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

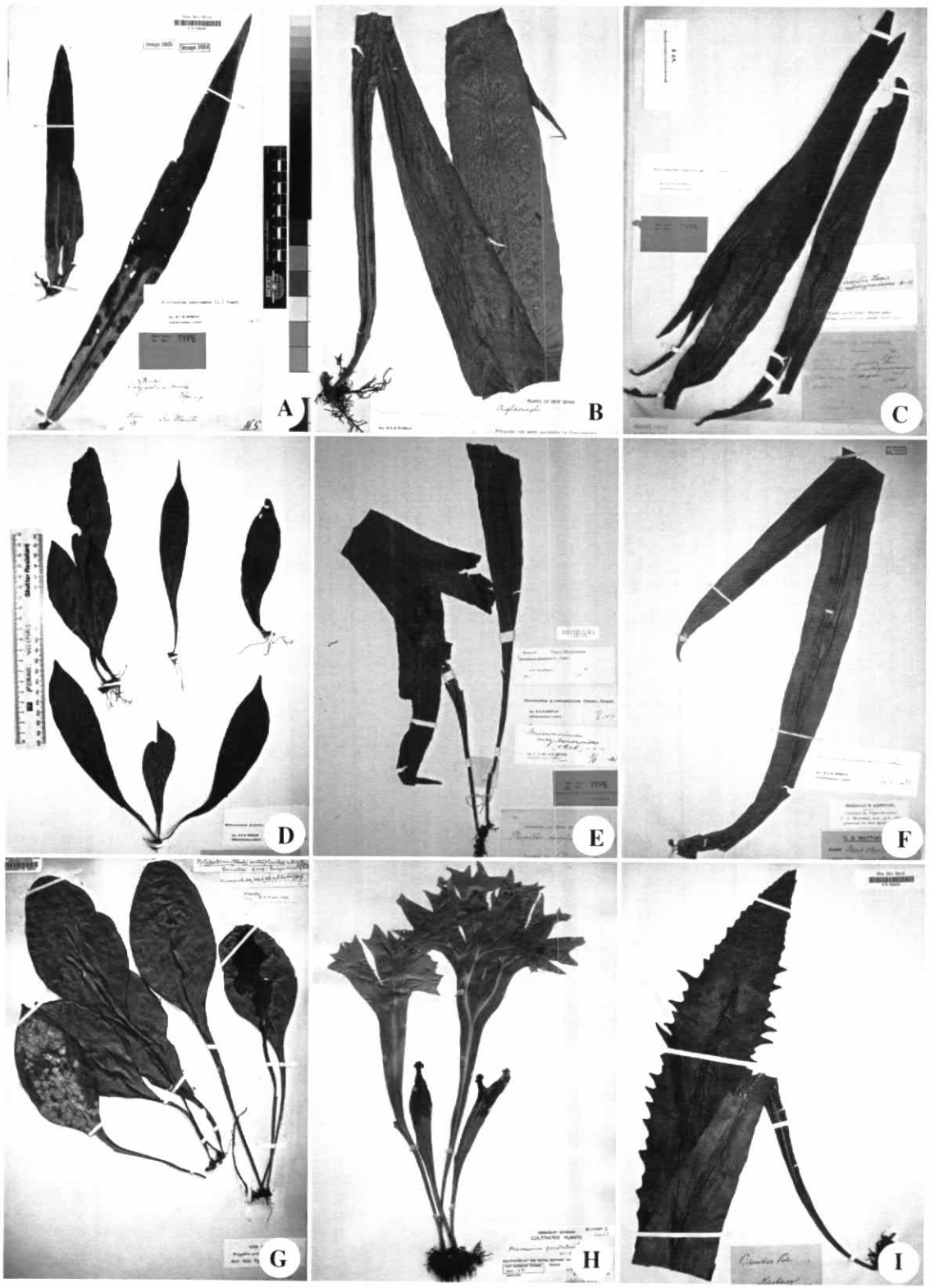

Figure 3.1 Herbarium Specimen of "*M. punctatum* complex". — A. *M. sessile*; B. *M. glossophyllum*; C. *M. punctatum* ssp. *subdrynariaceum*; D. *Polypodium tonkinens*;, E. *Pleopeltis megalosoides*; F. *P. irioides*; G. *P. ambiguum*; H. *M. punctatum* cv. *grandicep*; I. *M. punctatum* cv. *serratum*.

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*).

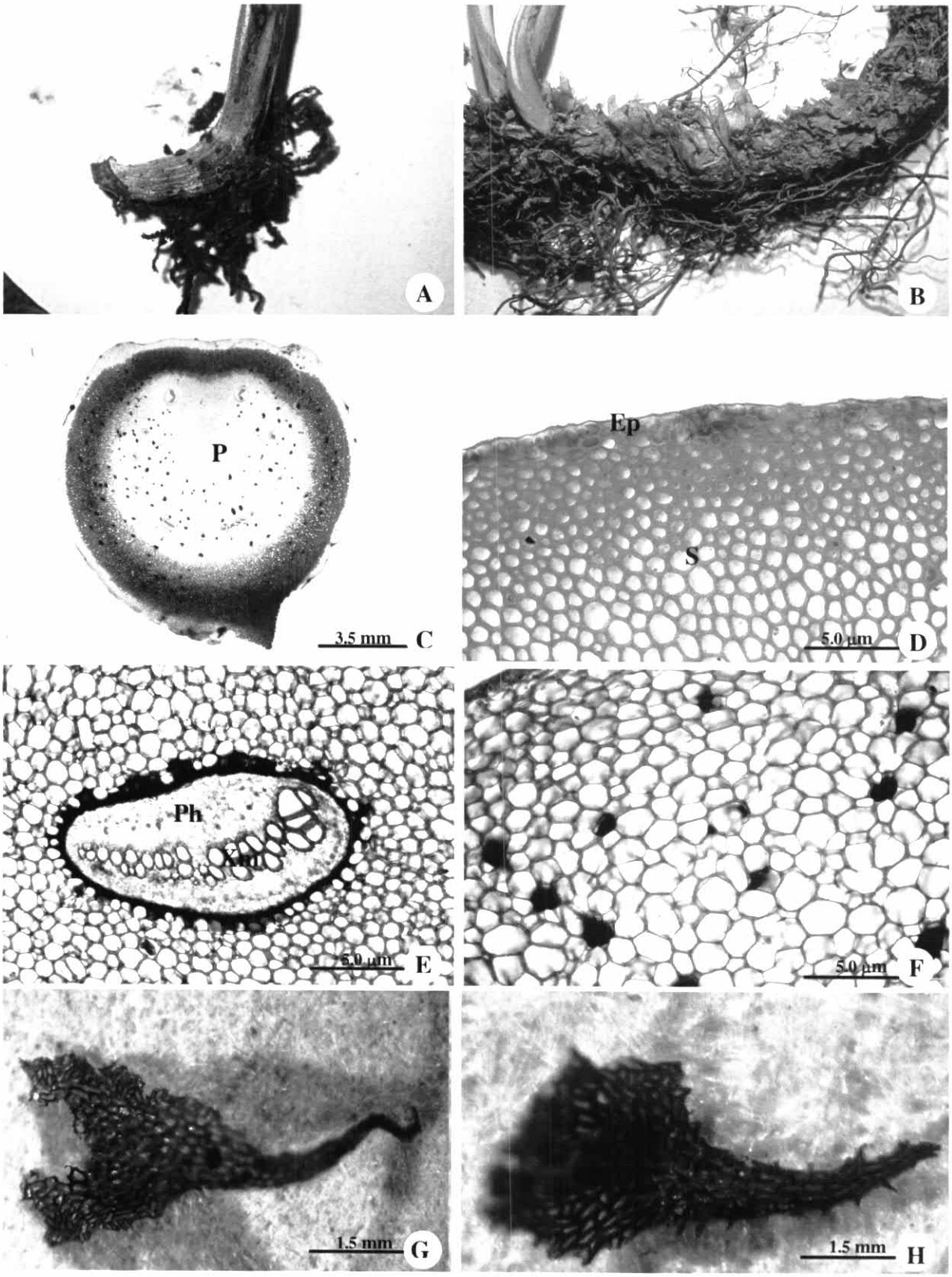

Figure 3.2 Rhizome and scale of "*Microsorum punctatum* complex".— A. waxy rhizome; B. rhizome covered by scales; C. rhizome in cross-section; D. collenchyma tissue; E. vascular bundle; F. sclerenchyma strand; G. entire marginal scale; H. dentate marginal scale. (Ep=epidermis, P=pith, Ph=phloem, S=sclerenchyma,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

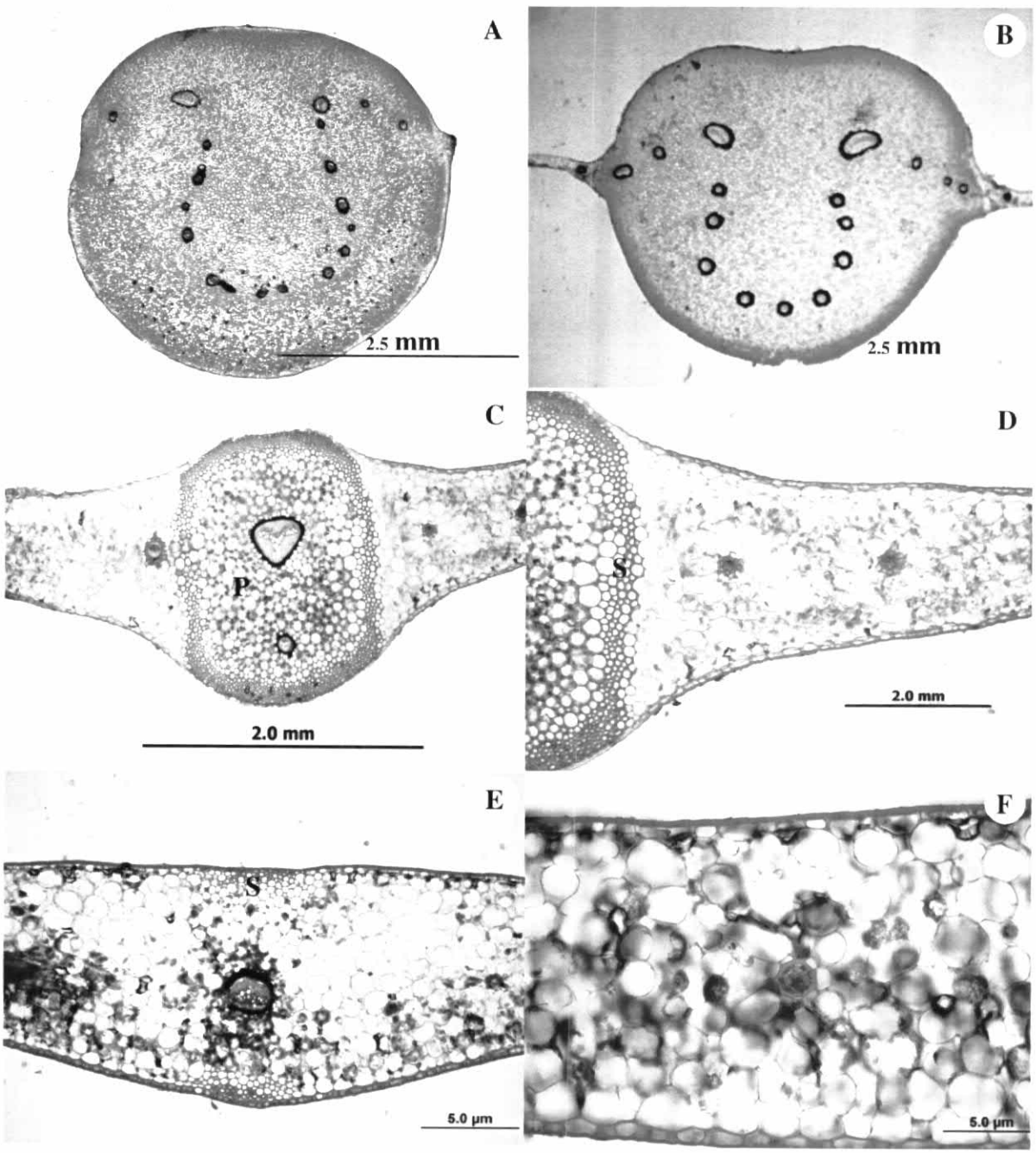

Figure 3.3 Stipe and midrib of "*M. punctatum* complex". — A-B. stipe in cross-section of *M. glossophyllum* and *M. punctatum*, respectively; C-D. midrib in cross-section of *M. punctatum*; E-F. midrib in cross-section of *M. siamense*. (P=pith, S=scerenchyma)

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.

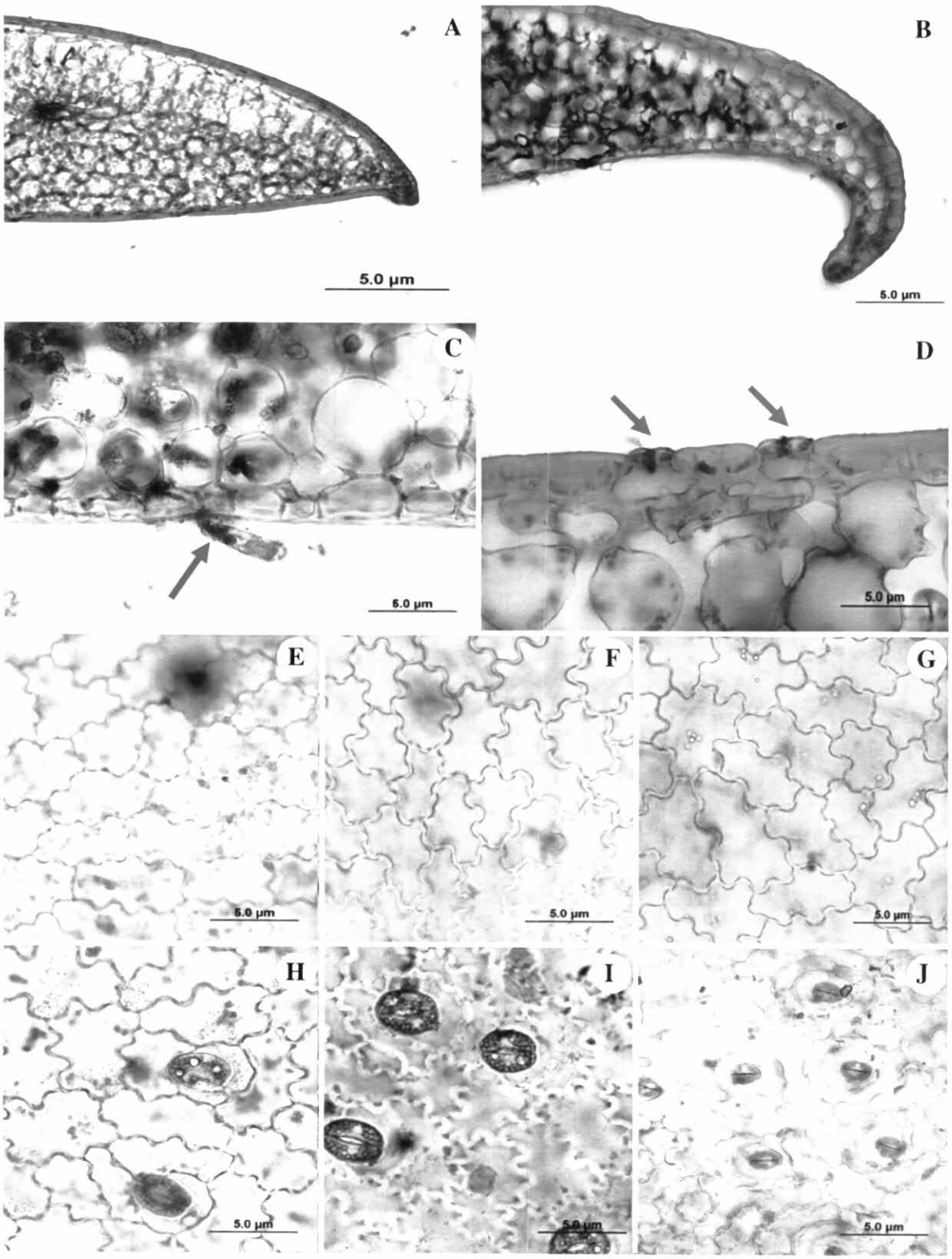

Figure 3.4 Stomata of "*M. punctatum* complex". — A-B. frond margin in cross-section of *M. thailandicum* and *M. siamense*, respectively; C. uniseriate hair with a glandular top-cell; D. guard cells are placed at the same level as the epidermal cell; E-G. epidermal cell; H-I. stomata.

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).

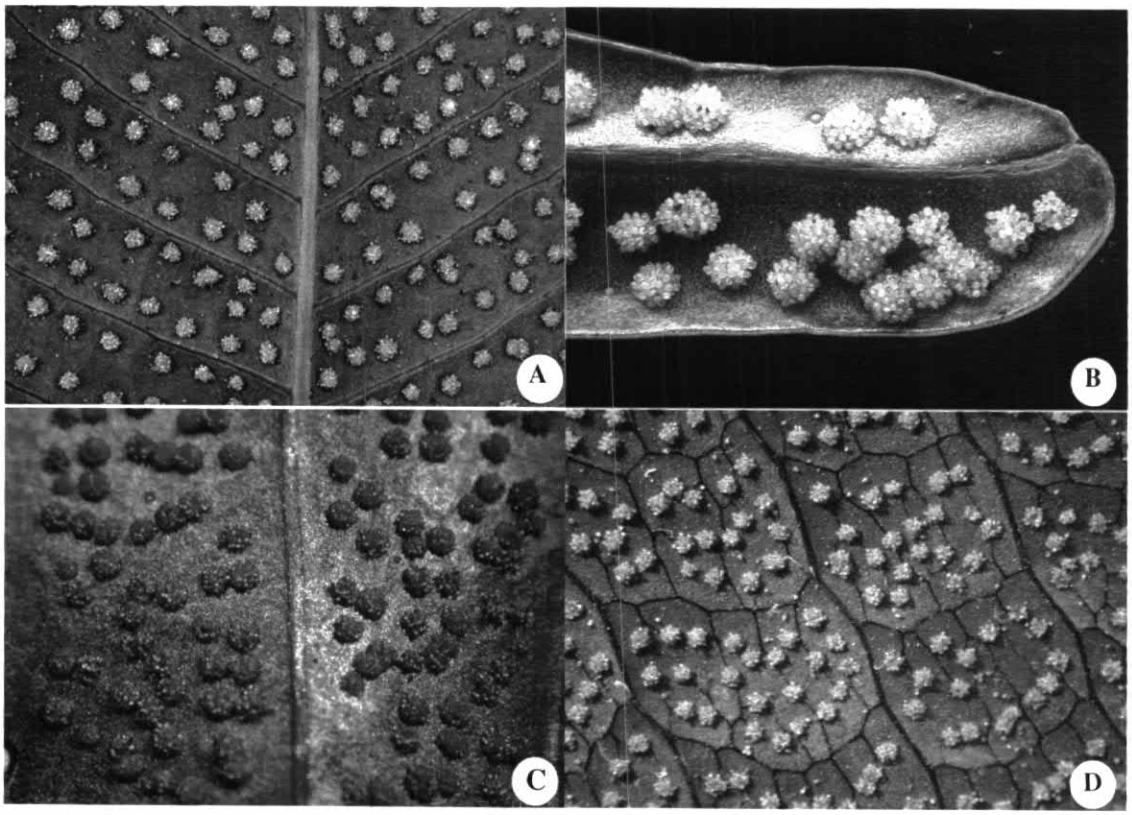

Figure 3.5 Sori distribution patterns of *M. punctatum* complex.— A. *M. membranaceum*; B. *M. thailandicum*; C. *M. punctatum*; D. *M. musifolium*.

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).

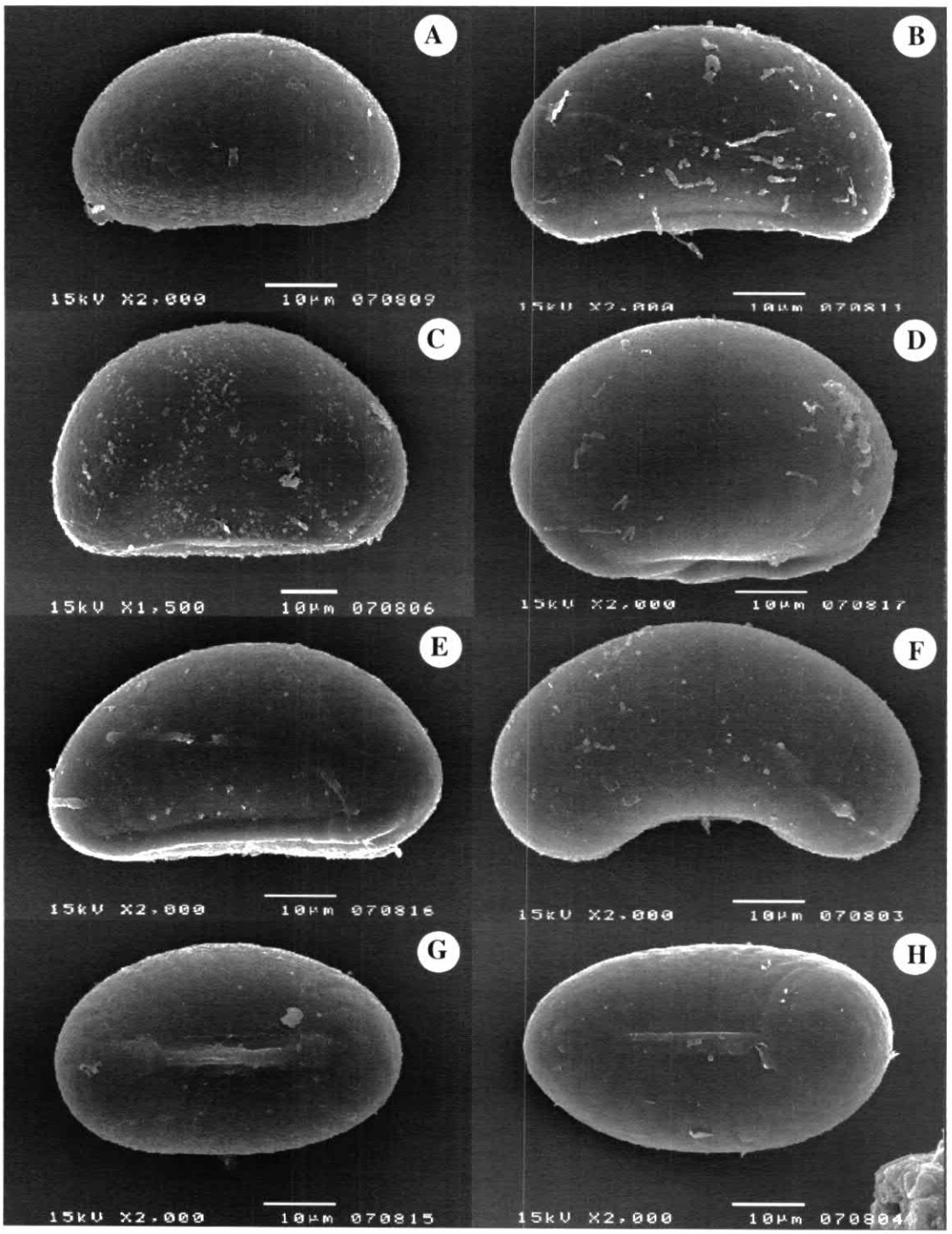

Figure 3.6 Spore of "the *M. punctatum* complex". — A. *Polypodium polycarpon*; B. *P. glabum*; C. *M. glossophyllum*; D. *P. irioides*; E. and G. *M. punctatum* ssp. *subirideum*; F. and H. *M. validum*.

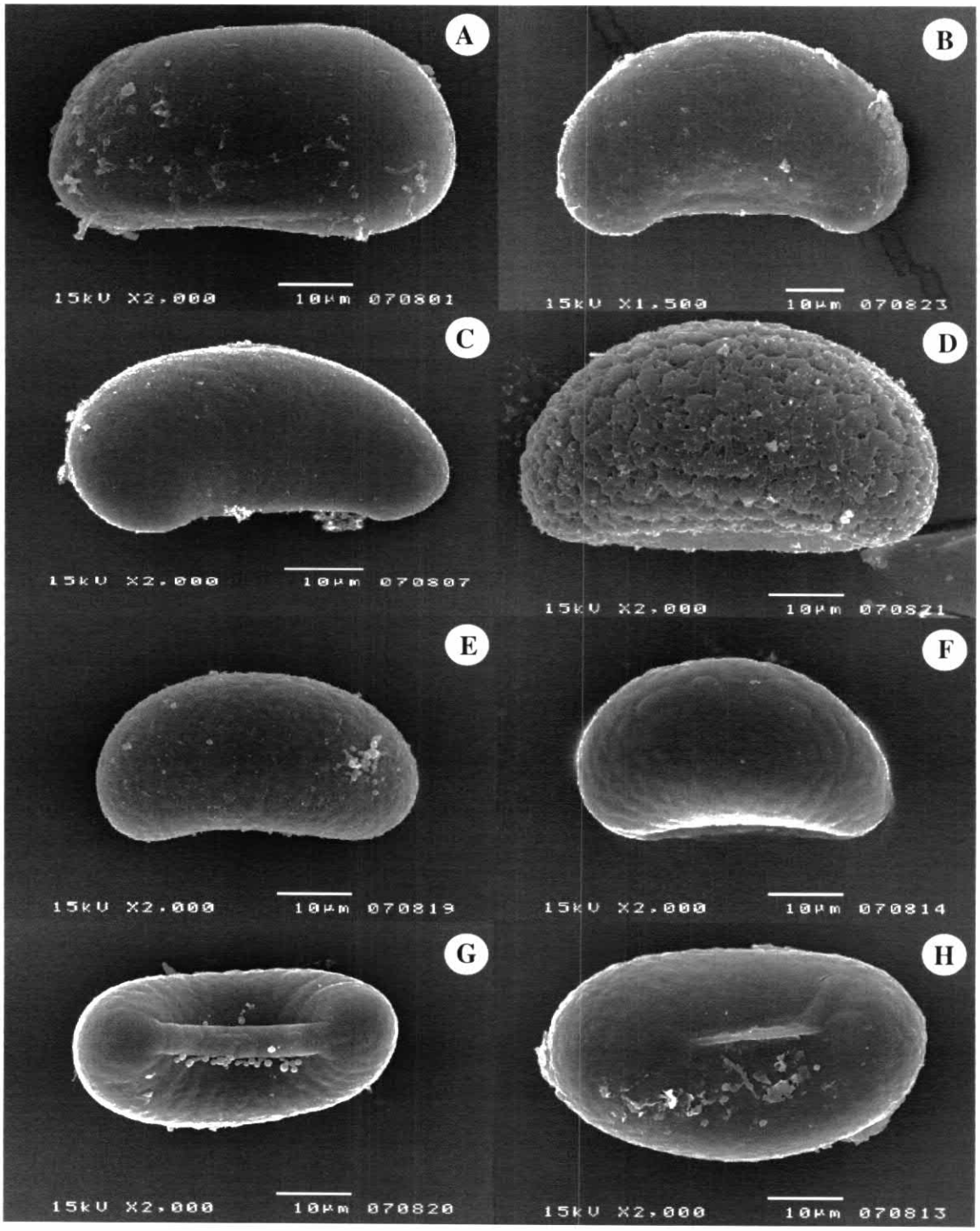

Figure 3.7 Spore of "the *M. punctatum* complex". — A. *M. whiteheadii*; B. *M. steerei*; C. *M. musifolium*; D. *M. membranaceum*; E. and G. *M. thailandicum*; F. and H. *M. punctatum*.

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; l*amina* 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.