. Collenchyma of most of species in the complex is only found in the midrib. It occurred as a subepidermis sheath surrounding the ground tissue (Fig. 3.3C), except in *M. musifolium* and *M. siamense* collenchyma was found as abaxial and adaxial subepidermis layer (Fig. 3.3E). In *Microsorum membranaceum* and *M. musifolium*, the vascular strands in cross section are always surrounded by collenchyma or party sclerenchyma sheaths. It look like a blacken sheath surrounded vascular tissue in cross section of rhizome, stipe, and midrib.

Of the *Microsorum punctatum* complex, *Microsorum punctatum* s. str. has the widest distribution and various forms (Bosman, 1991; Nooteboom, 1997; Bosman et al. 1998). However, these variations are more or less overlapping and should not recognize as separated taxa.

To sum up, it is clear that morphological and anatomical characters are useful to distinguish species in the *M. punctatum* complex.

CHAPTER 4

NUMERICAL STUDY

4.1 Introduction

Presently, taxonomic evidences for establishment of classification and phylogeny are gathered from various sources. All parts of a plant at all stages of its development can provide taxonomic characters, so taxonomic data must be assembled from many diverse disciplines i.e., comparative anatomy, embryology, palynology, cytogenetics, chemistry etc. (Jones and Luchsinger, 1987). Numerical taxonomy is the application of standard statistics and standard mathematics to solve the taxonomic problems (Clifford and Stephenson, 1975). The main objective of numerical methods is to simplify and portray degrees of relationship or similarity among any groups of organisms. This method utilizes many equally weighted characters and employs clustering and similar algorithms to yield objective grouping (Sneath and Sokal, 1973). In this thesis, Cluster Analysis (CA) and Canonical Discriminant Analysis (CDA) were used to solve classification problems in "*M. punctatum* complex".

Cluster Analysis (CA)

The aim of this numerical technique is to place individual specimens into groups (Sneath and Sokal, 1973). Moreover, cluster analysis is also used to identify a small number of groups such that elements belonging to a given group are, in some sense, more similar to each other than to elements belonging to other groups. Consequently, cluster analysis can be considered as another technique for data reduction (Dillon and Goldstein, 1984).

Canonical Discriminant Analysis (CDA)

Canonical discriminant analysis (CDA) is sometimes known simply as "canonical analysis". The differences between the groups can be established statistically by means of a test of the probability of the null hypothesis that all the groups of objects have the same centroid (multivariate mean). If there is only one measured variable, this is equivalent to the one-way analysis of variance for comparing two or more groups. The relationships between the groups can be assessed visually by means of a scatter plot in which the positions of the individuals or the group means or both are plotted on axes known as canonical axes (discriminant functions) which depend on the original observations and

are chosen by the analysis to best represent the differences between the groups (Lachenbruch and Goldstein, 1979).

Moreover, the numerical approaches by means of morphometric analyses were also carried out on many species complex and various plant to clarify the complexity of their taxonomic status, examples included Speer and Hilu (1998); Giussani (2000); Thomson (2000); Small and Hickey (2001); Casiva et al. (2002); Kim et al. (2003); Kidyue et al. (2005); Conceição et al. (2007).

4.2 Materials and Methods

4.2.1 Plant material

In this study about 1,500 dried herbarium specimens kept in the main herbaria in Thailand namely Forest Herbarium, National Park, Wildlife and Plant Conservation Department (BKF); the Prof. Kasin Suvatabhundbu Herbarium, Department of Botany, Chulalongkorn University (BCU) and European countries viz. B, BCU, BKF, BM, K, L, and P (herbarium abbreviations according to Holmgren & Holmgren, 1998) were examined. Seven hundred and seven complete specimens of 21 taxa were used (Table 4.1) in all analyses, each specimen was considered as an OTU (Operational Taxonomic Unit).

4.2.2 Character measurements

In total, 23 quantitative characters of both vegetative and reproductive structures of the collected specimens were studied and used in multivariate analyses (Table 4.1 and Fig. 4.1). The measurement of macroscopic quantitative character was carried out using a standard ruler or a digital caliper. While the microscopic characters were measured with an aid of the light microscope equipped with 10X lens coupled to micrometer disc and 10X objectives.

4.2.3 Data analysis

Twenty three quantitative characters (Table 4.2) and thirty seven qualitative characters (Table 3.2) were subjected to cluster and canonical discriminant analyses. Cluster analyses were carried out using the Gower similarity coefficient (Gower, 1971) and UPGMA clustering in the MVSP program (Kovach Computering Services, MVSP Plus, version 3.1). The characters used in the analysis were assumed to be of equal importance and were not weighted

Table 4.1 Herbarium which deposited specimen that used in this study of the *M. punctatum* (L.) Copel. complex

No.	Taxon	No. of specimen[1]	Herbarium[2]
1.	*Microsorum siamense* Boonkerd	6(1)	**L, BCU**
2.	*M. thailandicum* Boonkerd & Noote.	9 (2)	**L, BCU**
3.	*M. membranaceum* (Don.) Ching	86 (1)	**K**
4.	*M. glossophyllum* (Copel.) Copel.	67 (2)	B, K, L, and **P**
5.	*Pleopeltis megalosoroides* Alderw.	1 (1)	**L**
6.	*M. whiteheadii* A.R. Smith & Hoshizaki	5	**K,** BCU
7.	*M. steerei* Harr.	35 (1)	B, K, and **P**
8.	*P. playfairii* Baker	1 (1)	**K**
9.	*P. tonkinense* Baker	3 (3)	**P**
10.	*M. musifolium* (Blume) Ching	48 (3)	B, K, **L**, and P
11.	*M. punctatum* (L.) Copel.	302	B, K, L, and P
12.	*M. punctatum* ssp. *subirideum* H. Christ	3 (3)[3]	**L, P**[4]
13.	*M. punctatum* ssp. *subdrynariaceum* H. Christ	2 (2)	**L, P**
14.	*Polypodium irioides* Poiret	48 (1)	B, **K**, L, and P
15.	*M. validum* (Copel.) Ching	26 (3)	B, BM, **K**, L, and P
16.	*P. glabrum* Wall	10 (4)	**BM**, **K** and P
17.	*P. millisorum* Baker	5 (2)	BM, **K**
18.	*M. sessile* Fée	3 (3)	**B**
19.	*P. polycarpon* Cav.	40	B, BM, K, L, and P
20.	*M. neoquineense* Copel.	1	K
21.	*M. punctatum* (L.) Copel. cv. *seratum*	3 (1)	B and **L**
22.	*P. irioides* var. *lobatum* f. *cristatum* Bailey**	1	K
23.	*P. ambiguum* Blume**	1 (1)	**L**
	Total	**707**	

Note.---1.Only complete specimens of 21 taxa were used (Table 3.1) in all analyses; 2. herbarium abbreviations according to Holmgren & Holmgren, 1998 namely B = Botanischer Garten und Botanisches Museum Berlin-Dahlem (Berlin herbarium), Germany, BM = British Museum, England, BCU = The Prof. Kasin Suvatabhundbu Herbarium, Department of Botany, Chulalongkorn University, Thailand, BKF = Forest Herbarium, National Park, Wildlife and Plant Conservation Department, Thailand, K = Royal Botanic Gardens, Kew (Kew herbarium), England, L = Nationaal Herbarium Nederland (Rijkherbarium, Leiden), Netherlands, P = Museum National d' Histoire Naturelle (Paris herbarium), France; 3. Number of Type specimen of each species; 4. Herbarium keep Type specimen.

** = *Polypodium ambiguum* and *P. irioides* var. *lobatum* f. *cristatum* Bailey were included as population of *M. punctatum* (L.) Copel. In all analysis

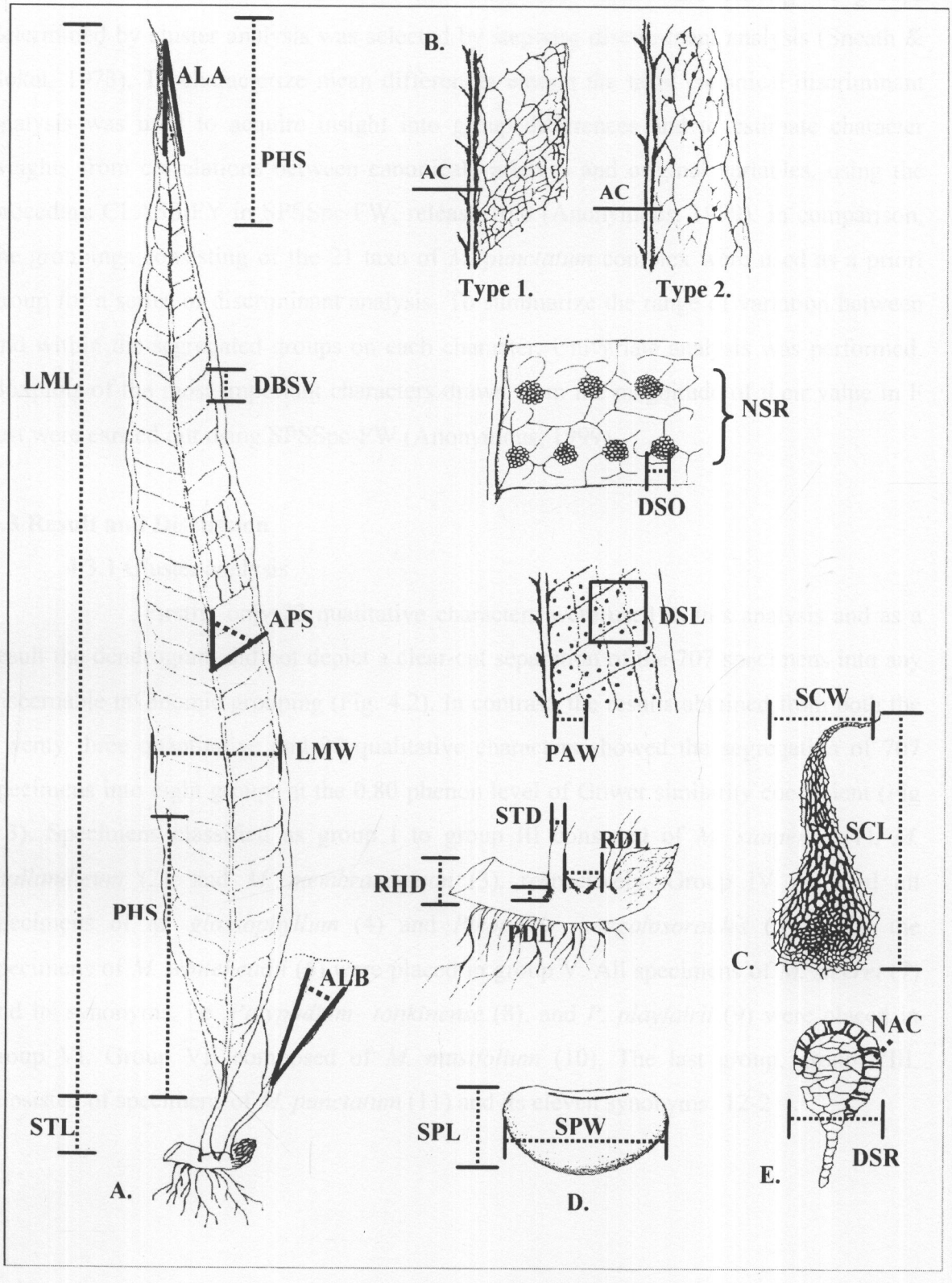

Figure 4.1 Measurement of rhizome, scale and frond part. --- A plant; B. venation; C. scale; D. spore; and E. sporangium.

A subset of characters that maximized differences among the groups determined by cluster analysis was selected by stepwise discriminant analysis (Sneath & Sokal, 1973). To characterize mean differences among the taxa, canonical discriminant analysis was used to acquire insight into group differences and to estimate character weights from correlations between canonical variables and original variables, using the procedure CLASSIFY in SPSSpc-FW, release 10.0 (Anonymous, 1999). In comparison, the groupings consisting of the 21 taxa of *M. punctatum* complex were used as a priori group for a series of discriminant analysis. To summarize the range of variation between and within the segregated groups on each character, Univariate analysis was performed. Boxplots of the most important characters drawn from the magnitude of their value in F test were carried out using SPSSpc-FW (Anonymous, 1999).

4.3 Result and Discussion

4.3.1 Cluster analysis

Firstly, only 23 qualitative characters were used in this analysis and as a result the dendrogram did not depict a clear-cut separation of the 707 specimens into any discernable taxonomic grouping (Fig. 4.2). In contrast, the results obtained from both the Twenty three quantitative and 37 qualitative characters showed the segregation of 707 specimens into eight groups at the 0.80 phenon level of Gower similarity coefficient (Fig 4.3). Specimens classified as group I to group III consisted of *M. siamense* (1), *M. thailandicum* (2), and *M. membranaceum* (3), respectively. Group IV included all specimens of *M. glossophyllum* (4) and *Pleopeltis megalosoroides* (5), while the specimens of *M. whiteheadii* (6) were placed in group V. All specimens of *M. steerei* (7) and its synonyms, i.e. *Polypodium tonkinense* (8), and *P. playfairii* (9) were placed in group VI. Group VII composed of *M. musifolium* (10). The last group, group VIII, consisted of specimens of *M. punctatum* (11) and its eleven synonyms (12-21).

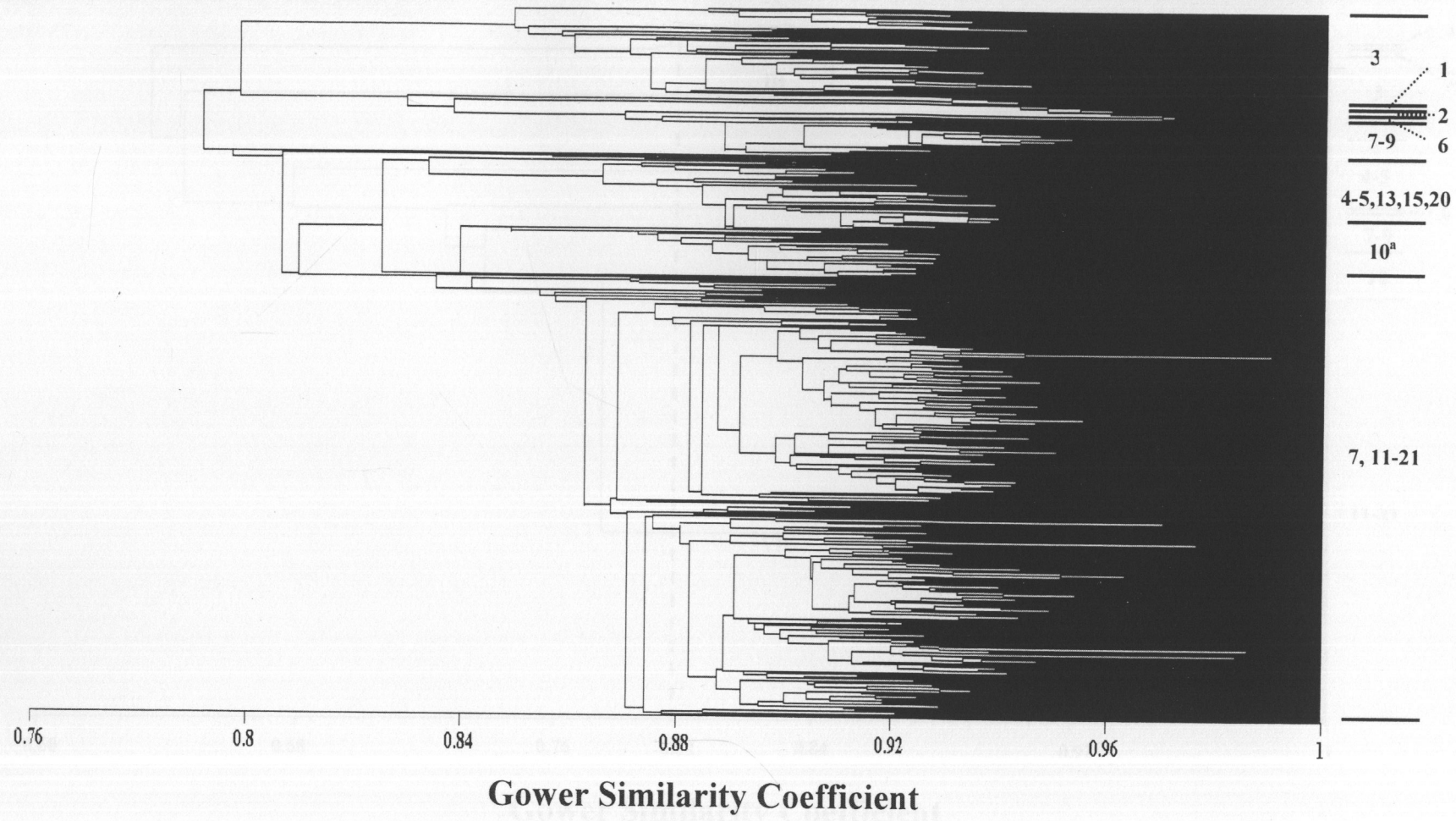

Figure 4.2 UPGMA clustering of 707 OTUs based on Gower Similarity Coefficient calculated between means only of 23 qualitative characters of the *M. punctatum* (L.) Copel. complex (a. numbers correspond to those in Table 4.1).

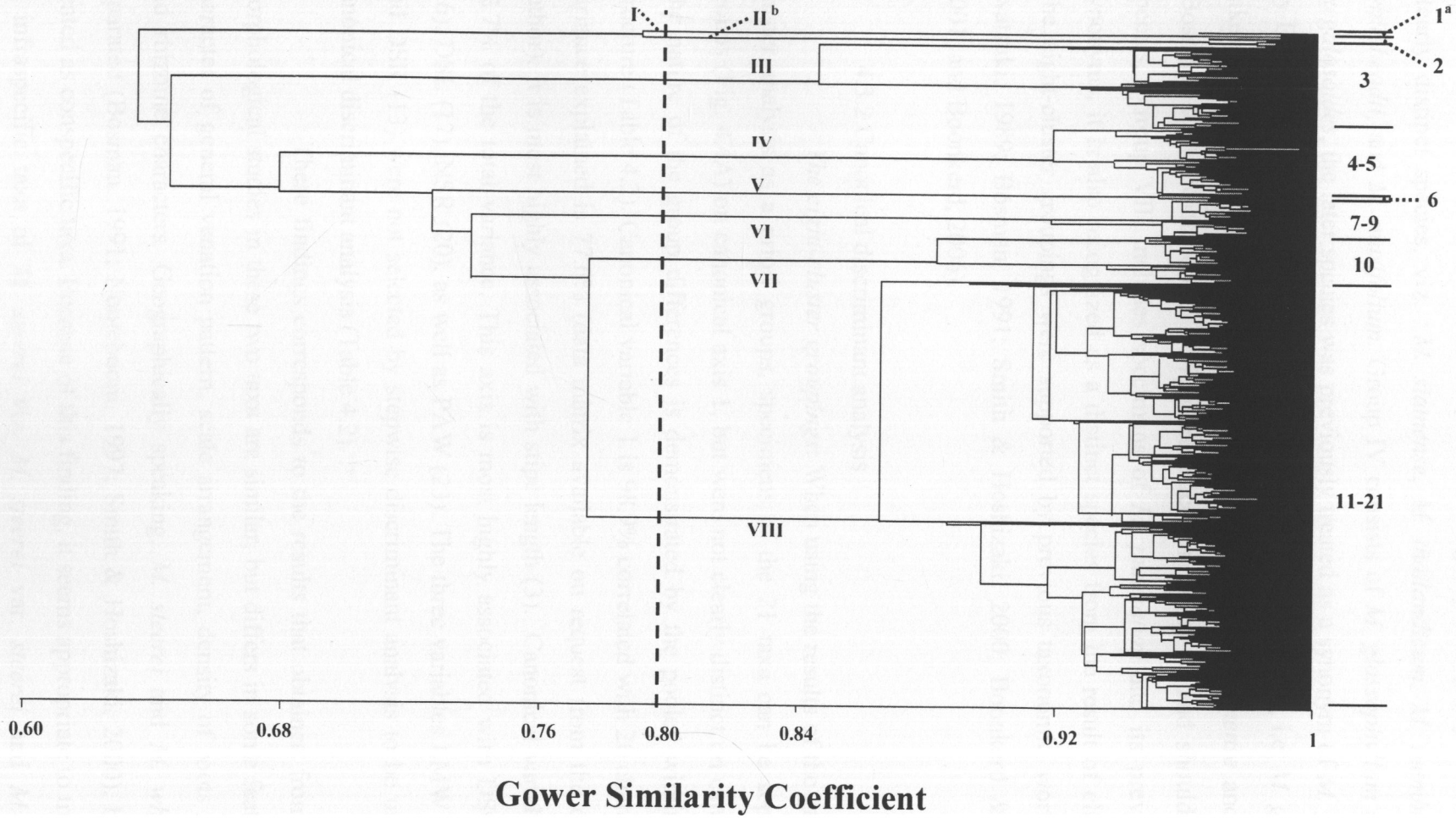

Figure 4.3 UPGMA clustering of 707 OTUs based on Gower Similarity Coefficient calculated between means of 23 qualitative and 35 quantitative characters of the *M. punctatum* (L.) Copel. complex (a. numbers correspond to those in Table 4.1; b. numbers correspond to those in Table 4.3).

It can be seen that the specimens of group I-III, group V and group VII are clearly distinct species, viz. *M. siamense*, *M. thailandicum*, *M. membranaceum*, *M. whiteheadii*, and *M. musifolium*. Group IV consists of *M. glossophyllum* and *Pleopeltis megalosoides*, the later species was previously treated as a synonym of *M. glossophyllum* by Bosman (1991). So, group IV should also be a distinct species, i.e. *M. glossophyllum*. Likewise, *M. steerei* and its two synonyms, *Polypodium tonkinense* and *P. playfairii* (Bosman, 1991; Nooteboom, 1997) were placed in group VI and should be a distinct species. Group VIII includes specimens of *M. punctatum* and its previously treated synonyms, it is also recognized as a distinct species from the result of cluster analysis. The eight-cluster groupings were supported by previous taxonomic work (Tagawa & Iwatsuki, 1989; Bosman, 1991; Smith & Hoshizaki, 2000; Boonkerd & Nooteboom, 2001; and Boonkerd, 2006).

4.3.2 Canonical discriminant analysis

The eight-cluster groupings: When using the results of the groupings from cluster analysis as a priori groups, specimens of the 21 taxa can be separated into 7 groups (Fig. 4.4A) on canonical axis 1, but were not clearly distinct on canonical axis 2. The nature of the group differences is demonstrated by the pooled within a canonical structure (Table 4.2). Canonical variable 1 is 98.9% correlated with 20 characters and the variance explained is 77.6% (data matrix available on request from the corresponding author). It is most highly associated with stipe length (3). Canonical variable 2 explains 12.7% of the total variance. This axis is most highly associated with DSO (12), SPW (16), DSL (17), NSR (20), as well as PAW (23). The three variables LMW (1), LML (2) and DSR (13) were not selected by stepwise discriminant analysis to be used in further canonical discriminant analysis (Table 4.2).

These findings corresponds to the results that obtained from our data on morphological studies in these two taxa are similar, but differs in some features, such as character of general venation pattern, scale arrangement, density of roots on rhizomes, and rhizome characters. Geographically speaking, *M. steerei* and *M. whiteheadii* are separated (Bosman, 1991; Nooteboom, 1997; Smith & Hoshizaki, 2000), but should be treated as conspecific taxa. Because of this finding, it seems appropriate to treat these taxa as infraspecific taxa of *M. steerei* viz. *M. steerei* var. *steerei* and *M. steerei* var. *whiteheadii*.

Table 4.2 Twenty three quantitative characters, with their methods of scoring used in this study of the *M. punctatum* (L.) Copel. complex. Univariate F values of the different characters used in the canonical discriminant analysis and pooled within canonical structure using (I) 8 clustering groupings and (II) 21 taxa groupings

No.	Abbreviation	Characters	Discriminant Function							
			I				II			
			F-value	Sign.	Axis 1	Axis 2	F-value	Sign.	Axis 1	Axis 2
1.	LMW [a]	Lamina width (mm)	45.43	0.00	-0.10	-0.12	54.11	0.00	-0.05	-0.17
2.	LML [a,b]	Lamina length (mm)	66.44	0.00	-0.03	0.10	33.07	0.00	-0.01	0.09
3.	STL	Stipe length (mm)	3535.79	0.00	**0.90** [c]	0.03	1250.81	0.00	**0.89**	0.06
4.	STD	Stipe diameter (mm)	78.83	0.00	-0.02	-0.04	41.42	0.00	-0.02	-0.06
5.	PDL	Phyllopodia length (mm)	48.53	0.00	-0.08	0.09	22.47	0.00	-0.08	0.09
6.	RHD [b]	Rhizome diameter (mm)	23.15	0.00	-0.05	-0.03	15.60	0.00	-0.03	-0.07
7.	RDL	Distance between closest phyllopodia (mm)	133.85	0.00	-0.11	0.20	56.10	0.00	-0.11	0.19
8.	SCL	Scale length (mm)	30.54	0.00	0.00	-0.19	13.08	0.00	0.00	-0.19
9.	SCW	Scale width (mm)	40.06	0.00	0.00	-0.12	19.01	0.00	0.00	-0.12
10.	ALA	Angle of frond apex	39.70	0.00	-0.08	-0.07	23.45	0.00	-0.08	-0.09
11.	ALB	Angle of frond base	116.77	0.00	-0.11	0.05	46.92	0.00	-0.11	0.04
12.	DSO	Diameter of sori (mm)	115.24	0.00	-0.01	**-0.39**	41.00	0.00	-0.01	-0.37
13.	DSR [a,b]	Diameter of sporangium (mm)	0.85	0.55	-0.01	-0.05	0.53	0.95	-0.01	-0.05
14.	NAC	Number of annulus cell	123.90	0.00	-0.04	-0.03	44.51	0.00	-0.04	-0.02
15.	SPL	Spore length (mm)	44.59	0.00	0.03	-0.20	17.51	0.00	0.03	-0.20
16.	SPW	Spore width (mm)	22.83	0.00	0.01	**-0.17**	9.37	0.00	0.01	-0.17
17.	DSL	Density of sori per cm^2	141.50	0.00	0.00	**0.42**	53.87	0.00	0.00	0.41
18.	PLS	Distance between lowest sori and frond base (mm)	73.53	0.00	-0.06	0.13	32.63	0.00	-0.06	0.13
19.	PHS [b]	Distance between highest sori and frond apex (mm)	9.44	0.00	-0.02	-0.07	7.33	0.00	-0.02	-0.03
20.	NSR	Number of sori rows between closest secondary vein	259.02	0.00	0.02	**0.58**	101.01	0.00	0.02	**0.58**
21.	DBSV	Distance between closest secondary vein (mm)	58.74	0.00	0.03	0.23	27.90	0.00	0.03	0.24
22.	APS	Angle between primary and secondary vein	123.11	0.00	-0.03	0.29	55.50	0.00	-0.03	0.32
23.	PAW	Primary areole width (mm)	141.39	0.00	-0.05	**-0.39**	49.69	0.00	-0.05	-0.38

Note.- 1. A character followed by a superscript letter indicates a character not selected by stepwise discriminant analysis to be used in further canonical discriminant analysis: a. I, b. II; 2. c. number in bold alphabet is the important variables which associated with each axis.

Regarding *M. membranaceum*, this species should be proposed as a distinct species according to the results of our study (Fig. 4.4A); although Nooteboom (1997) suggested that it might be a variety of *M. punctatum*.

The 21 taxa groupings: Twenty one taxa (Table 3.1) were used as a priori group. Nineteen out of twenty-three quantitative characters were used in this analysis (Table 4.2). The canonical variable 1 is 98.9.0% correlated with twenty three characters and the variance explained by it is 75.4%. It is most highly associated with stipe length (3). Canonical variable 2 accounted for 12.4% of the total variance which is the axis most highly associated with NSR (20). According to the stepwise discriminant analysis five variables, viz. LML (2), RHD (6), DSR (13), and PHS (19) were not selected for further use in canonical discriminant analysis (Table 4.2).

The canonical plot on two canonical axes (Fig. 4.4B) also shows the separation of specimens of the twenty-one taxa (Table 3.1) into 7 groups on canonical axis 1, but is not clearly distinct on canonical axis 2. Groups 1-4 are included *M. siamense*, *M. thailandicum*, *M. membranaceum*, and *M. musifolium*, respectively. Whereas group 5 is comprised of all specimens of *M. glossophyllum* and *Pleopeltis megalosoides*, this later species was previously treated by Bosman (1991) as a synonym of *M. glossophyllum*. Group 6 includes all specimens of *M. whiteheadii*, *M. steerei* and its two previous treated synonyms (Bosman, 1991; Nooteboom, 1997). Likewise group 7, the largest group, is composed of members of 11 previous treated synonyms of *M. punctatum* (Nooteboom, 1997).

Based on the results of cluster analyses together with canonical discriminant analyses, it is reasonable to segregate the 21 taxa of the *M. punctatum* complex into eight distinct taxa, viz. *M. siamense*, *M. thailandicum*, *M. membranaceum*, *M. glossophyllum*, *M. steerei* var. *whiteheadii*, *M. steerei* var. *steerei*, *M. musifolium*, and *M. punctatum*, respectively. Boxplots of the six most important characters that segregate these eight segregated taxa are presented in Fig. 4.4. It can be seen that stipe length (STL) is the most important character.

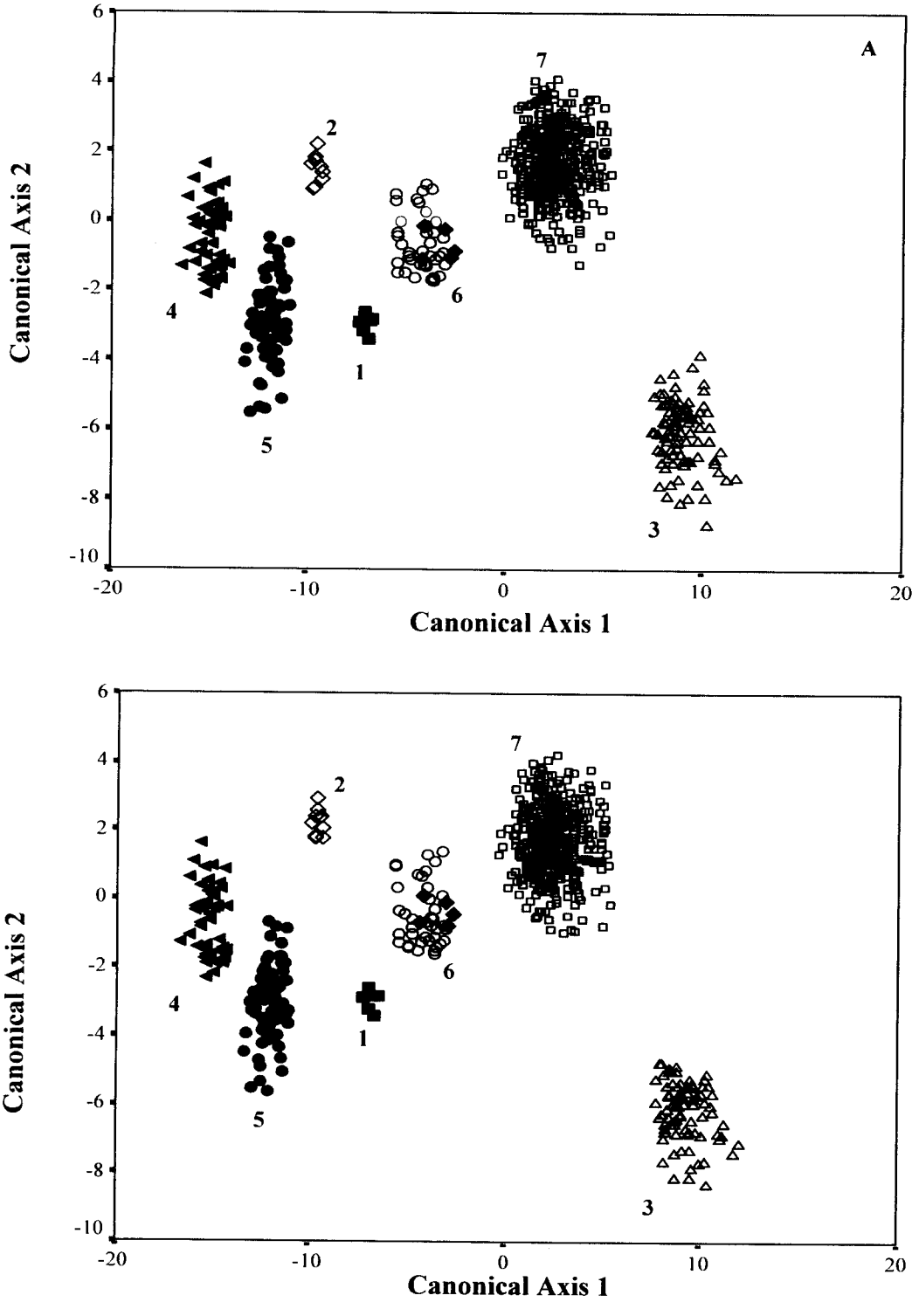

Figure 4.4 The ordination of 707 herbarium specimens of the *M. punctatum* (L.) Copel. complex --- A. 8 clustering groupings; B. 21 taxa groupings (1. *M. siamense* (■); 2. *M. thailandicum* (◊); 3. *M. membranaceum* (Δ); 4. *M. musifolium* (◄); 5. *M. glossophyllum* (●); 6. *M. steerei* var. *whiteheadii* (♦) and *M. steerei* var. *steerei* (○); 7. *M. punctatum* (□)).

4.3.3 classificatory discriminant analysis

Twenty out of twenty-three quantitative characters were determined by stepwise discriminant analysis to be important for discriminating between the eight segregated taxa (Table 4.2).

The linear discriminant function classification result shows 100.0% correctly classified. For this reason, the linear discriminant function (Table 4.3) can be used for identification of unnamed specimens in the *M. punctatum* complex. To employ the discriminant function in Table 4.3 for identification, multiply each character score by its coefficient in each column. The total in each column is calculated, and then the column with the highest total is the taxon to which the specimen belongs. This method of identification is different from traditional keys; however it can be applied in a complementary manner.

Table 4.3 Classification Function Coefficients of eight clustering groups obtained from cluster analysis based on 19 quantitative characters (I. *Microsorum siamense*; II. *M. thailandicum*; III. *M. membranaceum*; IV. *M. glossophyllum*; V. *M. steerei* var. *whiteheadii*; VI. *M. steerei* var. *steerei*; VII. *M. musifolium*; and VIII . *M. punctatum*)

Character	Categories							
	I	II	III	IV	V	VI	VII	VIII
STL	0.69	0.36	3.35	1.24	0.01	1.24	-0.43	2.3
STD	0.25	0.25	3.87	4.51	0.91	-2.5	0.87	1.4
PDL	0.09	-0.03	-5.04	-1.18	0.71	-0.59	1.18	-2.43
RHD	1.53	3.61	1.94	0.95	2.8	3.46	1.7	2.41
RDL	0.58	-0.02	0.06	0.05	1.47	0.54	1.34	0.92
SCL	0.98	2.53	2.28	-1.34	4.81	4.73	4.27	1.5
SCW	7.12	0.67	0.54	31.71	-4.21	-3.23	1.38	-0.25
ALA	0.52	-0.38	0.05	0.07	0.31	0.42	0.31	0.05
ALB	0.17	0.18	1.27	0.82	0.4	0.43	1.26	1.1
DSO	36.61	35.98	47.47	34.5	49.14	38.67	38.67	36.21
NAC	15.83	22.16	13.96	19.61	15.37	16.34	15.2	14.57
SPL	1.59	1.15	1.84	1.5	2.19	1.5	1.52	1.74
SPW	1.6	1.85	1.62	1.88	1.06	1.55	1.47	1.23
DSL	0.48	0.59	0.56	0.68	0.63	0.71	0.9	0.77
PLS	-0.01	-0.01	-0.01	-0.02	0.02	0	0.01	0.01
PHS	0.47	0.28	0.57	0.67	0.59	0.61	0.72	0.57
NSR	0.92	1.86	-0.31	0.84	0.84	1.03	1.3	1.84
DBSV	0.26	2.28	-0.27	0.56	0.13	0.59	0.97	0.39
APS	1.28	2.08	1.1	1.81	1.41	1.93	1.33	1.68
PAW	-2.01	-2.94	-0.45	-3.48	-1.57	-1.49	-0.08	-3.2
(Constant)	-314.1	-494.6	-530.25	-501.82	-350.24	-385.16	-335.05	-420.01

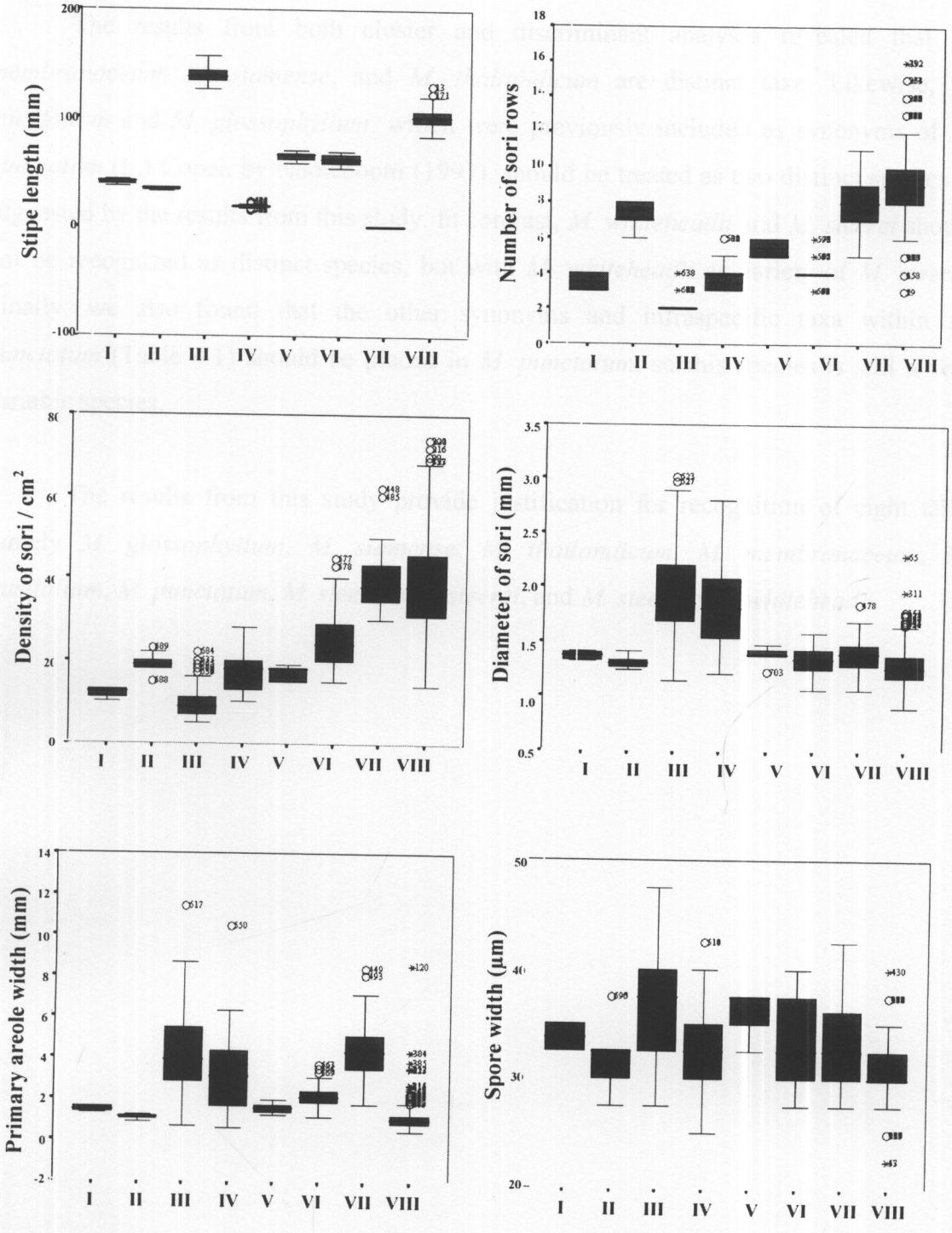

Figure 4.5 Boxplots of the six most important characters of the *M. punctatum* (L.) Copel — I. *Microsorum siamense*; II. *M. thailandicum*; III. *M. membranaceum*; IV. *M. glossophyllum*; V. *M. steerei* var. *whiteheadii*; VI. *M. steerei* var. *steerei*; VII. *M. musifolium*; and VIII. *M. punctatum*.

4.4 Conclusions

The results from both cluster and discriminant analyses revealed that *M. membranaceum*, *M. siamense*, and *M. thailandicum* are distinct taxa. Likewise, *M. musifolium* and *M. glossophyllum*, which were previously included as synonyms of *M. punctatum* (L.) Copel. by Nooteboom (1997), should be treated as two distinct species as suggested by the results from this study. In contrast, *M. whiteheadii* and *M. steerei* should not be recognized as distinct species, but with *M. whiteheadii* as variety of *M. steerei*. Finally, we also found that the other synonyms and infraspecific taxa within *M. punctatum* (Table 4.1) should be placed in *M. punctatum*, so this species is still a very variable species.

The results from this study provide justification for recognition of eight taxa, namely *M. glossophyllum*, *M. siamense*, *M. thailandicum*, *M. membranaceum*, *M. musifolium*, *M. punctatum*, *M. steerei* var. *steerei*, and *M. steerei* var. *whiteheadii*.

CHAPTER 5

RAPD STUDY

5.1 Introduction

The development of the polymerase chain reaction (PCR) had a significant impact in almost all areas of molecular biology (Saiki et al., 1988) and modifications of the basic procedure have allowed the development of numerous assays for detecting variation at the nucleotide level (Korner and Livak, 1989).

Over the years, detection of genetic diversity has progressed to assay molecular DNA variation. The molecular techniques are well suited to clarify problems of cryptic species. One of the most popular methods for the investigation of genetic diversity has recently been Random Amplified Polymorphic DNA (RAPD). With this technique, scientist can reveal a level of genetic variation useful for distinguishing populations and sometimes species, and typically with more variation data than isozyme (Swartz and Brunsfeld, 2002).

A genetic marker based on differences in DNA sequences between individuals generally detects more polymorphism than morphological and protein-based markers (Botstein et al. 1980, Tanksley et al. 1989). Compared to other methods for detecting DNA polymorphisms, this technique has the potential advantage for the investigation of rare plants. It is relatively inexpensive and technically straightforward for conducting experiments and does not requires prior knowledge of the genome (Wang et al., 2004). RAPD-PCR can be used to amplify certain segment of a genome by using a short arbitrary primer (Nilsson et al., 1998). However, care must be taken when using RAPD-PCR, as several factor have been reported to affect the reproducibility of the method (Hilton et al., 1997)

The *M. punctatum* complex is a variable species. The results from anatomical, morphological and numerical studies suggested that there are likely eight taxa in this complex. There is a need for more information and for an in-depth investigation to clarify the taxonomic status of the taxa in the complex, and to infer phylogenetic relationship in the *M. punctatum* complex and its related taxa. For this reason, a RAPD technique was used as an objective.

5.2 Material and Methods

5.2.1 Plant materials

Eight taxa of the *M. punctatum* complex were used for RAPD-PCR analyses. Forty one samples from 15 localities were collected (Table 5.1).

5.2.2 DNA Extraction

Fresh leaves were used for DNA extraction by a modified method of Doyle and Doyle (1987) which uses CTAB (Cetyltrimethylammonium bromide) method (Dellaporta, et al. 1983). The following procedure had been used in this study:

A: For preparation, set the microcentrifuge tube filled with 500 µl of modified CTAB extaction buffer and, preheat CTAB buffer in water-bath to 65 °C

1. Add liquid nitrogen to a pestle containing 0.1g of leaf material and grind to powder with a mortar. After that, transfer powder to extraction buffer in a microcentrifuge tube, vertex 10 second, and incubate at 65 °C for 20-30 minutes, mixing every 10 minutes.
2. Add 500 µl of chloroform-isoamylalcohol mixture, invert gently 5 times and incubate by gently shaking at room temperature. Centrifuge at 10,000 rpm at 4 °C for 10 minutes.
3. Transfer the supernatant into a new microcentrifuge tube. Add 0.1 volume of 3M Sodium acetate, mix, then add 0.6 volume iced-cold (-20 °C) isopropanol, mix by inverting.
4. Let the tube stand at -20 °C for 30 minutes and centrifuge at 10,000 rpm at 4 °C for 10 minutes. Gently discard the supernatant (be careful not to disturb DNA pellet).
5. Add 500 µl of ice-cold 75% ethanol, gently invert the tube several times. Centrifuge at 10,000 rpm for 5 minutes, discard the supernatant and allow the pellet to air-dry.
6. Dissolve DNA in 200 µl of sterile TE buffer and store then DNA solution at -20 °C until use.

B: To increase the purity of DNA sample, optional steps for some samples are listed below:

1. Add 1 µl of RNAase (20 µg/ml in concentration) to digest RNA and incubate the solution at 37 °C for 30-60 minutes.
2. Add 200 µl of Phenol:Chloroform mixture, invert gently. Centrifuge at 10,000 rpm at 4 °C for 10 minutes.
3. Add 500 µl of Chloroform:Isoamylalcohol mixture, invert gently. Centrifuge at 10,000 rpm at 4 °C for 10 minutes. Then transfer supernatant into a new microcentrifuge tube.
4. Add 0.1 volume of 3M sodium Acetate, mix, then, add 2 volume iced-cold (-20 °C) absolute ethanol, invert gently and let the tube stand at -20 °C for 30 minutes. Centrifuge at 10,000 rpm at 4 °C for 10 minutes. Gently discard the supernatant (be careful not to disturb DNA pellet)
5. Add 500 µl of iced-cold 75 % ethanol, gently invert the tube several times and allow the pellet to air-dry.
6. Dissolve the DNA in 50-100 µl of sterile TE buffer and store DNA solution at -20 °C until use.

5.2.3 RAPD Analysis

Genetic variation at the molecular level among eight taxa of the *M. punctatum* complex and outgroup was detected by RAPD markers. The materials used in this analysis are shown in Table 5.1.

Screening of primers

Forty four primers of arbitrary ten-oligonucleotide sequences (Operon Technologies, Alameda and Genset Oligos) were screened for selecting appropriate primers which gave polymorphic, scorable, reproducible DNA product (bands).

Table 5.1 Sampling sites and numbers of DNA samples of *M. punctatum* complex and outgroup taxa used in RAPD analysis.

Species	Locality	No. of Individual	Abbre-viations
M. whiteheadii	1. Sumatra, Indonesia	6	W1-6
M. siamense	1. Bannang Sata District, Yala, Thailand	4	S1-4
M. thailandicum	1. Chumphon, Thailand	5	T1-5
	2. Na Yong District, Trang, Thailand	1	T6
M. membranaceum	1. Doi Inthanon National Park, Chiang Mai, Thailand	1	B1
	2. Suan Phueng District, Ratchaburi, Thailand	1	B2
M. musifolium	1. Waeng District, Naratiwat, Thailand	6	M1-6
M. punctatum	1. Khao Chong Waterfall, Trang, Thailand	2	P1-2
	2. Khoa Nan Peak, Nakhon Si Thammarat, Thailand	2	P3-4
	3. Na Yong District, Trang, Thailand	2	P5-6
	4. Mindanoa, Phillipines	4	PP1-4
	5. Suan Phueng District, Ratchaburi, Thailand	1	P7
	6. Ton Prae Tong Waterfall, Phatthalung, Thailand	1	P8
P. irioides	1.Ton Prae Tong Waterfall, Phatthalung, Thailand	3	I1-3
	3. Khao Chong Waterfall, Trang, Thailand	3	I4-6
M. punctatum cv. *grandicep*	1. Suan Luang District, Bangkok, Thailand	3	G1-3
	2. Kan Na Yao District, Bangkok, Thailand	2	G4-5
M. punctatum cv. *serratum*	1. Pathum Wan District, Bangkok, Thailand	2	E1-2
	2. Kan Na Yao District, Bangkok, Thailand	3	E3-5
Total		41	

RAPD-PCR condition and electrophoresis

Amplification was carried out in a 20 µl volume in 0.2 ml PCR microtube using a PTC-100 Peltier Thermal cycle. The reaction mixture contained 1.25 mM of dNTPs, 10x Taq DNA polymerase buffer (QIAGEN, Leusden, Netherlands), 50 mM $MgCl_2$, 2.5 picomole primer, 5 ng DNA, and 1 unit of Taq DNA Polymerase (QIAGEN, Leusden, Netherlands). The amplification cycle was performed as follows: initial 3 minutes at 94°C denaturation; 40 cycles of 0.1 minutes at 94°C, 0.2 minutes at 38°C annealing, 1 minutes at 72 °C; and 7 minutes at 72 °C extension. The amplified products were subsequently run on gel or stored at 4 °C overnight before electrophoresis.

Amplified fragments were separated in 1 % agarose gel using 1x TAE buffer and were visualized and photographed using a gel documentation analysis set (BIO RAD), after staining with Ethidium bromide.

Table 5.2 Twenty one RAPD primers, primer sequences and numbers of amplified loci per primer.

Primer Code	Nucleotide sequence	No. of amplified loci
OPS-1	5′ CTACTGCGCT 3′	9
OPS-2	5′ CCTCTGACTG 3′	10
OPS-7	5′ TCCGATGCTG 3′	10
OPS-9	5′ TCCTGGTCCC 3′	12
OPS-10	5′ ACCGTTCCAG 3′	7
OPS-11	5′ AGTCGGGTGG 3′	10
OPS-12	5′ CTGGGTGAGT 3′	12
OPS-14	5′ AAAGGGGTCC 3′	7
OPS-16	5′ AGGGGGTTCC 3′	6
OPS-17	5′ TGGGGACCAC 3′	12
OPS-18	5′ CTGGCGAACT 3′	11
OPS-20	5′ TCTGGACGGA 3′	15
UBC-718	5′ GGGAGAGGGA 3′	10
UBC-722	5′ CCTCTCCCTC 3′	19
UBC-729	5′ CCCAACCCAC 3′	13
UBC-731	5′ CCCACACCAC 3′	15
UBC-734	5′ GGAGAGGGAG 3′	10
UBC-742	5′ CCTCCCTCCT 3′	14
UBC-785	5′ CACCCAACCA 3′	13
GEN-867	5′ CCTGACTCTC 3′	18
Total		233

5.2.4 Data Analysis

Only clearly appearing bands of RAPD products were scored for presence. The presence or absence of bands was coded as binary (1, 0) data respectively. A SIMQUAL module which computes genetic similarity coefficients for qualitative data was calculated according to Dice (1945). The similarity matrix obtained was employed to find clusters by the Unweighted Pair Group Method using Arithmetic Averages (UPGMA), using the SAHN-clustering module, and a dendrogram was produced using the TREE programs all in NTSYS-pc, version 2.10m (Rohlf, 2000).

5.3 Results

5.3.1 RAPD Analysis

RAPD profile

Only 20 of 44 RAPD primers (Table 5.2) of arbitrary ten-oligonucleotide sequence screened were selected and used to analyses the genomic DNAs of 41 individual plants from various localities of *M. punctatum* complex (Table 5.2). These 20 primers generated 233 clear and reproducible bands (RAPD loci), varying in size from 0.25 to 3 kb. The number of bands per primer ranged from 6 (in OPS-16) to 19 (in UBC-722) with an average of 11.65 bands/primer. The number of polymorphic RAPD loci per species ranged from 1 to 12 (Table 5.3). Polymorphism in RAPD banding patterns among *M. punctatum* complex and outgroup was detected. The number of polymorphic RAPD loci per species ranged from 1 to 12 and given the percentage of polymorphic loci as 36.33-57.51 %(Table 5.3). The result also showed that there were no or only a slight polymorphism among populations of the same species.

Of all 233 different RAPD loci, 114-119 (48.93-51.07%) of *M. whiteheadii*, 104-108 (44.64-46.35%) of *M. siamense*, 115-122(49.36-52.36%) of *M. thailandicum*, 116-117(49.79-50.21%) of *M. membranaceum*, 102-105(43.78-45.06%) of *M. musifolium* were polymorphic. The percentage of polymorphic loci of *M. punctatum*, *M. punctatum* from Philippines, *M. punctatum* cv. *grandiceps*, and *M. punctatum* cv. *serratum* is 119-124(51.07-53.22%), 131-134(36.33-57.51%), 107-117(45.92-50.21%) and 112-121 (48.07-51.93%), respectively. Finally, in *P. irioides* 118-125 bands (50.64-53.65%) were detected (Table 5.3).

Table 5.3 Number and percentage of DNA polymorphism in the *M. punctatum* complex.

Primer code	No. of amplified loci (a)	Species	No. of polymorphic fragments (b)	% polymorphic (b/a) x 100
OPS-1	9	*M. whiteheadii*	8	88.89
		M. siamense	8	88.89
		M. thailandicum	5	55.56
		M. membranaceum	5	55.56
		M. musifolium	7	77.78
		M. punctatum	5	55.56
		P. irioides	6	66.67
		M. punctatum from Philippines	6	66.67
		M. punctatum cv. *grandicaps*	6	66.67
		M. punctatum cv. *serratum*	5	55.56
OPS-2	10	*M. whiteheadii*	8	80.00
		M. siamense	9	90.00
		M. thailandicum	9	90.00
		M. membranaceum	8	80.00
		M. musifolium	8-9	80.00-90.00
		M. punctatum	9-10	90.00-100.00
		P. irioides	9-10	90.00-100.00
		M. punctatum from Philippines	8	80.00
		M. punctatum cv. *grandicaps*	8	80.00
		M. punctatum cv. *serratum*	8	80.00
OPS-7	10	*M. whiteheadii*	4	40.00
		M. siamense	1	10.00
		M. thailandicum	2	20.00
		M. membranaceum	2-3	20.00-30.00
		M. musifolium	2-3	20.00-30.00
		M. punctatum	2	20.00
		P. irioides	2-3	20.00-30.00
		M. punctatum from Philippines	5	50.00
		M. punctatum cv. *grandicaps*	4-5	40.00-50.00
		M. punctatum cv. *serratum*	2-3	20.00-30.00
OPS-9	12	*M. whiteheadii*	2	16.67
		M. siamense	4-5	33.33-41.67
		M. thailandicum	5	41.56
		M. membranaceum	1	8.33
		M. musifolium	2	16.67
		M. punctatum	1	8.33
		P. irioides	4-5	33.33-41.67
		M. punctatum from Philippines	4	33.33
		M. punctatum cv. *grandicaps*	5-7	41.56-58.33
		M. punctatum cv. *serratum*	6-7	50.00-58.33

Table 5.3 (continued)

Primer code	No. of amplified loci (a)	Species	No. of polymorphic fragments (b)	% polymorphic (b/a) x 100
OPS-10	7	*M. whiteheadii*	6-7	85.71-100.00
		M. siamense	6	85.71
		M. thailandicum	7	100.00
		M. membranaceum	6	85.71
		M. musifolium	5	71.42
		M. punctatum	6-7	85.71-100.00
		P. irioides	7	100.00
		M. punctatum from Philippines	6-7	85.71-100.00
		M. punctatum cv. *grandicaps*	5	71.42
		M. punctatum cv. *serratum*	6	85.71
OPS-11	10	*M. whiteheadii*	5	50.00
		M. siamense	1	10.00
		M. thailandicum	4-5	40.00-50.00
		M. membranaceum	4	40.00
		M. musifolium	2	20.00
		M. punctatum	4	40.00
		P. irioides	5	50.00
		M. punctatum from Philippines	4-5	40.00-50.00
		M. punctatum cv. *grandicaps*	3-4	30.00-40.00
		M. punctatum cv. *serratum*	3-4	30.00-40.00
OPS-12	12	*M. whiteheadii*	5	41.67
		M. siamense	5-6	41.67-50.00
		M. thailandicum	2	16.67
		M. membranaceum	9	75.00
		M. musifolium	4	33.33
		M. punctatum	5-6	41.67-50.00
		P. irioides	5-6	41.67-50.00
		M. punctatum from Philippines	6	50.00
		M. punctatum cv. *grandicaps*	3-4	25.00-33.33
		M. punctatum cv. *serratum*	3-5	25.00-41.67
OPS-14	7	*M. whiteheadii*	2	28.57
		M. siamense	2	28.57
		M. thailandicum	4	57.14
		M. membranaceum	3	42.85
		M. musifolium	2	28.57
		M. punctatum	4	57.14
		P. irioides	3	42.85
		M. punctatum from Philippines	2	28.57
		M. punctatum cv. *grandicaps*	3	42.85
		M. punctatum cv. *serratum*	2-3	28.57-42.85

Table 5.3 (continued)

Primer code	No. of amplified loci (a)	Species	No. of polymorphic fragments (b)	% polymorphic (b/a) x 100
OPS-16	6	*M. whiteheadii*	1	16.67
		M. siamense	1	16.67
		M. thailandicum	2	33.33
		M. membranaceum	2	33.33
		M. musifolium	2	33.33
		M. punctatum	3	50.00
		P. irioides	3	50.00
		M. punctatum from Philippines	3	50.00
		M. punctatum cv. *grandicaps*	3	50.00
		M. punctatum cv. *serratum*	2	33.33
OPS-17	12	*M. whiteheadii*	4	33.33
		M. siamense	4	33.33
		M. thailandicum	3	25.00
		M. membranaceum	3-4	25.00-33.33
		M. musifolium	3	25.00
		M. punctatum	4-5	33.33-41.67
		P. irioides	3	25.00
		M. punctatum from Philippines	5	41.67
		M. punctatum cv. *grandicaps*	3	25.00
		M. punctatum cv. *serratum*	3	25.00
OPS-18	11	*M. whiteheadii*	8-9	72.73-81.82
		M. siamense	9	81.82
		M. thailandicum	8	72.73
		M. membranaceum	8	72.73
		M. musifolium	9	81.82
		M. punctatum	8-9	72.73-81.82
		P. irioides	8-10	81.82-90.91
		M. punctatum from Philippines	8	72.73
		M. punctatum cv. *grandicaps*	8	72.73
		M. punctatum cv. *serratum*	8	72.73
OPS-20	15	*M. whiteheadii*	7	46.67
		M. siamense	5	33.33
		M. thailandicum	5	33.33
		M. membranaceum	3	20.00
		M. musifolium	3	20.00
		M. punctatum	6	40.00
		P. irioides	4-5	26.66-33.33
		M. punctatum from Philippines	9	60.00
		M. punctatum cv. *grandicaps*	10	66.67
		M. punctatum cv. *serratum*	8	53.33

Table 5.3 (continued)

Primer code	No. of amplified loci (a)	Species	No. of polymorphic fragments (b)	% polymorphic (b/a) x 100
UBC-718	10	*M. whiteheadii*	3	30.00
		M. siamense	3	30.00
		M. thailandicum	3	30.00
		M. membranaceum	4	40.00
		M. musifolium	5	50.00
		M. punctatum	5	50.00
		P. irioides	5	50.00
		M. punctatum from Philippines	5	50.00
		M. punctatum cv. *grandicaps*	6-7	60.00-70.00
		M. punctatum cv. *serratum*	5-6	50.00-60.00
UBC-722	19	*M. whiteheadii*	7	36.84
		M. siamense	5	26.31
		M. thailandicum	4	21.05
		M. membranaceum	10	52.63
		M. musifolium	8	42.10
		M. punctatum	7	36.84
		P. irioides	5-6	26.32-31.58
		M. punctatum from Philippines	9	47.37
		M. punctatum cv. *grandicaps*	4	21.05
		M. punctatum cv. *serratum*	7-8	36.84-42.10
UBC-729	13	*M. whiteheadii*	6	46.15
		M. siamense	7	53.85
		M. thailandicum	11	84.62
		M. membranaceum	7	53.85
		M. musifolium	9	69.23
		M. punctatum	9	69.23
		P. irioides	8	61.54
		M. punctatum from Philippines	8	61.54
		M. punctatum cv. *grandicaps*	6-8	46.15-61.54
		M. punctatum cv. *serratum*	8-9	61.54-69.23
UBC-731	15	*M. whiteheadii*	10	66.67
		M. siamense	10	66.67
		M. thailandicum	8	53.33
		M. membranaceum	10	66.67
		M. musifolium	10	66.67
		M. punctatum	12	80.00
		P. irioides	9-10	60.00-66.67
		M. punctatum from Philippines	7	46.67
		M. punctatum cv. *grandicaps*	6-9	40.00-60.00
		M. punctatum cv. *serratum*	8-10	53.33-66.67

Table 5.3 (continued)

Primer code	No. of amplified loci (a)	Species	No. of polymorphic fragments (b)	% polymorphic (b/a) x 100
UBC-734	10	*M. whiteheadii*	5	50.00
		M. siamense	2	20.00
		M. thailandicum	5-6	50.00-60.00
		M. membranaceum	4	40.00
		M. musifolium	3	30.00
		M. punctatum	4	40.00
		P. irioides	4	40.00
		M. punctatum from Philippines	4	40.00
		M. punctatum cv. *grandicaps*	4	40.00
		M. punctatum cv. *serratum*	4	40.00
UBC-742	14	*M. whiteheadii*	10	71.43
		M. siamense	10	71.43
		M. thailandicum	9-11	64.29-78.57
		M. membranaceum	9	64.29
		M. musifolium	8	57.14
		M. punctatum	9	64.29
		P. irioides	7-8	50.00-57.14
		M. punctatum from Philippines	12	85.71
		M. punctatum cv. *grandicaps*	5-6	35.71-42.86
		M. punctatum cv. *serratum*	8-9	57.14-64.29
UBC-785	13	*M. whiteheadii*	10	75.92
		M. siamense	11	84.62
		M. thailandicum	11	84.62
		M. membranaceum	8	61.54
		M. musifolium	9	69.23
		M. punctatum	11	84.62
		P. irioides	11	84.62
		M. punctatum from Philippines	12	92.31
		M. punctatum cv. *grandicaps*	7	53.85
		M. punctatum cv. *serratum*	12	92.31
GEN-867	18	*M. whiteheadii*	3	16.67
		M. siamense	2	11.11
		M. thailandicum	9	50.00
		M. membranaceum	9	50.00
		M. musifolium	2	11.11
		M. punctatum	5	27.78
		P. irioides	6-7	33.33-38.89
		M. punctatum from Philippines	8	44.44
		M. punctatum cv. *grandicaps*	9	50.00
		M. punctatum cv. *serratum*	6	33.33

Table 5.3 (continued)

Primer code	No. of amplified loci (a)	Species	No. of polymorphic fragments (b)	% polymorphic (b/a) x 100
Total	233	*M. whiteheadii*	114-119	48.93-51.07
		M. siamense	104-108	44.64-46.35
		M. thailandicum	115-122	49.36-52.36
		M. membranaceum	116-117	49.79-50.21
		M. musifolium	102-105	43.78-45.06
		M. punctatum	119-124	51.07-53.22
		P. irioides	118-125	50.64-53.65
		M. punctatum from Philippines	131-134	36.33-57.51
		M. punctatum cv. *grandicaps*	107-117	45.92-50.21
		M. punctatum cv. *serratum*	112-121	48.07-51.93

Phenetic Analysis of RAPD data

For cluster analysis a total 233 RAPD markers were included. The UPGMA dendrogram derived from Dice's (1945) similarity coefficient splitted the 41 specimens into six groups (Fig. 5.2) at 0.50 phenon level. Specimens classified as group 1 composed of *M. punctatum P. irioides M. punctatum* cv. *grandicaps* and *M. punctatum* cv. *serratum*. Specimens classified as group 2, 3, 4, 5, and 6 are *M. whiteheadii*, *M. siamense*, *M. membranaceum*, *M. thailandicum*, and *M. musifolium*, respectively.

The dendrogram demonstrates that two cultivars of *M. punctatum*, *M. punctatum* cv. *grandiceps* and *M. punctatum* cv. *serratum*, are distinct from *M. punctatum* and its synonyms though they have very closely relationship. Furthermore, *M. musifolium*, one of synonym of *M. punctatum* according to Nooteboom's system (1997), is clearly separated from *M. punctatum*. The taxon *M. membranaceum* which was pointed out that it might be a variety of *M. punctatum* by Nooteboom (1997), exhibited a genetic similarity with *M. siamese*, *M. whiteheadii* and *M. thailandicum*. Thus, *M. punctatum* and its synonym, included *M. musifolium* and *M. membranaceum*, is not a well defined group according to the RAPD data.

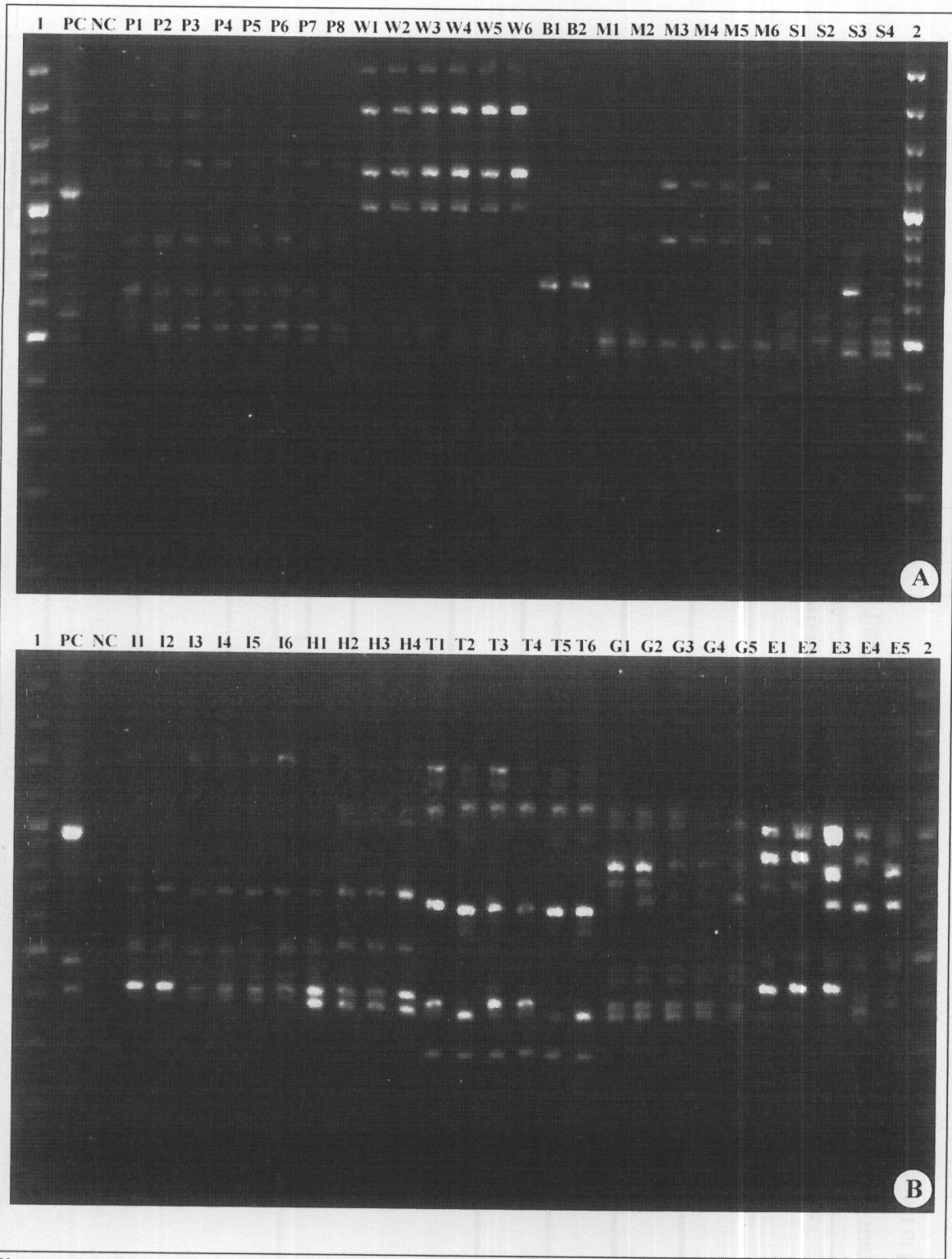

Figure 5.1 Examples of RAPD band profiles of the *M. punctatum* complex operated by primer OPS-20. Lane 1 and lane 2 (in A and B) are 1 kb DNA ladder. The abbreviation above each picture indicated populations of each species according to Table 5.1. The alphabet 'PC' and 'NC' is positive and negative control.

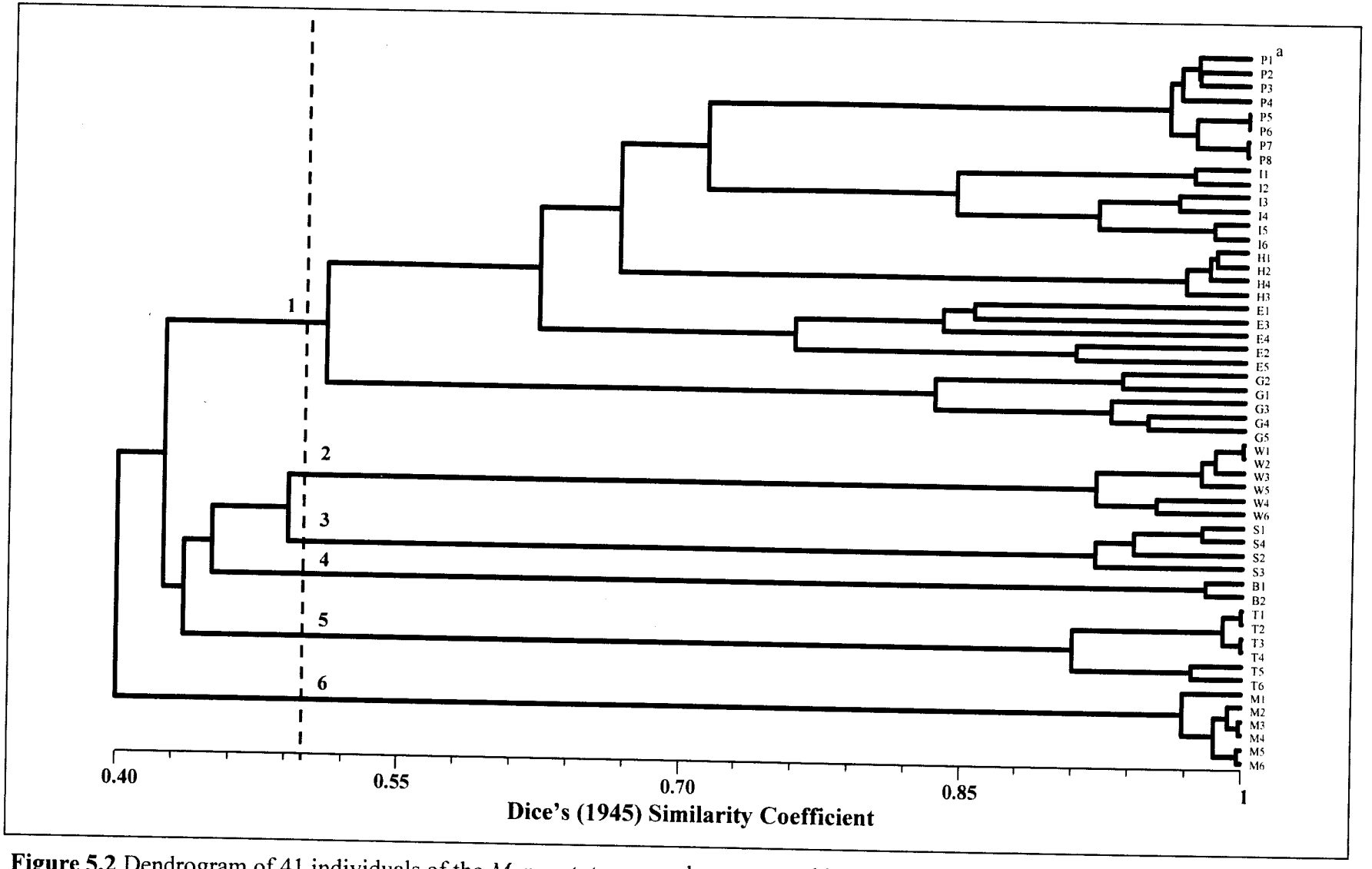

Figure 5.2 Dendrogram of 41 individuals of the *M. punctatum* complex generated by cluster analysis using UPGMA based on RAPD. (a. numbers correspond to those in Table 5.1).

5.4 Discussion and Conclusion

It can be seen (Fig. 4.1, 4.2 and 5.2) that both results from numerical and RAPD studies do not agree with the previous taxonomic classification of *M. punctatum* and its related taxa. Specimens classified as group 1 are similar to the result from cluster analysis of morphometric study.

Although *M. glossophyllum* and *M. steerei* were not included into this experiment due to technical limitation, conclusive results were obtained. The result from molecular analyses (RAPD-PCR) was congruent with both general morphological, anatomical and morphometric analyses. From UPGMA dendrogram derived from Dice's (1945) similarity coefficient in RAPD analysis, it can be seen that *M. punctatum* complex grouped into three groups viz. *M. punctatum* group, *M. thailandicum* group, and the last group comprised only one taxon, *M. musifolium*. The dendrogram also supported the genetically similarity between two locality of *M. punctatum*, from Thailand (P1-8) and Philippines (H1-4), and its synonym – *Polypodium irioides* (I1-6). The latter taxa were placed between *M. punctatum* from Thailand and Philippines (Fig 5.2). The result suggested that they are not significantly different genetically although from different locations. This finding corresponded with the results from the morphological study that pointed out that *P. irioides* is very similar to *M. punctatum* in many characters. It is reasonable to treat *P. irioides* as a synonym of *M. punctatum.*

Two cultivars of *M. punctatum*, *M. punctatum* cv. *grandiceps* (G1-5) and *M. punctatum* cv. *serratum* (E1-5), were placed in the same group with *M. punctatum*. It looks like *M. punctatum* cv. *serratum* have more genetic similarity with *M. punctatum* than *M. punctatum* cv. *grandiceps* (Fig 5.2). In addition, the morphological revealed that *M. punctatum* cv. *serratum* have same diagnostic characters with *M. punctatum* more than *M. punctatum* cv. *grandiceps*. Though these two varieties are close to *M. punctatum*, they still separate into independent subgroups. It thus makes sense to treat these two taxa as cultivars as in Nooteboom's taxonomic system.

Nooteboom published a classification system of microsoroid ferns in 1997. In this system, he included *M. musifolium* as synonym of *M. punctatum*. In contrast, the result from RAPD analysis agreed with both of the morphological and numerical studies that

separated *M. musifolium* from *M. punctatum*. This study did not support the previous classification of Nooteboom (1997) who proposed placing *M. musifolium* as synonym of *M. punctatum*. Here, *M. musifolium* is treated as a separate taxon following the classification system of Bosman (1991).

In the second group, *M. whiteheadii, M. siamense, M. membranaceum,* and *M. thailandicum*, displayed a close genetic relationships. They can be distinguished from each other at 0.54 of average taxonomic distance. It makes sense to propose these four taxa as independent species following the taxonomic classification system of Boonkerd (2006), Boonkerd, and Nooteboom (2001) and Smith and Hoshizaki (2000).

The characteristic of undulate frond margin, the diagnostic character to distinguish *P. irioides*, can be found in transplanted plants of *M. punctatum* preserved their anatomical, morphological and molecular characteristics in the greenhouse in dry season. The morphological difference due to stress was reported in many times (Tenhunen et al., 1985; Chen and Chen, 2005; Reudink et al. 2005; Masood and Abraham, 2006). So, it made sense to propose the hypothesis that this morphological difference might be due to stress.

The results from this study revealed that genetic variation can be detected in the same taxon that grows in different localities such as *M. punctatum* collected from Thailand and Philippines. The later plants have differed in some morphological character with the former such as fronds size and color. Frond of Philippines plants is thinner and dark green though reach sunlight in open areas, while *M. punctatum* collected from Thailand have thicker and light green fronds in same condition. This study and morphometric analyses indicated that *M. punctatum* collected in Thailand and Philippines are quite morphologically different from each other was supported by molecular data. It can be concluded that the morphological difference among taxa might come from the genetic variation (Paris, 1989; Li and Haufler, 1999).

Finally, according to RAPD data, the *M. punctatum* complex should be divided into independent taxa namely *M. whiteheadii, M. siamense, M. musifolium, M. membranaceum, M. thailandicum*, and *M. punctatum*; and also recognize two cultivars, *M. punctatum* cv. *serratum* and *M. punctatum* cv. *grandiceps*.

CHAPTER 6

PHYLOGENETIC STUDY

6.1 Introduction

Recent studies in fern systematics have improved not only our understanding in the relationships of ferns (Hasebe et al., 1995; Pryer et al., 1995 and Pryer et al., 2001) but also the phylogenetic relationships within major groups of derived ferns (Schneider , 2003). Unraveling the phylogeny of major derived fern groups, such as asplenioid, dryopteroid, and polygrammoid ferns, is of particular interest because these ferns are important components of current tropical vegetation. Comparisons of the phylogenetic patterns of ferns and angiosperms are a crucial component in our efforts to understand the evolution of modern floras. Polygrammoid ferns are not only one of the most species-rich groups of ferns but they also stand out among vascular plants as one of the most diverse and abundant groups of vascular epiphytes in tropical and subtropical forests, particularly in the paleotropics (Benzing, 1990).

Polypodiaceae sensu Smith et al. (2006) are a monophyletic lineage of leptosporangiate ferns comprising the Grammitidaceae and Polypodiaceae sensu Kubitzki (1990), for which the temporary name "polygrammoid ferns" was employed until recently. Polypodiaceae are a pantropical fern family, adapted to diverse ecological conditions (dry or wet, epiphytic or terrestrial habitats), and show a diverse range of global distribution patterns.

So far, microsoroids and grammitids are the most taxonomically difficult groups in Polypodiaceae (Schneider et al., 2004). Microsoroid ferns, with approximately 15 species, are the second most species rich representatives of the polygrammoid clade in the African region. The microsoroid lineage has its diversity center in S.E. Asia and is absent from the neotropics (except for *M. scolopendrium* (Burm. f.) Copel., which is introduced). Several species also occur in or are endemic to the African region (Janssen et al., 2007).

Bosman (1991) and Janssen et al. (2007) have proposed the phylogenetic hypothesis based on morphological and molecular data for the microsoroid fern. Bosman (1991) pointed out that their are four monophyletic subgroups in microsoroid fern based

on morphological data. The first subgroup composed of *M. glossophyllum*, *M. punctatum* and *M. steerei*. While *M. musifolium* and *M. spectrum* formed sister group for each other and belonged to subgroup two. The third subgroup consisted of *M. membranaceum*, *M. lastii* and *M. leandrianum*. In contrast, Janssen et al. (2007) stated that *M. musifolium* is sister group of *M. punctatum* with 100% bootstrap support in phylogenetic study using molecular data from DNA sequencing. The result additively showed that *M. membranaceum* and *M. punctatum* united in a polytomy.

6.2 Materials and Methods

Data Set

Data sets were compiled from 21 taxa of the complex (Table 4.1) from the following herbaria BCU, BKF, BM, K, B, L and P. The followings are characters scoring for morphological data matrix.

1. Appearance of rhizome surface:
   (0) not waxy
   (1) at least sometimes waxy under the scales or often waxy appearance of rhizome surface
2. Roots density:
   (0) more or less high
   (1) low
3. Differentiation of vascular bundle sheaths:
   (0) vascular bundle sheaths with parenchymatous tissue
   (1) vascular bundle sheaths with collenchymatous or occasionally partly sclerenchymatous tissue
   (2) exclusively sclerenchymatous tissue
4. Number of sclerenchyma strands:
   (0) < 100
   (1) ≥ 100
5. Type of attachment of scales:
   (0) pseudopeltate
   (1) peltate
6. Density of scales:
   (0) densely set

(1) apically densely set

7. Scale arrangement:

(0) appressed

(1) slightly spreading

(2) distinctly spreading

(3) erect

8. Scales margin:

(0) entire

(1) dentate to denticulate

9. Presence of hyaline marginal region on rhizome scales:

(0) absent

(1) present

10. Scales shape (average index):

(0) < 2.5

(1) $\geq 2.5 < 3.0$

(2) $\geq 3.0 < 3.5$

(3) ≥ 3.5

11. Indumentum type of central region of scales:

(0) dark black on central region or glabrous

(1) bearing multiseptate hairs at least when young

(2) long unicellular hairs

12. Scales translucence:

(0) opaque and blackish

(1) translucent and brownish

13. Phyllopodia distinctness:

(0) distinct

(1) obscure

14. Frond type of dissection:

(0) simple

(1) pinnatatifid

15. Frond color when living:

(0) light to dark green

(1) iridescent blue-green

16. Stipe character:
    (0) winged for considerable part or not winged
    (1) narrowly winged
17. Presence of indumentum:
    (0) with only scales
    (1) with a few scales and short glandular hairs
    (2) with only short glandular hairs
    (3) glabrous
18. Presence of stipe:
    (0) present
    (1) absent or obscure
19. Frond shape:
    (0) linear
    (1) elliptic
    (2) obovate
    (3) ovate
20. Margin of lamina:
    (0) entire
    (1) entire-undulate
    (2) serrate
21. Midrib character:
    (0) slightly raised or raised on both surface
    (1) raised on lower surface, grooved on upper surface
22. Venation general pattern:
    (0) all veins forming a mesh of areoles
    (1) areoles 1- or 2-serial, second row often with 2 or 3 included veinlet
    (2) the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger, areoles in a row between two veins
    (3) connecting veins forming a row of about equally sized areoles between two adjacent veins and no prominent veinlet situated parallel to the veins
23. Visibility of veins:
    (0) all veins distinct

(1) all veins indistinct or secondary and smaller veins more or less immersed and vague (at least in living specimen)

24. Branching of included free veinlet:

(0) free veinlet recurrent

(1) free veinlet simple and once-forked

(2) free veinlet simple, once or twice forked

25. Visibility of hydathodes:

(0) distinct

(1) indistinct

26. Position of sori to surface of lamina:

(0) superficial

(1) slightly sunken

27. Sori limitation to the apical part of the lamina:

(0) absent or sometimes present

(1) always present

28. Sori shape:

(0) round

(1) slightly round or elongate

29. Sori distribution pattern:

(0) uniseries

(1) forming into 2-4 irregular rows parallel to each pair of secondary veins

(2) forming more than 3-10 (-15) irregular rows parallel to each pair of secondary veins

(3) mostly irregularly scattered on simple free or on 2 or 3 connecting veins

30. Presence of sori in the very marginal areoles:

(0) never present

(1) sometimes present

31. Presence of sori in the costal areoles or on their bordering veins:

(0) absent in costal areoles

(1) sometimes present in costal areoles

(2) always present in costal areoles

32. Spore surface:
    (0) cristate
    (1) plain to slightly verrucate
    (2) irregularly rugate

Phylogenetic tree construction

The maximum parsimony analyses (MP) have been conducted using PAUP* 4.0b10 (Swofford, 2000) with the following search strategy: heuristic MP analyses with 1,000 random taxon-addition replicates with TBR branch swapping (to completion), holding all most-parsimonious trees in memory. Both equal and unequal weighted parsimony analyses were performed with unordered characters. Unequal weighted MP analyses employed a weighting scheme generated for each data set based on empirical observation of relative observed nucleotide substitutions. In searches where more than one tree was recovered, we summarized the results using strict consensus methods implemented in PAUP* 4.0b10. Relative support for recovered clades was calculated using non-parametric bootstrap methods (Felsenstein, 1985) based on heuristic bootstrap searches implemented in PAUP* 4.0b10, 1,000 bootstrap replicates each based on a heuristic random addition search with 10 replicates, TBR swapping, and holding all trees in memory. Results were summarized as majority rule consensus trees if more than one tree was found. Two species of *Goniophlebium subauriculatum* (Blume) Presl and *Lepisorus longifolius* (Blume) Holtt. were selected to be outgroup of the *M. punctatum* complex (Kreier and Schneider, 2006; Janssen et al., 2007).

6.3 Result

Phylogenetic tree

The parsimony analysis resulted in 19 equally most parsimonious trees, with a length of 102 steps, Consistency index (CI) = 0.4902, Homoplasy index (HI) = 0.5098, CI excluding uninformative characters = 0.4694, HI excluding uninformative characters = 0.5306, Retention index (RI) = 0.5806, Rescaled consistency index (RC) = 0.2846. Four topologies could be recognized (Fig. 6.1 and 6.2). All of these four topologies yield similar four clades included clade I (*M. siamense*, *M thailandicum*), clade II (*M.*

glossophyllum, *Pleopeltis megalosoides*), Clade III (*M. punctatum* and 10 synonym), and clade IV (M. steerei and 2 synonym, sometime *M. whiteheadii*).

The differences between these four topologies are the early branching clade or the basal most clade and relationship among clades mention above. First topology showed (Fig. 6.1A) clade I was the basal most clade and *M. whiteheadii* was the second basal most clade. Whiles *M. whiteheadii* was sister to clade IV in topology 2 (Fig. 6.1B). In addition, *M. musifolium* formed as sister to *M. membranaceu*m and was not the basal most position in both topology 1 and topology 2. In contrast, *M. membranaceu*m and *M. musifolium* are not sister taxa and are basal most position in topology 3 and 4 (Fig. 6.2a and 6.2B). The second basal most clade of these two topologies was clade II. *M. whiteheadii* was sister to clade IV in topology 4 (Fig. 6.2B) or clade I in topology 4 (Fig. 6.2B)

The majority rule consensus tree was built and shown as Figure 6.3. *M. membranaceu*m and *M. musifolium* are also not sister taxa and are basal most positioning the majority rule consensus tree with 53% bootstrap support. All clades as mention before (clade I-IV) still recognized with generally high bootstrap support. Clade I consisted of two independent taxa, *M. siamense* and *M thailandicum*. Clade II, *M. glossophyllum* was sister to its synonym, *Pleopeltis megasoides*. Clade III comprised of *M. punctatum* and 10 synonyms. The last clade, clade IV composed of *P. playfairii* was sister to *M. steerei-P. tonkinense* clade. Specially, *M. whiteheadii* formed polytomy to clade I-IV. The monophyly of ingroup in all of these topologies was supported by only one character.

The strict consensus (Fig. 6.4) formed polytomy with 3 recognizable clades (moderate to high bootstrap support). The first clade (63% bootstrap support), *M. whiteheadii* was the first taxon to diverse, followed by *M. steerei-P. tonkinense-P. playfairii* clade. In that clade, *P. playfairii* was basal most position with 78% bootstrap support while *M. steerei* was sister to *P. tonkinense* with 60% bootstrap support. The second clade (71% bootstrap support) consisted of two independent taxa, *M. siamense* and *M. thailandicum*. The last clade (93% bootstrap support) was of *M. glossophyllum* and its synonym, *Pleopeltis megalosoroides*.

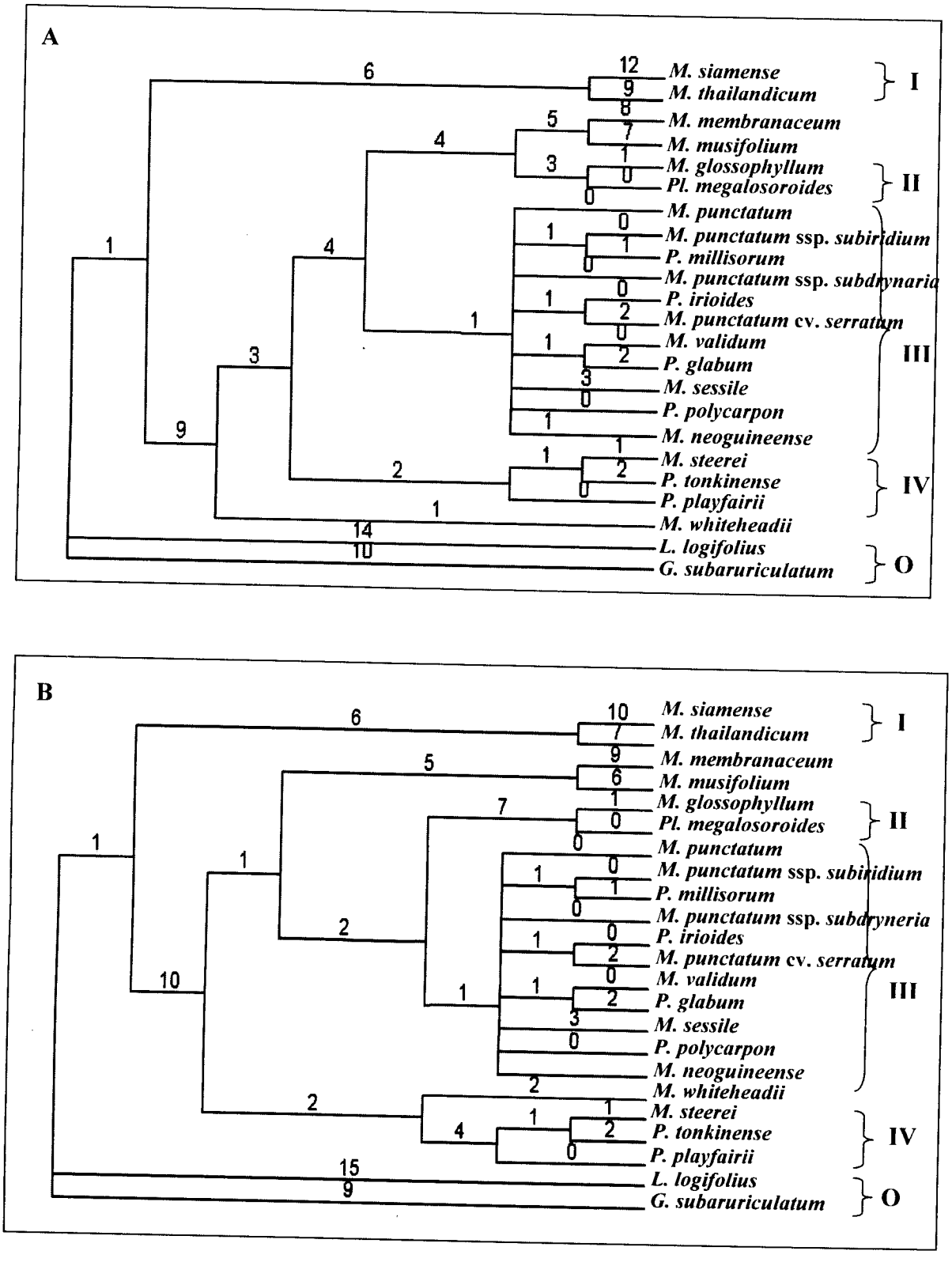

Figure 6.1 The first two topologies of the consensus trees of "the *M. punctatum* complex" base on morphological characters. The clade indicated by "O" was outgroups. I, II, III and IV were groups for discussion. Number was branch-length.

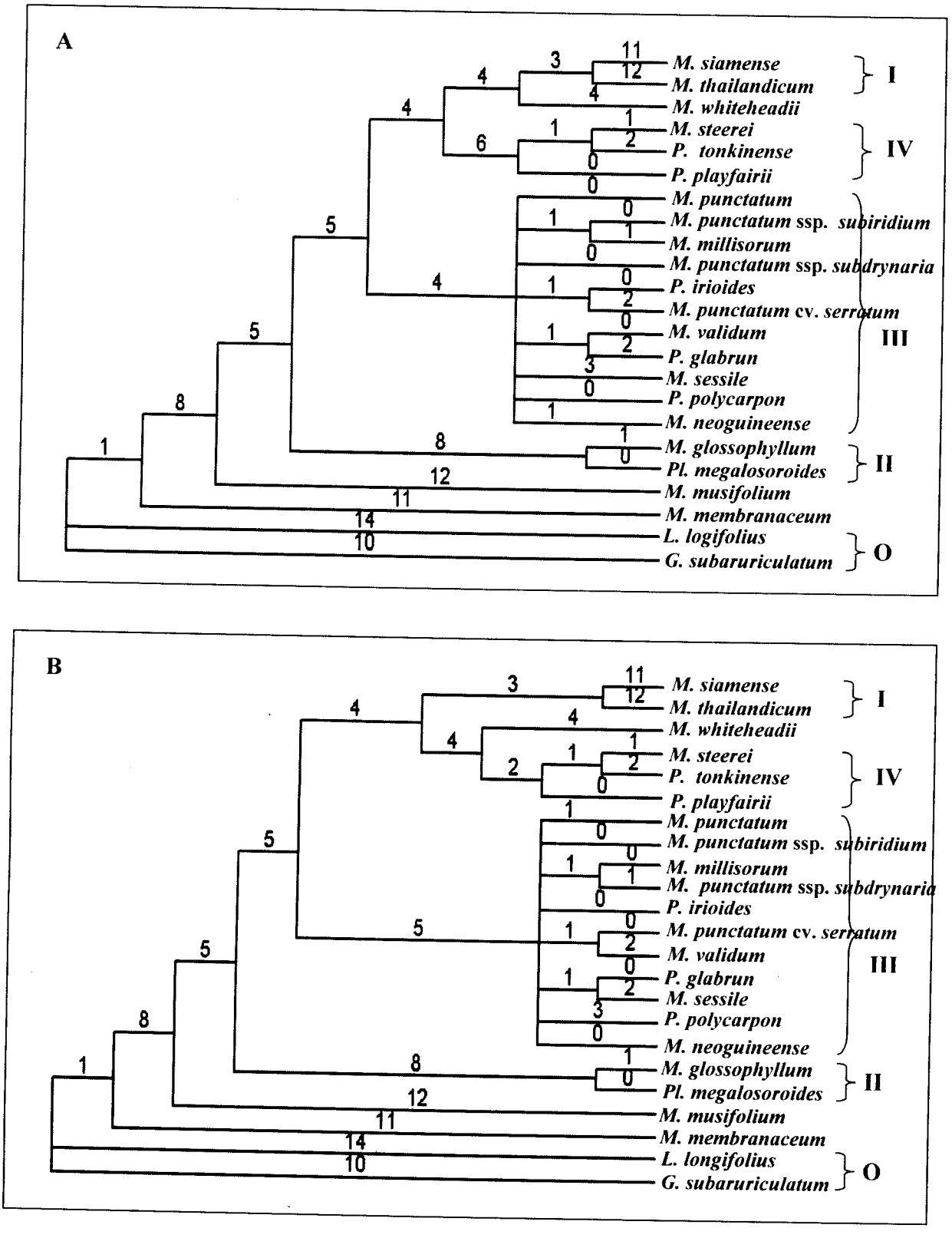

Figure 6.2 The last two topologies of the consensus trees of "the *M. punctatum* complex" base on morphological characters. The clade indicated by "O" was outgroups. I, II, III and IV were groups for discussion. Number was branch length.

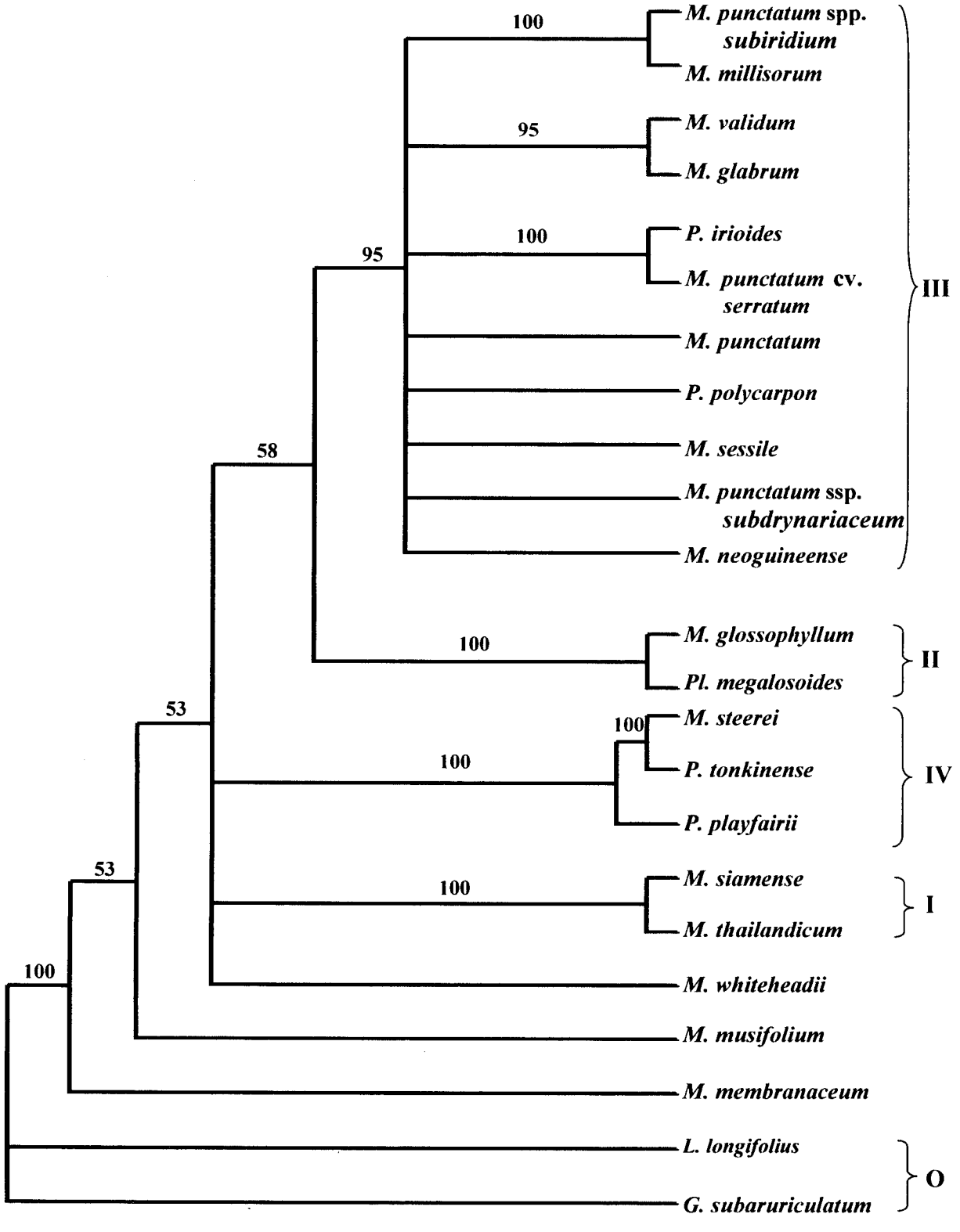

Figure 6.3 The majority rule consensus tree of "the *M. punctatum* complex" base on morphological characters. The clade indicated by "O" was outgroups. Number was majority rule 50%.

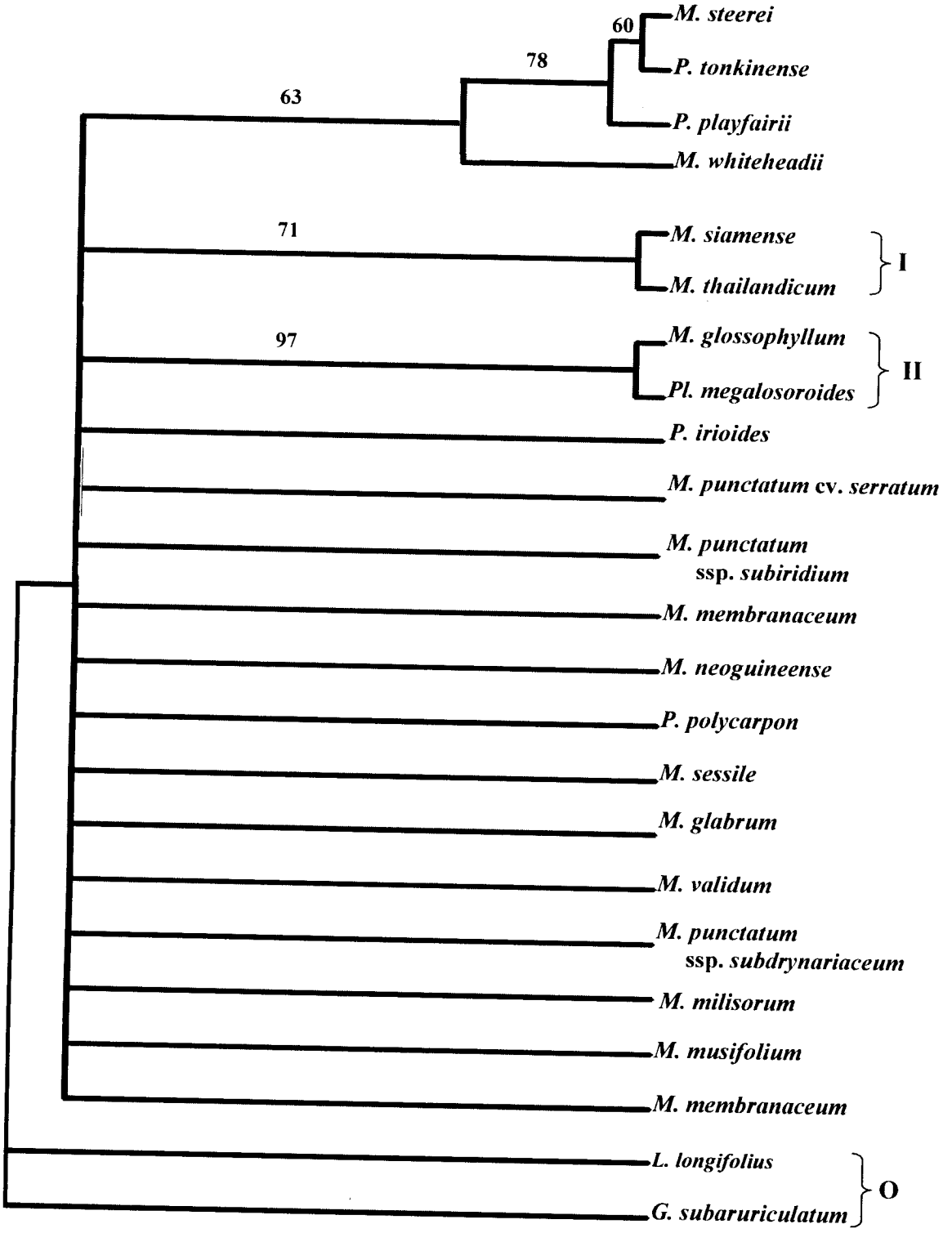

Figure 6.4 The strict consensus tree of "the *M. punctatum* complex" base on morphological characters. The clade indicated by "O" was outgroups. Number was bootstrap value from 100 replicates.

6.4 Discussion and Conclusion

The phylogenetic trees from morphological and anatomical data set showed little resolution in the consensus trees due to ambiguous characters support. The relationships among taxa and clades may not fully be inferred. The two early branching positions on the majority rule consensus tree were *M. membranaceum* and *M. musifolium* though they formed polytomy in strict consensus trees.

The result of this study also corresponded to Bosman (1991) who studied in this genus from the result of cladistic analysis base on morphological data matrix of genus *Microsorum* using three well-know computer program namely CAFA, PAUP, and HENNIG86. She proposed three monophyletic subgroups viz. A) *M. glossophyllum, M. punctatum* and *M. steerei*, B) *M. musifolium*, and C) *M. membranaceum*. Four clades from morphological phylogeny were reliable from the majority rule consensus tree in this study. *M. membranaceum* and *M. musifolium* were at the most basal position of monophyletic clade of *M. glossophyllum, M. punctatum* and *M. steerei*. In addition, *M. siamense* and *M. thailandicum*, a described species from southern Thailand, were culmed in this monophyletic clade like *M. whiteheadii*, described species from Java, Indonesia.

Clade I showed the relationship of two independent taxa. *M. siamense* was sister to *M. thailandicum* with highly bootstrap support (71%) in strict consensus tree. Its relationships might be reliable due to having shared some morphological characters, i.e. iridescent blue color in living fronds, narrowly winged stipe, and presence of uniseriate paraphyses in sori. Both of them are lithophytic plant on limestone. This finding agreed with Boonkerd (2006) who noted that *M. thailandicum* is very closely related to *M. siamense* but differences in its smaller fronds blade, number of sporangium annulus cell and character of hydathodes. Moreover, Boonkerd and Nooteboom (2001) also pointed out that *M. thailandicum* differs from *M. punctatum* and *M. steerei* in the iridescent blueish fronds and in sporangium annulus having more cells.

Clade II was always appeared in all trees. This clade also received 97% bootstrap support in consensus tree. Therefore, this clade might be reliable. *M. glossophyllum* was very closely related to *Pleopeltis megalosoroides* with most morphological characters except in only rhizome scale index character.

Clade III consisted of 11 taxa of *M. punctatum* and 10 synonyms followed previous classification system (Bosman, 1991; Nooteboom, 1997). This finding agreed with Bosman et al. (1998) that *M. punctatum* was variable species due to its great variation in morphological characters. The possible morphological synapomorphies of this clade are many morphological characters, i.e. differentiation of vascular bundle sheaths, phyllopodia distinctness etc.

Clade IV was always appeared in all trees like clade II. This clade showed 78% bootstrap support in strict consensus tree. Thus this clade might be reliable. The possible morphological synapomorphies of this clade are appearance of rhizome surface, density and spreading of scales, presence of indumenta and sori limitation to the apical part of the lamina. Moreover, M. steerei was more closely related to *P. tonkinense* than *P. playfairii* due to their sharing some common morphological character, i.e. fronds shape, sori position to surface of lamina.

The result of phylogenic study agreed with morphological and morphometric studies that *M. glossophyllum* is a distinct species from *M. punctatum*. This finding is not in agreement with Nooteboom (1997) classification of the microsoroid ferns in reducing *M. glossophyllum* to synonym of *M. punctatum*.

In many aspects, however, these results seem to agree with Bosman (1991), Bosman et al. (1998), Smith and Hoshizaki (2000), Boonkerd and Nooteboom (2001), Boonkerd (2006) view of microsoroid ferns classification to separated *M. whiteheadii, M. thailandicum*, and *M. siamense* from *M. punctatum*.

CHAPTER 7

GENERAL CONCLUSION

The *Microsorum punctatum* (L.) Copel. complex (Polypodiaceae) is a common species which has a widespread distribution in various forest types of temperate and tropical parts of the Old World. It was found that the taxonomic status of this species is still dubious due to its great variations in frond-form, sizes, and venation patterns. Because of these this plant group was called as a species complex (Bosman, 1991; Nooteboom, 1998). The finding of additional new species in this plant group (Smith & Hoshizaki, 2000; Boonkerd and Nooteboom 2001; Boonkerd, 2006) made doubtful taxonomic relationships among these species. In this research, *M. punctatum* (L.) Copel. complex is represented by 21 taxa according to Nooteboom (1997), Boonkerd and Nooteboom (2001), and Boonkerd (2006). To solve this problem morphological, anatomical, molecular studies as well as morphometric analyses were carried out.

It is evident that eight species, namely *M. glossophyllum*, *M. siamense*, *M. thailandicum*, *M. membranaceum*, *M. musifolium*, *M. punctatum*, *M. steerei*, and *M. whiteheadii* are members of the *M. punctatum* (L.) Copel. complex based on the morphological and anatomical studies. The following characters, i.e. frond color, stipe, venation pattern, rhizome scale and habitat are taxonomically useful to distinguish these taxa.

Two techniques of numerical taxonomy were used to investigate the variation of morphological and anatomical characters of taxa in the *M. punctatum* complex based on both qualitative and quantitative characters. The results suggested that *M. membranaceum*, *M. siamense*, and *M. thailandicum* are distinct taxa. Likewise, *M. musifolium* and *M. glossophyllum*, which were previously treated as synonyms of *M. punctatum* (L.) Copel. by Nooteboom (1997), are additional two distinct species based on the results of morphometric analyses. In contrast, it was found that *M. whiteheadii* and *M. steerei* are rather close and should not recognize as distinct species. These findings corresponds to the results obtained from morphological studies that *M. whiteheadii* and *M. steerei* are similar, but differ in some features, such as character of general venation pattern, scale arrangement, density of roots on rhizomes, and rhizome characters.

Geographically speaking, *M. steerei* and *M. whiteheadii* are separated (Bosman, 1991; Nooteboom, 1997; Smith & Hoshizaki, 2000), but should be treated as conspecific taxa. Because of this finding, it seems appropriate to treat these taxa as infraspecific taxa of *M. steerei* viz. *M. steerei* var. *steerei* and *M. steerei* var. *whiteheadii*.

In addition, the six most important characters that have separated the eight species are stipe length, number of sori rows between adjacent secondary veins, sori diameter, sori density, primary-areole width, and spore width. According to the results from numerical study two new combinations are given here.

M. steerei (Harr.) Ching

M. steerei (Harr.) Ching, *Bull. Fan Mem. Inst. Biol.* 4: 306. 1933. -- *Polypodium steerei* Harr., J. Linn. Soc., Bot. 16. (1877) 32. –**Type:** *Steere s.n.* (lecto, proposed by Price (1982) 202; holo MICH; iso K!), -10-1873, Taiwan, Ape's Hill, Takow

a. var. **steerei**

M. steerei (Harr.) Ching, *Bull. Fan Mem. Inst. Biol.* 4: 306. 1933. – *Polypodium steerei* Harr., J. Linn. Soc., Bot. 16. (1877) 32. – **Type:** *Steere* s.n. (lecto, proposed by Price (1982) 202; holo MICH; iso K!), -10-1873, Taiwan, Ape's Hill, Takow

Popypodium tokinensis Baker, *J. Bot.* 28 (1890) 266. – **Type:** *Balansa 148* (holo K!; iso P!), Tonkin, Takeuin, ner Quang Yen.

Polypodium playfairii Baker, *Ann. Bot. (Oxford)* 5. (1891) 474. – **Type:** *Playfair 383*, (holo K!), Taiwan, Ape's Hill.

b. var. **whiteheadii** (A.R. Sm. & Hoshiz.) S. Petchsri, *comb. & stat. nov.*

Microsurum whiteheadii A.R. Sm. & Hoshiz., Novon 10(4) (2000) 411. – **Type:** *Reggie Whitehead s.n.* (holo UC; iso BO, L!, MO, US), 2-6-1997, Sumatra, Weastern Sumatra, Prov. Paya Khambu, Koto District, 30 Km from Bukit Tinggi, 900 m.

Finally, it is also found that the other synonyms and infraspecific taxa within *M. punctatum* (Table 2.1) should be placed in *M. punctatum*, so this species is still a very variable species.

The RAPD technique was used to detect the genetic diversity of taxa in the *M. punctatum* complex. The result from this study pointed out in the same direction and in agreement with both general morphological, anatomical studies and morphometric analyses. It was also found that the two varieties of *Microsorum punctatum*, i.e *M. punctatum* var. *serratum* and *M. punctatum* var. *grandiceps* are still recognized.

In conclusion, based on the overall results from this study the *M. punctatum* (L.) Copel. complex comprise of eight taxa namely *M. glossophyllum* (Copel.) Copel., *M. siamense* Boonkerd, *M. thailandicum* Boonkerd & Noot., *M. membranaceum* (D. Don) Ching, *M. musifolium* (Blume) Copel., *M. punctatum* (L.) Copel., *M. steerei* (Harr.) Ching var. *steerei*, and *M. steerei* (Harr.) Ching var. *whiteheadii* (A.R. Sm. & Hoshiz.) S. Petchsri. In all, *Microsorum punctatum* is still a very variable species.

According to the great variations in many characters viz. frond form, size, and venation patterns, distribution of sori on lamina and geographical distribution; it is reasonable to tentatively classify the variations of the complex in to 8 taxa. The following is a simplified key to identify taxa and its description in the *M. punctatum* (L.) Copel. complex based on the output obtained from DELTA (Dallwitz et al., 1993).

Key to taxa

1a. Living fronds iridescent blue-green 2

1b. Living fronds light to dark green 3

2a. Midrib slightly raised on both surfaces; rhizome scales peltate; frond elliptic, base narrowly angustate; hydathodes distinct ..... **1. *M. siamense***

2b. Midrib grooved on the upper surface; rhizome scales pseudopeltate; frond narrowly elliptic to linear, base attenuate; hydathodes indistinct ..... **2. *M. thailandicum***

3a. Scale margins entire, clathrate with hyaline margins; rhizome not white waxy 4

3b. Scale margins denticulate or dentate, clathrate throughout or opaque; rhizome often waxy, at least sometimes waxy under the scales 5

4a. Stipe present, up to 10 cm long; lamina membranaceous, base narrowly angustate; scales distinctly or slightly spreading, pseudopeltate, central region with long hair; sori forming into 2-4 irregular rows ..... **3. *M. membranaceum***

4b. Stipe absent or indistinct; lamina firm-herbaceous or subcoriaceous, base truncate to obtuse; scales appressed, peltate, central region glabrous; sori forming more than 4 irregular rows ..... **7. *M. musifolium***

5a. Lamina usually shorter than 40 cm long, with only short glandular hairs 6

5b. Lamina up to 100 cm long, with only scales, or with a few scales and short glandular hairs 7

6a. Rhizome white waxy, phyllopodia obscure; stipe distinct, less than 2 mm diam., lamina base narrowly angustate ..... **6. *M. steerei* var. *steerei***

6b. rhizome not white waxy, phyllopodia distinct; stipe absent or obscure, up to 5 mm diam., lamina base attenuate ..... **5. *M. steerei* var. *whiteheadii***

7a. Scales opaque and blackish, more than two cell layers thick; stipe less than 2 cm long; lamina with only scales; all veins distinct; sorus present in costal areoles .... **4. *M. glossophyllum***

7b. Scales translucent and brownish, one cell layer thick; stipe up to 12 cm long; lamina with a few scales and short glandular hairs; all veins more or less immersed and vague (at least in living specimen); sorus absent in costal areoles ..... **8. *M. punctatum***

1. *Microsorum siamense* Boonkerd Blumea 51 (2006) 143. **Type:** Yala, Thailand, *P.V. Fern 1* (holotype, L; isotype, BCU)

Rhizome creeping, 3.3–4.1 mm diam., approximately cylindrical, not white waxy, bundle sheaths not differentiated, vascular bundles in cylinder 9–10, sclerenchyma strands 60–240, roots densely set; scales peltate, densely set, widest near the base, slightly spreading, ovate to triangular, up to 1.3 by 3.1 mm, margin denticulate, apex acuminate to slightly caudate, clathrate throughout, dark black on central region. *Phyllopodia* more or less distinct, 2.8–3.9 mm apart; stipe present, 35.3–44.3 mm long, 2.4–2.6 mm diam., raised on lower surface, slightly raised on upper surface. *Fronds* monomorphous, subcoriaceous, iridescent blue-green in color when living; lamina elliptic, up to 13.6 by 4.2 cm, index 3.1–3.8, widest about or above the middle of leaf length, base narrowly angustate, the stipe more or less winged, margin entire undulate, apex acute to long acuminate, with short glandular hairs, scales and acicular hairs absent. *Venation pattern*:

connecting veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, all veins or secondary and smaller veins more or less immersed and vague, free veinlet simple and once- forked, angle between primary and secondary vein 28–32 -degree. *Sori* mostly irregularly scattered on simple free or on 2 or 3 connective veins; round, superficial, usually occupying the upper (apical) half portion of the lamina, 10–13 cm^2, 1.3–1.4 mm diam., absent in the marginal areoles, occasionally present in costal areoles; paraphyses present, sporangium annulus 20–22 –celled; indurated cells 11–14. *Spores* concavo-convex, yellow, 32.5–35 by 50–52.5 μm.

Distribution. — Endemic, Thailand (Yala).

Ecology. — In rock crevices on moist rock of limestone hill in semi-shade, 100 m altitude.

Specimens examined. — Thailand: P.V. fern 1 (BCU, L); S.P. 20, 26, 39, 60, 62 (BCU).

2. *Microsorum thailandicum* Boon. & Noot. Blumea 46 (2001) 581. **Type:** Chumphon, Thailand, *T. Boonkerd 1442* (holotype, L; isotype, BCU).

Rhizome 4.9–5.2 mm diam., approximately cylindrical, not white waxy; bundle sheaths not differentiated, vascular bundles in cylinder 8–12, sclerenchyma strands 40–245, roots densely set. Scales pseudopeltate, sometimes some peltate, densely set, widest near the base, slightly spreading, ovate, or triangular, 2.6–3.3 mm long, 1–1.2 mm wide, margin denticulate, apex acuminate to slightly caudate, clathrate throughout, dark black on central region. *Phyllopodia* more or less distinct, 3–3.9 mm apart; stipe present, 10.8–13.2 mm long, 2.7–3.2 mm diam., raised on lower surface, grooved on upper surface. *Fronds* monomorphous, subcoriaceous, iridescent blue-green in color when living; lamina narrowly elliptic, 21.3–39.6 cm long, 1.2–1.9 cm wide, index 12.8–25.2, the widest indistinct, base attenuate, the stipe more or less winged, margin entire, apex long acuminate, with short glandular hairs, scales and acicular hairs absent. *Venation pattern*: the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger areoles in a row between two veins like in type 1, all veins or secondary and smaller veins more or less immersed and vague (at least in living specimen), free veinlet simple or once- or twice- forked, angle between primary and secondary vein 47–52 –degree. *Sori* mostly irregularly scattered on simple free or on 2 or

3 connective veins; round or slightly elongate, slightly immersed, usually occupying the upper (apical) half portion of the lamina, 15–23 cm^2, 1.2–1.4 mm diam., absent in the marginal areoles, occasionally present in costal areoles; paraphyses present; sporangium annulus 20–31 -celled; indurated cells 15–20. *Spores* concavo-convex, yellowish hyaline, 27.5–37.5 by 42.5–47.5 μm.

Distribution. — Endemic, Thailand (Chumphon).

Ecology. — In rock crevices on rather dry rock-ceiling of limestone hill in semi-shade, 250–300 m altitude. Its blue leaf iridescence is still retained when was introduced to a home garden where it is not really in deep shade as was in natural habit.

Specimens examined. — Thailand: Boonkerd 1442 (BCU, L); S.P. 2, 6, 49, 64, 71, 88, 120, 121 (BCU).

3. *Microsorum membranaceum* (D. Don) Ching Bull. Fan Mem. Inst. Biol. 4 (1933) 309; — *Polypodium membranaceum* D. Don, Prodr. Fl. Nepal. (1825) 2. — [*Colysis membranacea* J. Sm., Cult. Ferns (1857) 11 nom. Illeg.] — **Type:** *Wallich* (K!, iso B!), Nepal.

Polypodium hymenodes Kunze, Linnaea 23 (1850) 279/319. — **Type:** *Kunze* s.n. (B!), cult. Leipzig.

Polypodium membranaceum var. *grandifolium* Aldrew., Malayan Ferns (1909) 649. — **Type:** *Wallich 282* (holotype K!; isotype, BM!), Nepal.

Rhizome 2.3–9.8 mm diam., approximately cylindrical or dorso-ventrally flattened; not white waxy; bundle sheaths not differentiated, vascular bundles in cylinder 15–20; sclerenchyma strands 54–97, roots densely set; *scales* pseudopeltate, apically densely set, otherwise more or less sparsely set, slightly spreading, ovate, or triangular, up to 8 by 2.5 mm, margin entire, apex acute, clathrate except the hyaline marginal region, central region bearing multiseptate hairs at least when young. *Phyllopodia* more or less distinct, 0.8–5.3 mm apart; stipe present, 0.6–4.4 mm long, 2.6–7.2 mm diam., raised on lower surface, slightly raised on upper surface. *Fronds* monomorphous or slightly dimorphous; membranaceous, light to dark green in color; lamina narrowly elliptic to narrowly ovate; 18.9–94.9 by 1.4–13.8 cm, index 4–13.6, widest below or about the middle of leaf length; base narrowly angustate, the stipe winged for considerable part, margin entire, apex acuminate, with short glandular hairs, scales and acicular hairs absent. *Venation pattern*: connecting veins forming a row of about equally sized areoles between two adjacent vein

and no prominent veinlet situated parallel to the veins, or the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger areoles in a row between two veins like in type 1; all veins distinct, free veinlet simple or once to twice- forked, angle between primary and secondary vein 13–31 -degree. *Sori* forming into 2–4 irregular rows parallel to each pair of secondary veins, round or slightly elongate; superficial or slightly immersed on the whole surface of the lamina, or usually occupying the upper (apical) half portion of the lamina, 5–22 cm^2, 1.1–3.2 mm diam., absent in the marginal areoles, generally present in costal areoles; paraphyses absent; sporangium annulus 16–23 -celled; indurated cells 10–13. *Spores* concavo-convex, yellow, 27.5–47.5 by 47.5–82.5 μm.

Distribution. — Nepal; Sikkim; Bhutan; India; Srilanka; Burma; S China; Taiwan; N Thailand; N Laos; N Vietnam; Philippine.

Ecology. — Epiphyte, epilithic, or terrestrial in evergreen or deciduous forest, 600–2,600 or up to 4,000 m altitude.

Specimens examined. — Burma: Lace 4894 (K); Topping 4200 (K) — Ceylon: Abeysiri 55 (K); Beddome 339 (K); Gardner 1298 (K); Hooker 1145 (K), 1298 (K); Skinner 4828 (K); Sledge 543 (K), 832 (K); Walker 25 (K), 1834 (K) — Himalaya: Barnerji et al. 1313 (K), 2604 (K), 26957 (K); Henry 339 (K); Jalconer 68 (K); Konar 56 (K); Stachey & Winterbottom 1 (K); Stewart 21169 (K); Treutler 661 (K); Trotter 246 (K), 730 (K); Watt 101087 (K) — India: Jati 10 (K); Beddome 67 (K), 101 (K), 159 (K), 177 (K); Clarke 21388 (K), 27186 (K), 33720 (K); Gamble 14409 (K), 14870 (K); Haines 5379 (K); Jacquemont 11600 (K); Madhusoodanan CU29683 (K); Manickam 31442 (K); Mooney 128 (K); Nair 51452 (K); Narasimtan 165111 (K); Ramamoorthy 256 (K); Saldanha 421 (K), 641 (K), 717 (K), 820 (K), 14457 (K), 14800 (K), 15068 (K), 17959 (K) — Indo-China: Matthew 1967 (K) — Nepal: Bliss 41 (K), 51 (K), 189 (K); Gamble 1925 (K), 4847 (K); J.J. 6061 (K); Keke 902 (K); Khasya 1867 (K); Khwaunju 1259 (K); Maddine 1867 (K), Mense 343 (K) — Philippines: Elmer 5873 (K), 8367 (K); Merrill 11691 (K) — Sikkim: Balker 339 (K), Gamble 884 (K), 4000 (K), 6366 (K), 6367 (K), 9699 (K); Sinchal 339 (K); Treutler 661 (K) — Thailand: Garrett 59 (K), H.B.G. Garrett 59b (K), 591 (K); Larsen et al. 2314 (K); Iwatsuki 9600 (K), 15642 (K); Shimizu 10102 (K); Smith 1187 (K) — Vietnam: Balansa 1990 (K).

4. *Microsorum glossophyllum* (Copel.) Copel. Gen. Fil. (1947) 196. — **Type:** *King 388*, (BM, P), Papua New Guinea, Mt Gewagewa. — *Pleopeltis megalosoroides* Aldrew., Nova Guinea 14 (1924) 39. — **Type:** *Lam 1365* (holotype, L; isotype, B), Papua New Guinea, near Doorman River.

Rhizome 4.1–11.7 mm diam., approximately cylindrical, often waxy, at least sometimes waxy under the scales; bundle sheaths collenchymatous, vascular bundles in cylinder 10–20, sclerenchyma strands 53–94, roots very densely set (forming a thick mat); *scales* pseudopeltate, densely set, widest near the base, slightly spreading, ovate, 2.3–5.9 mm long, 0.8–1.7 mm wide, margin denticulate, apex acuminate, opaque, central region glabrous. *Phyllopodia* more or less distinct, 2.6–10.2(–25.8) mm apart; stipe present, 1.1–14.6 mm long, 2.1–7.9 mm diam., raised on lower surface, slightly raised on upper surface. *Fronds* monomorphous, firm-herbaceous; *lamina* narrowly obovate to broad oblanceolate, 49.3–146.2 cm long, 3.2–14.8 cm wide, index 6.8–16.9, widest about or above the middle of leaf length, base narrowly cuneate, the stipe winged for considerable part, margin entire, apex acute to acuminate, with a few scales, short glandular hairs and acicular hairs absent. *Venation pattern*: connecting veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, all veins distinct, free veinlet simple and once- forked, angle between primary and secondary vein 21–42 -degree. *Sori* mostly irregularly scattered on simple free or forming irregular rows parallel to each pair of secondary veins; round, superficial, absent from the basal parts for 1/5–4/5 of total length of lamina; 10–30 cm^2, 1.2–2.8 mm diam., absent in the marginal areoles, occasionally present in costal areoles; paraphyses absent; sporangium annulus 18–23 -celled; indurated cells 13–16. *Spores* plano-convex to concavo-convex, hyaline, 25–42.5 by 45–70 μm.

Distribution. — Papua New Guinea (Type); Irian Jaya; Solomon Is.

Ecology. — Epiphyte (low) or terrestrial, rarely epilithic on limestone, volcanic rocks, brown loam and clay. Often nest-forming, 80–2,800 m altitude.

Specimens examined. — *Microsorum glossophyllum* (Copel.) Copel. — Irian Jaya: Johns 7995 (K); Leeuwenberg 9853 (K); McDonald 3829 (K); Widjaja 4293 (K) — Papua New: Blackwood 188 (K); Braithwaite 4721 (K), 4866 (K, P); Brass 11319 (K), 23055 (K), 24483 (K), 29549 (K), 29786 (K), 30498 (K), 31569 (K), 32403 (K); Bulmer 103837 (K); Carr 7660 (B), 13015 (B), 13340 (K); Clemens 7133 (B); Conn & Kairo 152 (K); C. King 388 (P, **type**); Croft 151 (K), 203 (L), 451 (L), 533 (L), 568 (K), 1728

(K), 65719 (L); Leland et al. 65641 (L); Edelfelt 220 (P); Flenley 2084 (K); Vinas 5974 (K), 60249 (K); Gay 1086 (K); Hoogland et al. 6877 (K); van Mettenius 276 (B); Nakaike 408 (K); Parris 7751 (K), 9251 (K), 9479 (K); Sand 1780 (K); Veldkamp & Stevens 5911 (K), 6793 (L); Verdcourt 5113 (K); Vink 16534 (L), 17568 (L); Wakefield 1437 (K); Walker 548 (K); Whitmore 1045 (K); Womersley 6820 (K), 11092 (K) .

Pleopeltis megalosoides Alderw. — Papua New: Lam 1365 (L, **type**).

5. *Microsorum steerei* var. *whiteheadii* (A.R. Sm. & Hoshiz.) S. Petchsri, *comb. & stat. nov*

***Microsorum whiteheadii* A.R. Sm. & Hoshiz.** Novon 10 (2000) 411. — **Type:** *Whitehead* s.n. (isotype, L), Sumatra, near Paya Khumbu.

Rhizome 5.2–6 mm diam., approximately cylindrical or dorso-ventrally slightly flattened; not white waxy; bundle sheaths not differentiated, vascular bundles in cylinder 50–80; sclerenchyma strands 98–113, roots very densely set (forming a thick mat); *scales* pseudopeltate, moderately densely set, brown; slightly spreading, ovate; 3.1–5 mm long, 1.5–3.2 mm wide, margin erose-denticulate to dentate, apex attenuate, clathrate throughout, central region glabrous, or central region bearing multiseptate hairs at least when young. Phyllopodia obscure, 4.2–5.2 mm apart; stipe absent or indistinct, 0.2–1 mm long, 5.4–6.2 mm diam., not or slightly raised on both surfaces. *Fronds* monomorphous, coriaceous, light to dark green in color; lamina broad to narrowly oblanceolate; 27.6–35.5 cm long, 5.5–7.9 cm wide, index 3.1–5.8, widest about or above the middle of leaf length, base attenuate, the stipe more or less winged; margin entire, apex round to acute, or acuminate, with short glandular hairs, scales and acicular hairs absent. *Venation pattern*: the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger areoles in a row between two veins like in type 1; all veins or secondary and smaller veins more or less immersed and vague (at least in living specimen); free veinlet simple and once- forked, angle between primary and secondary vein 38–45 -degree. *Sori* mostly irregularly scattered on simple free on the whole surface of the lamina or forming irregular rows parallel to each pair of secondary veins; round, superficial, 14–19 cm^2, 1.2–1.8 mm diam., absent in the marginal areoles, generally absent in costal areoles; paraphyses present; sporangium annulus 19–28 -celled; indurated cells 18–20. *Spores* concavo-convex, yellow, 27.5–37.5 by 42.5–47.5 μm.

Distribution. — Western Sumatra (type).

Ecology. — Growing on limestone outcrops, 900 m altitude.

Specimens examined. — Sumatra: Whitehead s.n. (L, **type**), Kampu 1, 2, 3, 4, 5 (BCU).

6. *Microsorum steerei* var. *steerei* (Harr.) Ching Bull. Fan Mem. Inst. Biol. 4 (1933) 306; — **Type:** *Steere* s.n., (K; isotype, P), Taiwan, Ape's Hill, Takow.

Polypodium tonkinense Baker, J. Bot. 28 (1890) 266. — **Type:** *Balansa 107, 148* (K; isotype, P), Tonkin, near Quan Yen.

Polypodium playfairii Baker, Ann. (London) 5 (1891) 474. — **Type:** *Playfair 383* (K), Taiwan, Ape's Hill.

Rhizome 2.5–7.1 mm diam., approximately cylindrical, often waxy, at least sometimes waxy under the scales; bundle sheaths not differentiated, vascular bundles in cylinder 10–15; sclerenchyma strands 50–100, roots densely set; *scales* pseudopeltate, apically densely set, otherwise more or less sparsely set; distinctly spreading, ovate, or triangular; 2.1–5.9(–23.7) mm long, 0.7–1.4 mm wide, margin denticulate, apex acuminate, clathrate throughout, central region glabrous. Phyllopodia distinct, 1.7–7.9 mm apart; stipe present, 0.6–22.6 mm long, 0.5–2.7 mm diam., sharply raised on upper and lower surface. *Fronds* monomorphous or slightly dimorphous, subcoriaceous, light to dark green in color; lamina narrowly elliptic to narrowly obovate to linear; 16.2–56.1 cm long, 1.9–6.5 cm wide, index 4–19.4, widest about or above the middle of leaf length or indistinct, base narrowly angustate, the stipe winged for considerable part, margin entire, apex acuminate, with short glandular hairs, scales and acicular hairs absent. *Venation pattern*: connecting veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, all veins or secondary and smaller veins more or less immersed and vague (at least in living specimen), free veinlet simple or once- or twice- forked, angle between primary and secondary vein 34–61 -degree. *Sori* mostly irregularly scattered on simple free or forming irregular rows parallel to each pair of secondary veins; round, superficial or slightly immersed, on the whole surface of the lamina or usually occupying the upper (apical) half portion of the lamina, 10–60 cm^2, 1.1–1.6 mm diam., absent in the marginal and costal areoles; paraphyses absent; sporangium annulus 18–24 -celled; indurated cells 10–17. *Spores* concavo-convex, hyaline, 27.5–40 by 42.5–55 μm.

Distribution. — Taiwan; China; Vietnam.

Ecology. — By slope, under wood on limestone, 100–200 m altitude.

Specimens examined. — *M. steerei* (Harr.) Ching — China: Christensen 1339 (BM), 3418 (BM); Henry 1895 (K), 1951 (K); Kew H1905/85 (K); Suruhoe 82 (K) — Formosa: B20 0091734 (B) — Hanoi, Vietnam: d' Alleizette herb s.n. (P)— Kew, England: Edward 38 (K), 2411 (K); Kew A.D. 19 (K) — Philippines: Loher 867 (K); Vidal 4041 (K) — Tokin, Vietnam: Balansa 45 (P), 70 (P); Bon 1274 (P), Cadière 30 (P), 99 (P); Christ 1940 (P); Giesenhagen 1910 (P); Pételot (Colani) 1339 (P), 1789 (BM), 4871 (P), 4911 (P) — Taiwan: Balansa 198 (K), J.B. Steere (s.n.) (P, **type**).

P. tokinense Baker — Tokin, Vietnam: Balansa 107 (P, **type**), 148 (P, **type**).

P. playfairii Baker — Taiwan: Playfair 383 (K, **type**).

7. *Microsorum musifolium* Copel. Univ. Calif. Publ. Bot. 16 (1929) 112; *Polypodium musifolium* Blume, Enum. Pl. Javae (1828) 134. *Pleopeltis musifolium* T. Moore, Index Filic. (1857) lxxviii. *Drynaria musifolia* J. Sm., Cult. Ferns (1857) 14. — **Type:** *Blume* (s.n.), (holotype, L!), Java, near Buitenzorg.

Rhizome 3.8–10.8 mm diam., approximately cylindrical or dorso-ventrally slightly flattened, not white waxy; bundle sheaths collenchymatous, vascular bundles in cylinder 19–20, sclerenchyma strands 45–87, roots very densely set (forming a thick mat); *scales* peltate, apically densely set, otherwise more or less sparsely set, appressed, ovate, 2.1–4.5 mm long, 1–2.1 mm wide, margin entire, apex acuminate, with hyaline marginal region, central region glabrous. Phyllopodia distinct; 2.4–8.8 mm apart; stipe absent or indistinct, 0.8–12.7 mm long, 2.8–9.1 mm diam., raised on lower surface, slightly raised on upper surface. *Fronds* monomorphous, (firm-)herbaceous, light to dark green in color; lamina narrowly obovate to broad oblanceolate, 43.4–138.1 cm long, 5.3–13.6 cm wide, index 5.9–16.4, widest about or above the middle of leaf length, base truncate to obtuse, the stipe more or less winged, margin entire, apex acute to acuminate, with a few scales and short glandular hairs, acicular hairs absent. *Venation pattern*: connecting veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, all veins distinct, free veinlet simple and once- forked, angle between primary and secondary vein 21–44 -degree. *Sori* mostly irregularly scattered on simple free on the whole surface of the lamina or usually occupying the upper (apical) half portion of the lamina, or forming irregular rows parallel to each pair of secondary veins; round, superficial, 15–62 cm^2, 1.1–1.8 mm diam., absent in the marginal areoles,

occasionally present in costal areoles; paraphyses absent; sporangium annulus 17–20 - celled; indurated cells 13–15. *Spores* concavo-convex, hyaline, 27.5–42.5 by 37.5–57.5 μm.

Distribution. — Southern Burma; Peninsular Malaysia; Singapore; Sumatra; Java; Borneo; Philippines: Luzon, Catanduanes and Mindanao; Papua New Guinea.

Ecology. — Primary rain forest, in stream beds or near streams. Low epiphyte or epilithic, 100–900 m altitude. Often cultivated in botanical gardens.

Specimens examined. — Borneo: Elmer 20871 (P); Enders 4022 (L); Hose 1827 (K), 1894 (K); Inder 4022 (K); Iwatsuki et al. 3252 (K); Jaman 4036 (K); Kato et. al B3252 (L) — Java: Bernardi 234 (B); Blume s.n. (L, **type**); Zollinger 3005 (P) — Malay Peninsula: Henderson 19708 (BM) — Malaysia: Beddome 1911 (K); Henderson 19704 (K); King 192 (K); Littke 469 (L); Matthew 1928 (K); Yapp 575 (K); Turneau 836, 905 (K); Unesco Limestone Exp. 635 (K) — Papua New: Lauterbach 567 (P); Ledermann 7695 (B), 8549 (B), 8743 (B); Schlechter 2764 (B) — Philippines: Copeland 1537 (B); EBL 1537 (P); Elmer 10500 (K, L); Vanoverberg 3678 (P) — Sarawak: Elmer 20871 (K); Gay 132 (K); Hancook 342 (K); Hose 1827 (BM); J.Smith 1859 (BM) — Singapore: Corner 30247 (K); Haniff 21028 (K) — Sumatra: Brooks 357 (BM); Hancook 1892 (K); Lau 1796 (K); de Wide & de Wide 12385 (L).

8. *Microsorum punctatum* (L.) Copel. Univ. Calif. Publ. Bot. 16 (1929) 111; — *Acrostichum punctatum* L., Sp. Pl. ed. 2 (1763) 1524. — *Polypodium punctatum* Sw., J. Bot. (Schrader) 1800 (1801) 21, non Thunb. (1784). — *Phymatodes lingalata* C. Presl, Tent. Pterid. (1836) 198. — *Pleopeltis punctata* Bedd., Suppl. Ferns S. India (1876) 22. — **Type:** *Fothergill* s.n. (n.v., not found in LINN), China.

Polypodium polycarpon Sw., J. Bot. (Schrader) 1800 (1801) 21, non. Illeg., non Cavanilles. — **Type:** *Thumberg* s.n. (n.v.), Java.

Polypodium polycarpon Cav., Descr. Pl. (1801) 246. *Niphobolus polycarpus* Spr., Syst. Veg. 4 (1827) 45. — *Phymatodes polycarpa* C. Presl, Tent. Pterid. (1836) 198, t. 8 f. 19. — *Pleopeltis polycarpa* T. Moore. Index Filic. (1857) Ixxviii. — *Microsorum polycarpon* Tardieu, Fl. Madag. Fam 5 (1960) 114. — **Type:** *Née* s.n. (n.v.).

Polypodium irioides Poiret., Encycl. 5 (1804) 513. — *Phymatodes irioides* C. Presl, Tent. Pterid. (1836) 196. — *Microsorum irioides* Fée, Mém. Foug. 5. Gen. Filic. (1852)

268, t. 20B. — *Pleopeltis iriodes* T. Moore, Index Filic. (1857) lxxviii. — **Type:** *Beddome 48/341* (isotype, K; photo B, BM), India, *Commerson* (holotype, P), Mauritius.

Polypodium ambiguum Blume, Enum. Pl. Javae add. (1828) 125 — **Type:** *Reinwardt* (L).

Polypodium glabrum Wall. ex Roxb., Calc. J. Nat. Hist. 4 (1844) 482, non Burman (1768). — **Type:** *Wallich 281* (isotype, BM, K), India, near Calcutta, *J.Smith* (K), India.

Phymatodes sessilis C. Presl, Tent. Pterid. (1863) 198. — *Polypodium sessile* Kaulf. ex Kunze, Bot. Zeit. (Berlin) 6 (1848) 116. — *Microsorum sessile* Fée, Mém. Foug. 5. Gen. Filic. (1852) 268. — *Pleopeltis sessilis* T. Moore, Index Filic. (1857) lxxvii. — **Type:** *Sieber 31* (B, BM, K) Mauritius.

Polypodium millisorum Baker, J. Linn. Soc. Bot. 15 (1877) 109. — *Pleopeltis millisora* Alderw., Bull Dép. Agric. Indes Néerl. 27 (1909) 8. — **Type:** *Moseley 3412* (BM, K), Little Kai Is.

Polypodium validum Copel. In Perkins, Fragm. Fl. Philipp. 3 (1905) 191. — *Microsorum validum* Ching, Bull. Fan Mem. Inst. Biol. 4 (1933) 295. — **Type:** *Copeland 973* (n.v.), Philippine, Mindanao, Davao.

Polypodium punctatum ssp. *subdrynariaceum* H. Christ, Bull. Herb. Boissier 2 (1906) 994. — *Polypodium punctatum* var. *subdrynariaceum* Alderw., Malayan Ferns (1909) 654. — **Type:** *Ridley 8935* (holotype P), Sreangoon, near Singapore.

Polypodium punctatum ssp. *subirideum* H. Christ, Bull. Herb. Boissier 2 (1906) 994. — *Polypodium punctatum* var. *subirideum* Alderw., Malayan Ferns (1909) 654. — *Microsorum subirideum Copel., Gen. Fil. (1947) 197.* — **Type:** *Elmer 5884* (holo K, iso P), Philippine, Castilla, Baguio, Benguet, *Endert 1506* (L), Java.

Polypodium antrophyoides Alderw., Bull Dép. Agric. Indes Néerl. 18 (1908) 22. — **Type:** *Forbes 3119* (BM), Sumatra, Palembang.

Polypodium neoguineenese Copel., Philipp. J. Sci. 6, Bot. (1991) 89. — *Pleopeltis neoguineenesis* Alderw., Malayan Ferns Suppl. 1 (1917) 390. — *Microsorum neoquineense* Copel., Gen. Fil. (1947) 196. — **Type:** *King 335, Thomas 11536* (lectotype, K; photo BM), Papua New Guinea, Lakekamu.

Rhizome 2.2–11.1 mm diam., approximately cylindrical, often waxy, at least sometimes waxy under the scales; bundle sheaths not differentiated, vascular bundles in cylinder 15–20, sclerenchyma strands 50–100, roots very densely set (forming a thick mat); *scales* pseudopeltate, sometimes peltate, apically densely set or otherwise more or

less sparsely set, slightly spreading, narrowly ovate to ovate or triangular, 1.5–5.6 mm long, 0.7–2.6 mm wide, margin dentate to denticulate, apex acuminate, clathrate throughout to subclathrate, central region glabrous. Phyllopodia more or less distinct, 1.6–13.7 mm apart. *Fronds* monomorphous or slightly dimorphous, firm-herbaceous to subcoriaceous or coriaceous, light to dark green in color; lamina narrowly elliptic to narrowly ovate to narrowly obovate to broad to narrowly oblanceolate to linear; 27.9–171.9 cm long, 2.6–19.2 cm wide, index 5.6–28.8, widest about or above the middle of leaf length, base cordate to narrowly angustate, the stipe winged for considerable part, margin entire, or entire undulate, or sinuate (in cultivar variety), apex rotundate to acuminate, with a few scales and short glandular hairs, acicular hairs absent; stipe present, 0.7–9.9 mm long, 2.5–7.9 mm diam., sharply raised on upper and lower surface. *Venation pattern*: connecting veins forming a row of about equally sized areoles between two adjacent vein and no prominent veinlet situated parallel to the veins, or the first connecting vein forming one row of small primary costal areoles parallel to the costa, other larger areoles in a row between two veins like in type 1; all veins or secondary and smaller veins more or less immersed and vague (at least in living specimen), free veinlet simple and once- forked, angle between primary and secondary vein 20–56 -degree. *Sori* mostly irregularly scattered on simple free veins on the whole surface of the lamina, or forming irregular rows parallel to each pair of secondary veins, round, superficial, 14–74 cm^2, 0.9–2.3 mm diam., absent in the marginal areoles, generally absent in costal areoles; paraphyses absent; sporangium annulus 10–26 -celled; indurated cells (10–)12–18(–20). *Spores* plano-convex, hyaline, or yellowish hyaline, 22.5–37.5 by 40–65 μm.

Distribution. — Paleotropics and subtropics.

Ecology. — Epiphyte, but also epilithic or terrestrial in various types of forest, sometimes in savannah but also in wet places in streambeds, up to 2,800 m altitude.

Specimens examined. — *M. punctatum* (L.) Copel. — Annobon Is.: Skinn 283 (K) — Australia: Melville et al. 3669 (K); Wall 11232 (K) — Cameroon: Fris et al 7120 (K); Hepper 8682 (K); Mildbraed 4426 (B); Preuss 2 (B), 309 (B); Sermolli 5232 (K), 7219 (K), 7244 (K) — Congo: Anton Cupffert 337 (K); Leonard 1618 (K) — China: Esquirol 3601 (P); Rosenstock 99678 (B); Ying 1657 (K) — Christmas Is.: Andrews 108 (K); Allen 7 (K), 173 (K); Mitchell 132 (K), 154 (K); du Puy 7 (K); Wace 4 (K), 42 (K) — Ethiopia: Meyer 7997 (K) — Fiji: Brownlie 1304 (L); Seemann 728 (L) — Gabon: Jeffrey 208 (K); Louis et al. 950 (P) — Ivory Coast: Viane 16 (K) — Java: Mousset 166

(P) — Kenya: Lucas 230 (K) — Madagascar: M.R. Decary 17754 (P); Perrier 1747 (P) — Micronesia: Bryan 1114 (K), 1167 (K) — Nigeria: J.M. Baker 84 (K) — Nongowa: Bakshi 207 (K) — Papua New Guinea: Brass 29373 (L); Croft 1129 (K); Forster 10852 (K); Hartley 11536 (L); Holttum 15702 (K, L); Jermy (& Rankin) 8220 (K); Leeuwenberg 10647 (L); Sand 2695 (K); Schlechter 16304 (P); Walker 7884 (L) — Philippines: Elmer 8263 (L) — Polynesian: B20 0099749 (B) — South Africa: B20 0099 607 (B), Rudatis 1369 (P) — Sulawesi: Bünnemeijer 12427 (L); de Joncheere 1325 (L); T.G. Walker T12316 (L) — Sumatra: Lütjeharms 4750 (L), 5151 (K), 5159 (K) — Tahiti: Forsberg 14149 (K); Savatier 987 (P); Sloover P195 (P); Vesco 1847 (P) — Tanzania: Bidgood 4775 (K); Balslev 342 (K) — Thailand: Bunk 384 (K); H.B.G. Garrett 288 (P); Murata et. al. 16387 (K) — Uganda: Dümmer 472 (K); Faden 69-946 (K); Glokudler 288 (K), 1458 (K), 1488 (K); Hafashimane 357 (K); Jackson 123 (K); Lecerber 2042 (K), 2617 (K); Sangster 630 (K); Tweedie 2432 (K) — Vietnam: Cadière 98 (K).

M. punctatum ssp. *subirideum* Christ — Borneo: Ashton 19060 (K) — Cameroon: Tchinaye 89 (K) — Fiji: Brownlie 16074 (L) — Java: van Balgooy 4628 (K); Botavae 74 (K); Endert 15062 (L, **type**); Inaeteay 173 (K); de Vriese & Teijsmann 32 (K), 325 (K) — Madagascar: Humblot 666 (L); Perrier 6149 (P) — Malaccan: No. 908302245 (L) — Malay Peninsular: Ernst 11045 (K); Hooker 526 (K); G. King's collector (= Kunstler) 5069 (K); Parrell 11385 (K); Ridley 1917 (K) — McLucas: van Borssom Waalkes 3228 (K); Buwalda 4159, (K) 6052 (K); Parris 11051 (K), 11720 (K); Robinson 1954 (K) — Papua New Guinea: Brass 25458 (K, L); Carr 12148 (K); Croft 61266 (K), 61160 (K); Floyd 5682 (K); Køie 1149 (K); Kulong 11582 (L); Sands 2118 (K); Schodde 3026 (L); Stevens 58710 (K) — Philippines: Castro 5910 (L); Copeland 1535 (B), 1776 (P); Elmer 5884 (K, P, **type**), 7854 (P, L), 7991 (K), 10920 (K), 16863 (K); Gutierrez 117367 (K); Hatierg 171 (K); Ramos 14779 (K) — Sarawak: Christensen 529 (BM); Parris 6900 (K) — Solomon Is.: Jarrett 68 (L) — Tahiti: Braithwaite 2570 (P) — Thailand: van Beusekom et al. 258 (B, BM); Phengkai 683 (B); Christensen 529; Floto 7237 (K); Hennipman 3065 (BM); Larsen 2597 (K), 5078 (K); Murata et al.17674 (K); Tem 11209(K).

M. punctatum ssp. *subdrynariaceum* Christ — Annobon Is.: Melville 2023 (K) — Australia: Coveny & Hind 6900 — Bali: Holstvoogd 772 (L), 844 (L) — Borneo: Ashton 19060 (K); Combes 4097; Ismail 2744 (K) — Burma: Dickason 7637 (L); Wallace 191 (K) — Ceylon: Hooker 3799 (K) — China: Cavalerie (& Fortunat) 4012 (P); Fung 20053 (K); Rochers 2634 (P) — Congo: Ben 438; Gutzwiller 1305 (K); Thollon 1304 (P)

— Fiji: Brownlie 8454 (L) — Gabon: Leeuwenberg 13492 (K) — Guinea: Caruallo 2279 (K), 4235 (K); Jacques-Fe'lix 864 (P) — India: Abraham 666 (K); Bhargava et al. 2836 (L); Cusclah 17347 (K); Gough 3243 (K), 6055, 8289, 16350; Jarrett 766 (K), 784(K); J.D.H. 750 (K), 2223 (K); Manickam 31220 (K); Miller 1364 (K); Mooney 2383 (K); Paush 1931 (K); Piggott 2103 (K); Saldaha 12517 (K), 16392 (K); Sunanda 9557 (K); Tessier 19067 (K) — Irian Jaya: Nooteboom 5915 (K); Sands 6730 (K) — Ivory Coast: Viane 828 (K) — Java: Bakhuizen van den Brink 5739 (L); Hooker 1867 (K); Matthew 1928 (K); Mousset 20; Pleyte 57, 265; Rosenstock 20 (K); de Vriese 26 (K); Zollinger 1028 (K) — Kenya: Verdcourt 3919 (K) — Kl. Soenda Eil.: Elbert 913 (L) — Liberia: Wide 3876 (K) — Malay Peninsular: Castel 1961 (K); Piggott 2973 (K); Rodin 177 (K), 245 (K), 569 (K); Ridley 6554 (K); Turneau 904 (K); Yapp 296 (K) — Malaysia: Littke 394 (L) — Mayetta: Pascal 923 (P) — McLucas: van Borssom Waalkes 3053 (K), 3288 (K), 3238 (K); Buwalda 6052 (K); Eyma 3254 (K) — New Caledonia: Franc 11448 (P) — New Hebrides: Braithwaite 2306 (L), 2570 (L); Bourdy 306 (L, **type**); Savi 340 (L) — Nigeria: Chapman 3132 (K) — Reunion: Cadet 3824 (P) — Papua New Guinea: Croft 199 (K), 61578 (L), 65453 (K); Darbyshire 624 (K); Gay 405 (K), 1031 (K); Hoogland 10588 (K); Lam 1108 (K); van Royen 3474 (K); Schultze 104 (B)— Philippines: Elmer 22330 (K); Foxworthy 42135 (B); Ramos 973 (P); Ridsdale 5567 (K); Wenzel 2611 (B) — Sarawak: Kandau 62458 (K); Paul 64665 (K) — Singapore: Ridley 8935 (P, **type**) — Solomon Is.: Braithwaite 43701 (L); Brass 2756 (P); Beer 7768 (L); Whitmor 4321 (L) — South Africa: Schelpe 5032 (P) — Sulawesi: Coode 6251 (L); Darnaedi 2107 (K); Hennipman 5112 (L), 5462, 5981 (L) — Sumatra: Buwalda 6978 (K); Darnaedi 71 (K); Surbeck 1082 (L) — Tahiti: Braithwaite 4136 (P) — Taiwan: Tagawa 1853 (K) — Tanzania: Balslev 342 (K)— Thailand: Garrett 288 (K); Iwatsuki et al. 10900 (K); Larsen 3096 (K) — Timor: Bloembergen 3424 (K) — Uganda: Hafashimane 26 (K); Katendo 1187 (K); Thomas 145 (K) — Vietnam: Pételot (Colani) 2898 (P), 4101 (B); M. Semesle 580 (P); Tsiang 29192 (K), 36090 (K); Vieillard 459 (P).

Polypodium irioides Poiret — Australia: Heward 183 (K); Hooker 1820 (K) — Borneo: Korthals 113 (B), 973 (B) — Bhutan: Nuttall 1867 (K) — Ceylon: Beckett 648 (B) — China: Henry 10899 (B, K); Lungchow 83 (K); Matthew 1907 (K); Tutcher 10771 (K) — Congo: Louis 1417(K), 1932 (K) — India: B 200099652 (B); Beddome 48/341 (K); I.S. Gamble 16350 (K); Wallich 1837 (P) — Java: Jati 875 (L); Korthals 148 (L), 527 (L), 684 (L); Slanse 6314 (L); Zollinger 935 (B) — Malay Peninsular: Castel 15193

(K); Chusan 1847 (K); Hose 4823 (K); Matthew 2 (K), 4 (K) — Mauritius Is.: M. Boivin 891 (P), 1014 (P) — New Caledonia: Deplanche 198 (L); Germain 40 (L); Moore 30 (L); Noumea 29 (L), 199 (L); Pancher 506 (P) — Nigeria: Jones 16952 (K) — Papua New: Avon 370 (L) — Sarawak: Cooks 1909 (K) — Tahiti: Hooker 1867 (K); Maire 10775 (K); Moseley 6447 (K); Vieillard 10775 (K) — Uganda: Thomas 1369 (K) — Vietnam: Braker 4136 (P); Phustouve 34 (K).

M. validum (Copel.) Ching — Kl. Soenda Eil.: Schmitz 5169A (L), F7 (L) — Malaccan: Lam 3717 (L) — Philippines: Copland 275 (BM), 15356 (B); Elmer 9946 (BM, K, L), 13813 (L), 13598 (B, BM, K, P); Mathew 1928 (K); Merrill 7331 (P); Ramos 14862 (P), 31419 (P); USC 288 (L); Wenzel 1216 (BM)— Sumatra: Jacobson 10 (L); Lütjeharms 4990 (K).

P. glabrum Wallich — India: Wallich 281 (BM, K, **type**) — Ivory cost: Chevalier 21088 (P) — Java: R.B. le lunde 156 (P) — Malay Peninsular: Hooker 1803 (K); Maitban 281 (K) — Mozambique: Rehmann 8674 (P) — New Caledonia: Pancher 186 (P).

P. millisorum Baker — McLucas: Moseley 3412 (K, **type**) — Papua New: Brass 24220 (BM); Moseley 1874 (BM0; Wakefield 1435 (BM).

M. sessile Kze. — Mauritius: Sieber (Syn. Fil.) 31 (B, BM, K).

P. polycarpon Cav. — Cameroon: Bos 4106 (K); Leeuwenberg 5032 (P), 6651 (B, K, P); Thorold 28 (BM), 87 (K)— Comoro Is.: Benson 106 (BM), 1293 (BM) — Congo: de Néré 332 (P), 1412 (P); Wide et al. 3734 (B) — India: Bhargava et al. 6356 (K) — Ivory Cost: Leeuwenberg 1785 (K), 2542 (K) — Liberia: Linder 759 (K); Deighton 6056 (P); Wide et al. 3734 (K, P) — Madagascar: Barrett & Dorr 201 (P)— McLucas: Buwalda 6052 (L) —Micronesia: Hutchison 1139 (K) — Nyasaland: Chapeua 581 (BM) — Nigeria: Wright 4 (K) — Sierraleone: Deighton 6056 (K) — South AF: Chase 5220 (BM); Melsetter 46915 (BM); Mitchell 378 (BM); Schelpe 5032 (BM), 5225 (BM) — Tanganyika: Glover 263 (K) — Uganda: Dawkins 389 (K) — Thailand: Bloembergen 18 (K); den Hoed 909 (K); Kostermans 59 (K).

M. punctatum (L.) Copel. cv. *serratum* — Borneo: Endert 2358 (L).

M. neoquineense Copel. — Papua New: Thomas 11536 (K, **type**).

REFERENCES

Anonymous. 1999. SPSS for windows Release 10.0, Standard version [Computer software], Chicago, SPSS Inc.

Barrington, D.S. 1985. The present evolutionary and taxonomic status of the fern genus *Polystichum*: The 1984 botanical society of America pteridophyte section symposium. *Am. Fern J.* 75: 22-28.

Bartolozzi, F., Warburton, M.L., Arulsekar, S., and Gradziel T.M. 1998. Genetic characterization and relatedness among Californian almond cultivars and breeding lines detected by randomly amplified polymorphic DNA (RAPD) analysis. *J. Amer. Soc. Hort. Sci.* 123: 381-387.

Benzing, D.H., 1990. *Vascular Epiphytes.* Cambridge University Press, Cambridge.

Boonkerd, T. and H.P. Nooteboom. 2001. A new species of *Microsorum* (Polypodiaceae) from Thailand. *Blumea* 46: 581-583.

Boonkerd, T., Pechsri, S. and Baum, B.R. 2005. A phenetic study of *Cassia* sensu lato (Leguminosae-Caesalpinioideae: Cassieae: Cassiinae) in Thailand. *Pl. Syst. Evol.* 252: 153–165.

Boonkerd, T. 2006. A New Species of Microsorum (Polypodiaceae) from Thailand. *Blumea.* 51(1): 143-145.

Bosman, M.T.M. 1991. *A monograph of the fern genus Microsorum (Polypodiaceae).* Leiden, Rijksherbarium/Hortus Botanicus.

Bosman, M.T.M., Hovenkamp, P.H. and Nooteboom, H.P. 1998. Microsorum Link. In: C. Kalkman, H.P. Nooteboom (eds.), *Flora Malesiana.* Ser. II, 3: 90-131. Leiden, Rijksherbarium/Hortus Botanicus.

Botstein, D., White, R.L., Skolnick, M. and Davis, R. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-331.

Budke, J.M., Hickey, R.J. and Heafner, K.D. 2005. Analysis of morphological and anatomical characteristics of *Isoetes* using *Isoetes tennesseensis*. *Brittonia* 57: 167–182.

Buczkowska, K. 2004. The genus *Calypogeia* Raddi (Jungermanniales, Hepaticae) in Poland, biometrical analysis of morphological and anatomical variation. *Nova Hedwigia.* 78: 121-146.

Casiva, P.V., Saidman, B.O., Vilardi, J.C. and Cialdella, A.M. 2002. First comparative phenetic studies of Argentinean species of *Acacia* (Fabaceae), using morphometric, isozymal, and RAPD approaches1. *Am. J. Bot.* 89:843-853.

Chen, C.-C. and Chen, Y.-R. 2005. Study on laminar hydathodes of Ficus formosana (Moraceae) I. Morphology and ultrastructure. Bot. Bull. Acad. Sin. 46: 205-215

Conceição, A.S., Queiroz, L.P., Lambert, S.M., Pereira, A.C.S. and Borba, E.L. 2008. Biosystematics of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* (Leguminosae-Caesalpinioideae) based on allozyme and morphometric analyses. *Pl. Syst. Evol.* 270: 183-207.

Dallwitz, M.J., Paine, T.A. and Zurcher, E.J. 1993. User's Guide to the DELTA System: a General System for Processing Taxonomic Descriptions. 4th edition, available on the internet at http://biodiversity.uno.edu/delta/.

Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. A plant DNA minipreparation: Version II. *Plant Mol. Biol. Rep.* 1: 19.

Dice, L.R., 1945: Measurement of the amount of ecological association between species. *Ecology*. 26: 295-302.

Dillon, W.R. and Goldstein, M. 1984. *Multivariate analysis. methods and applications.* New York, John Wiley and Sons.

Dirlewanger, E., Duha, S., Viruel, M.A., and Saunier, R. 1998. Identification of peach varieties using molecular markers. *Acta. Hort.* 465: 69-77.

Doyle, J.J. and Doyle J.L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

Geesink. R and Kornet, D.J. 1989. Speciation and Malesian Leguminosae. In L.B. Holm-Nielsen et al. *Tropical forests: botanical dynamics, speciation and diversity.* 135-151. London.

Ghiselin, M.T. 1975. A radical solution to the species problem. *Syt. Zool.* 23: 536-544.

Giussani, L.M. 2000. Phenetic similarity patterns of dioecious species of *Poa* from argentina and neighboring countries. *Ann. Missouri Bot. Gard.* 87:203-233.

Gower, J.C. 1971. A general coeflicient of similarity and some of its properties. *Biometrics*. 27: 857-871.

Hanafy, M.F., Henry, T.W. and Domier, L.L. 1996. Use of random amplified polymorphic DNA (RAPD) for identification of *Gaeumannomyces* species. *Soil Bio and Biochem.*. 28: 703-710.

Hasebe, M. et al. 1995. Fern phylogeny based on rbcL nucleotide sequences. *Am. Fern J.* 85, 134–181.

Hilton, A.C., Banks, J.G., and Penn, P.C. 1997. Optimization of RAPD for fingerprinting *Salmonella*. *Appl. Microbiol.*.24: 243-248.

Hogbin, P.M. and Crisp, M.D. 2003. Evoltion of the coastal neospecies *Zieria prostrate* (Rutaceae) and its relationship to the *Zieria smithii* species complex. *Australian Syst. Bot.* 16: 515-525.

Holmgren, P.K. and N.H. Holmgren. 1998 [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Holtum, R.E. 1949. *A revised flora of Malaya II: Ferns of Malaya.* Govt. Printer. Singapore.

Holttum, R.E. 1954. (revised 1965). *Ferns of Malaya. A revised flora of Malayam II.* Incl. Appendix by I. Manton, Cytological notes on 100 species of Malayan ferns. Govt. Printer. Singapore.

Hull, D.L. 1978. A matter of individuality. *Philosophy. Sci.* 45: 335-360.

Janssen, T., Kreier, H.-P., and Schneider, H. 2007. Origin and diversification of African ferns with special emphasis on Polypodiaceae *Brittonia,* 59: 159-181.

Kidyue, M., Boonkerd, T., Thaithong, O. and Seelanan, T. 2005. Numerical Taxonomy of the *Hoya parasitica* (Asclepiadaceae) complex in Thailand. *Nat. His. J. Chulalongkorn Univ.* 5:47-59.

Kim, C., Shin, H. and Choi, H. 2003. A phenetic analysis of *Typha* in Korea and far east Russia. *Aquat. Bot.* 75: 33-43.

Korner, J.S. and Livak, K.J. 1989. Mutation detection using nucleotide analogs that alter electrophoretic mobility. *Nucleic Acids Res.* 17: 7779-7783.

Kreier, H.-P., and Schneider, H. 2006. Phylogeny and biogeography of the staghorn fern genus *Platycerium* (Polypodiaceae, Polypodiidae). *Am.J. Bot.* 93: 217–225.

Lachenbruch, P.A. and Goldstein, M. 1979. Discriminant analysis. *Biometric.* 35: 69-85.

Li, W. and Haufler, C.H. 1999. Genetic variation, breeding system, and pattern of diversification in Hawaiian *Polypodium* (Polypodiaceae). *Syst. Bot.* 24: 339-355.

Lisek, A., Korbin, M. and Rozpara, E. 2006. Using simply generated RAPD markers to Distinguish between sweet cherry (*Prunus avium* L.) cultivars. *Fruit Ornam. Plant Res.* 14: 53-59

Masood, A. and Abraham, G. 2006. Physiological response of *Azolla pinnata* plants to salinity stress. *Roum. Biotechnol. Lett.* 11: 2841-2844.

Motley, T.F. and Morden, C.W., 2001. Utility of RAPD markers in evaluating the status of the Hawaiian tree fern *Cibotium xheleniae*. *Pacific Sci.* 55: 145-155.

Nei, M. and Li, W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Nat. Academy of Sci., USA.* 76: 5269-5273.

Nilsson, J., Svensson, B., Ekelund, K. and Christiansson, A. 1998. A RAPD-PCR method for large-scale typing of *Bacillus cereus*. *Appl. Microbiol..*27: 168-172.

Nooteboom, H.P. 1997. The microsoroid ferns (Polypodiaceae). *Blumea.* 42: 261-395.

Ortiz A., Renaud R., Calzada I., and Ritter E. 1997. Analysis of plum cultivars with RAPD markers. *J. Hort. Sci.* 72: 1-9.

Pangua, E., Quinilla, L.G. and Pajaron, S., 2006. Taxonomic relevance of the gametophytic generation in a strictly rupicolous fern group: *Asplenium seelosii* s.l. *Bot. J. Linn. Soc.* 151: 375–386.

Paris, C.A. 1989. Cryptic species, species delimitation and taxonomic practice in the homosporus ferns. *Am. Fern J.* 79: 46-54.

Piesschaert, F., Jansen, S., Jaimes, I., Robbrecht, E. and Smets, E. 2001. Morphology, antomy, and taxonomic position of *Pagameopsis* (Rubiaceae-Rubioideae). *Brittonia.* 53:490-504.

Pryer, K.M., Smith, A.R. and Skog, J.E., 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and rbcL sequences. *Am. Fern J.* 85: 202–282.

Pryer, K.M. et al. 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature.* 409: 618–622.

Regalado, L. and Sânchez, C. 2001. Spore morphology as a taxonomic tool in the delimitation of three *Asplenium* L. species complexes (Aspleniaceae: Pteridophyta) in Cuba. *Grana* 41: 107-113.

Reudink, M.W., Joshua, P.S., Xu, B., Cunkelman, A. and Ronald, A.B. 2005. A Comparison of Physiological and Morphological Properties of Deciduous and Wintergreen Ferns in Southeastern Pennsylvania. *Am. Fern J.* 95: 45-56.

Rohlf, F.J. 1998. *NTSYS-pc version 2.0k Numerical taxonomy and multivariate analysis system*. Setauket, N. Y.: Software.

Rossello, J.A., Cebrian, M.C., and Mayol, M. 2002. Testing Taxonomic and Biogeographical Relationships in a Narrow Mediterranean Endemic Complex (Hippocrepis balearica) using RAPD Markers. *Ann. Bot.* 89(3):321-327.

Saiki, R.K. et al., 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*. 239: 487-91.

Salgado, E. 2004. Taxonomic and momenclature notes on Phillippine ferns II: three new names in *Aspinium*. Edinburgh J. Bot. 61: 185-195.

Sano, R., Takamiya, M., Kurita, S., Ito, M. and Hasebe, M. *2000. Diplazium subsinuatum* and *Di. tomitaroanum* should be Moved to *Deparia* According to Molecular, Morphological, and Cytological Characters. 113: 157-163.

Schneider, H. et al. 2003. Unraveling the phylogeny of polygrammoid ferns (Polypodiaceae and Grammitidaceae): exploring aspects of the diversification of epiphytic plants. *Mol. Phylogenet. Evol.* 31: 1041-1063.

Seeprasert, T., 2006. *Molecular taxonomic studies on Polygonium L. in Northeastern Thailand.* Master Degree Thesis. Department of Biologu, Khon Kaen University.

Shimada T., Hayama H., Haji T., Yamaguchi M., and Yoshida, M. 1999. Genetic diversity of plums characterized by random amplified polymorphic DNA (RAPD) analysis. *Euphytica*. 109: 143-147.

Small R.L. and Hickey R.J. 2001. Systematics of the Northern Andean *Isoëtes karstenii* Complex. *Am. Fern J.* 91: 41-69.

Smith, A.R. and Hoshizaki, B.J., 2000. *Microsorum whiteheadii* (Polypodiaceae), an attractive new species from Sumatra. *Novon.* 10: 411-414.

Sneath, P.H.A. and Sokal, R.R. 1973. *Numerical taxonomy: The principles and practice of numerical classification.* San Francisco, W. H. Freeman and Company.

Starr, J.R. and Ford, B.A. 2001. The taxonomic and phylogenetic utility of vegetative anatomy and fruit epidermal silica bodies in *Carex* section *Phyllostachys* (Cyperaceae). *Can. J. Bot.* 79: 362–379.

Swartz, L.M. and Brunsfeld, S.J. 2002. The morphological and genetic distinctness of *Botrychium mingananse* and *B. crenulatum* as Assessed by Morphometric analysis and RAPD markers. *Am. Fern J.* 92: 249-269.

Swofford, D.L. 1998. *PAUP*: phylogenetic analysis using parsimony.* Sinauer Associates, Sunderland, Massachusetts, USA.

Szweykowski, J., Buczkowska, K., and Odrzykoski, I. J. 2005. *Conocephalum salebrosum* (Marchantiopsida, Conocephalaceae) – a new Holarctic liverwort species. *Pl. Syst. Evol* 253:133-158

Tagawa, M. and Iwatsuki, K. 1989. Polypodiaceae. In: T. Smitinand and K. Larsen (eds.), *Flora of Thailand.* 3(1): 486-580. Bangkok, Chutima Press.

Tanksley, S. D., Young, N. D., Paterson, A. H., and Bonierbale, M. W. 1989. RFLP mapping in plant breeding – new tools for an old science. *Bio Technol.* 7: 257-264.

Tenhunen, J. D., Lange, O.L., Harley, P.C., Beyschlag, W. and Meyer, A. 1985. Limitations due to water stress on leaf net photosynthesis of *Quercus coccifera* in the Portuguese evergreen scrub. *Oecologia.* 67: 23-30.

Thomson, J.A. 2000. Morphological and Genomic Diversity in the Genus *Pteridium* (Dennstaedtiaceae). *Annals of Botany 85.*77-99.

Thomson, J.A., Chikuni, A.C., and McMaster, C.S. 2005. The taxonomic status and relationships of bracken ferns (*Pteridium*: Dennstaedtiaceae) from sub-Saharan Africa. *Bot J. Linn. Soc.* 148:311–321.

Wang, T., SU, Y., LI, X., ZHENG, B., CHEN, G., and ZENG, G. 2004. Genetic structure and variation in the relict populations of *Alsophila spinulosa* from southern China based on RAPD markers and cpDNA atpB-rbcL sequence data. *Hereditas.* 140: 8-17.

Warburto, M.L. and Bliss, F.A. 1996. Genetic diversity in peach (*Prunus persica* L. Batch) revealed by randomly amplified polymorphic DNA (RAPD) markers and compared to inbreeding coefficients. *J. Amer. Soc. Hort. Sci.* 121: 1012-1019.

Ward, J. M. 1993. Systematics of New Zealand *Inuleae* (Compositae-Asteraceae) 1. A numerical phenetic study of the species of *Raoulia. New Zealand J. Bot.* 31: 21-28.

Wiley, E.O.1981. *Phylogenetics, The theory and practice of phylogenetic systematics.* John Wiley and Sons, New York.

APPENDIX

APPENDIX

Table 4.4 Means and standard deviation of 23 quantitative characters of the eight clustering groupings of the *M. punctatum* (L.) Copel. complex.

Cha-	I		II		III		IV		V		VI		VII		VIII	
racter	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE
LMW	37.63	3.33	16.30	2.34	72.64	26.81	78.10	11.75	68.93	10.83	39.33	10.58	96.53	16.45	58.94	21.12
LML	130.62	3.11	304.54	52.37	536.25	184.31	970.13	157.08	386.16	62.33	314.65	70.38	831.55	156.73	747.08	222.75
STL	40.68	2.31	34.19	1.05	138.66	6.54	19.23	1.71	64.84	4.44	62.34	4.61	0.47	0.12	101.8	7.53
STD	2.49	0.11	2.92	0.18	4.75	0.97	5.31	1.28	5.84	0.34	1.54	0.36	5.75	1.04	4.55	0.96
PDL	3.19	0.39	3.41	0.32	2.67	0.88	5.87	1.45	4.63	0.45	3.28	1.15	6.13	1.25	4.48	1.45
RHD	3.63	0.32	4.93	0.25	5.56	1.47	7.48	1.56	5.71	0.35	4.99	0.88	6.96	1.44	5.82	1.38
RDL	4	0.20	1.63	0.19	2.83	1.32	11.51	2.80	5.11	0.54	3.48	1.64	12.45	2.99	8.57	2.56
SCL	2.6	0.27	3.02	0.20	4.42	1.34	4.01	0.64	4.23	0.75	4.03	1.03	3.64	0.40	3.31	0.63
SCW	1.24	0.06	1.15	0.08	1.46	0.36	1.25	0.18	2.78	0.39	1.04	0.19	1.42	0.22	1.20	0.27
ALA	19.33	0.82	7.33	1.41	14.25	4.36	18.88	3.57	18.4	2.97	16.71	5.53	23.57	5.84	13.86	4.67
ALB	2.5	0.55	1.78	0.83	2.02	1.09	6.01	1.56	8.00	1.00	1.88	1.75	19.34	6.88	4.88	3.94
DSO	1.37	0.04	1.31	0.06	1.93	0.39	1.85	0.41	1.37	0.10	1.33	0.13	1.39	0.15	1.28	0.17
DSR	0.18	0.01	0.18	0.01	0.19	0.02	0.19	0.02	0.18	0.01	0.16	0.03	0.16	0.01	0.17	0.15
NAC	20.83	0.75	28.33	1.80	19.41	1.76	19.78	0.97	26.00	2.00	21.56	1.37	18.90	0.75	19.25	1.00
SPL	51.25	1.37	45.22	1.89	58.75	8.14	57.97	4.68	51.5	2.85	50.64	4.16	47.71	4.89	51.67	4.13
SPW	33.75	1.37	31.94	3.70	36.60	5.57	32.53	3.94	36.00	2.24	33.27	3.98	33.33	3.62	31.38	3.10
DSL	12	1.26	19.11	2.37	9.87	3.78	37.34	4.63	17.00	1.87	25.97	7.92	39.71	7.13	38.70	10.93
PLS	59.44	2.81	138.91	17.19	196.78	113.64	517.69	144.79	124.61	16.03	156.98	43.55	430.14	137.82	383.64	120.66
PHS	11.22	1.15	5.01	1.75	16.79	6.07	17.66	7.57	16.57	2.41	16.13	7.84	19.33	4.85	14.35	6.63
NSR	3.33	0.52	7.22	0.67	2.24	0.46	4.01	0.87	5.40	0.55	4.10	0.88	8.08	1.47	9.29	2.01
DBSV	7.1	0.64	16.22	0.63	10.03	2.36	8.66	1.68	11.64	1.75	9.05	1.87	13.27	1.95	13.45	2.84
APS	29.5	1.52	49.78	1.72	21.51	3.57	29.10	4.84	41.00	2.65	44.73	6.04	27.74	3.60	33.98	5.46
PAW	1.46	0.13	1.09	0.11	4.14	1.97	3.11	1.73	1.55	0.29	2.16	0.59	4.35	1.52	1.10	0.55

Table 4.5 Summary of canonical discriminant function of eight clustering groups based on 23 quantitative characters.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation	Wilks' Lambda	Chi-square	df	Sig.
1	43.628	77.6	77.6	.989	.000	6290.994	140	.000
2	7.141	12.7	90.3	.937	.005	3662.521	114	.000
3	2.761	4.9	95.2	.857	.041	2211.437	90	.000
4	1.469	2.6	97.8	.771	.154	1294.813	68	.000
5	.791	1.4	99.2	.665	.380	669.409	48	.000
6	.297	.5	99.8	.478	.681	266.016	30	.000
7	.133	.2	100.0	.342	.883	86.148	14	.000

Table 4.6 Summary of canonical discriminant function of 21 taxa based on 23 quantitative characters.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation	Wilks' Lambda	Chi-square	df	Sig.
1	46.351	75.4	75.4	.989	.000	7195.815	380	.000
2	7.616	12.4	87.8	.940	.001	4549.504	342	.000
3	3.267	5.3	93.1	.875	.011	3072.092	306	.000
4	1.656	2.7	95.8	.790	.048	2076.731	272	.000
5	.949	1.5	97.3	.698	.129	1406.509	240	.000
6	.686	1.1	98.4	.638	.251	948.607	210	.000
7	.368	.6	99.0	.519	.423	590.102	182	.000
8	.288	.5	99.5	.473	.579	374.949	156	.000
9	.108	.2	99.7	.312	.746	201.275	132	.000
10	.062	.1	99.8	.242	.826	130.810	110	.086
11	.044	.1	99.9	.206	.878	89.536	90	.494
12	.028	.0	99.9	.166	.916	59.853	72	.846
13	.019	.0	99.9	.136	.942	40.787	56	.937
14	.016	.0	100.0	.126	.960	28.017	42	.952
15	.011	.0	100.0	.104	.975	17.048	30	.972
16	.008	.0	100.0	.088	.986	9.582	20	.975
17	.004	.0	100.0	.061	.994	4.239	12	.979
18	.002	.0	100.0	.046	.998	1.647	6	.949
19	.000	.0	100.0	.016	1.000	.175	2	.916

Table 4.7 Cross-validation summary with the linear discriminant function of the *M. punctatum* complex. (I = *Microsorum siamense*, II = *M. thailandicum*, III = *M. membranaceum*, IV = *M. glossophyllum*, V = *M. steerei* var. *whiteheadii*, VI = *M. steerei* var. *steerei*, VII = *M. musifolium*, and VIII = *M. punctatum*

From taxa	Classified in taxa							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
M. siamense	6	0	0	0	0	0	0	0
M. thailandicum	0	9	0	0	0	0	0	0
M. membranaceum	0	0	86	0	0	0	0	0
M. glossophyllum	0	0	0	68	0	0	0	0
M. steerei var. *whiteheadii*	0	0	0	0	5	0	0	0
M. steerei var. *steerei*	0	0	0	0	0	39	0	0
M. musifolium	0	0	0	0	0	0	48	0
M. punctatum	0	0	0	0	0	0	0	446
Total correctly classified	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**

BIOGRAPHY

Mr. Sahanat Petchsri was born on May 11, 1978, in Phatthalung Province. He was graduated in Biology from Faculty of Science, Prince of Songkla University in 1999. In 2002, he received his Master of Science in Botany from the Department of Botany, Faculty of Science, Chulalongkorn University, then continued his study in Biological Science Ph.D. Program, Faculty of Science, Chulalongkorn University from 2003-2008.