

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 Computing 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.	<i>Microsorium siamense</i> Boonkerd	6(1)	L, BCU
2.	<i>M. thailandicum</i> Boonkerd & Noote.	9 (2)	L, BCU
3.	<i>M. membranaceum</i> (Don.) Ching	86 (1)	K
4.	<i>M. glossophyllum</i> (Copel.) Copel.	67 (2)	B, K, L, and P
5.	<i>Pleopeltis megalosoroides</i> Alderw.	1 (1)	L
6.	<i>M. whiteheadii</i> A.R. Smith & Hoshizaki	5	K, BCU
7.	<i>M. steerei</i> Harr.	35 (1)	B, K, and P
8.	<i>P. playfairii</i> Baker	1 (1)	K
9.	<i>P. tonkinense</i> Baker	3 (3)	P
10.	<i>M. musifolium</i> (Blume) Ching	48 (3)	B, K, L, and P
11.	<i>M. punctatum</i> (L.) Copel.	302	B, K, L, and P
12.	<i>M. punctatum</i> ssp. <i>subirideum</i> H. Christ	3 (3) ³	L, P ¹
13.	<i>M. punctatum</i> ssp. <i>subdrynariaceum</i> H. Christ	2 (2)	L, P
14.	<i>Polypodium irioides</i> Poiret	48 (1)	B, K, L, and P
15.	<i>M. validum</i> (Copel.) Ching	26 (3)	B, BM, K, L, and P
16.	<i>P. glabrum</i> Wall	10 (4)	BM, K and P
17.	<i>P. millisorum</i> Baker	5 (2)	BM, K
18.	<i>M. sessile</i> Fée	3 (3)	B
19.	<i>P. polycarpon</i> Cav.	40	B, BM, K, L, and P
20.	<i>M. neoquineense</i> Copel.	1	K
21.	<i>M. punctatum</i> (L.) Copel. cv. <i>seratum</i>	3 (1)	B and L
22.	<i>P. irioides</i> var. <i>lobatum</i> f. <i>crisatum</i> Bailey**	1	K
23.	<i>P. ambiguum</i> 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 Suvatabhundu 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. *crisatum* Bailey were included as population of *M. punctatum* (L.) Copel. In all analysis

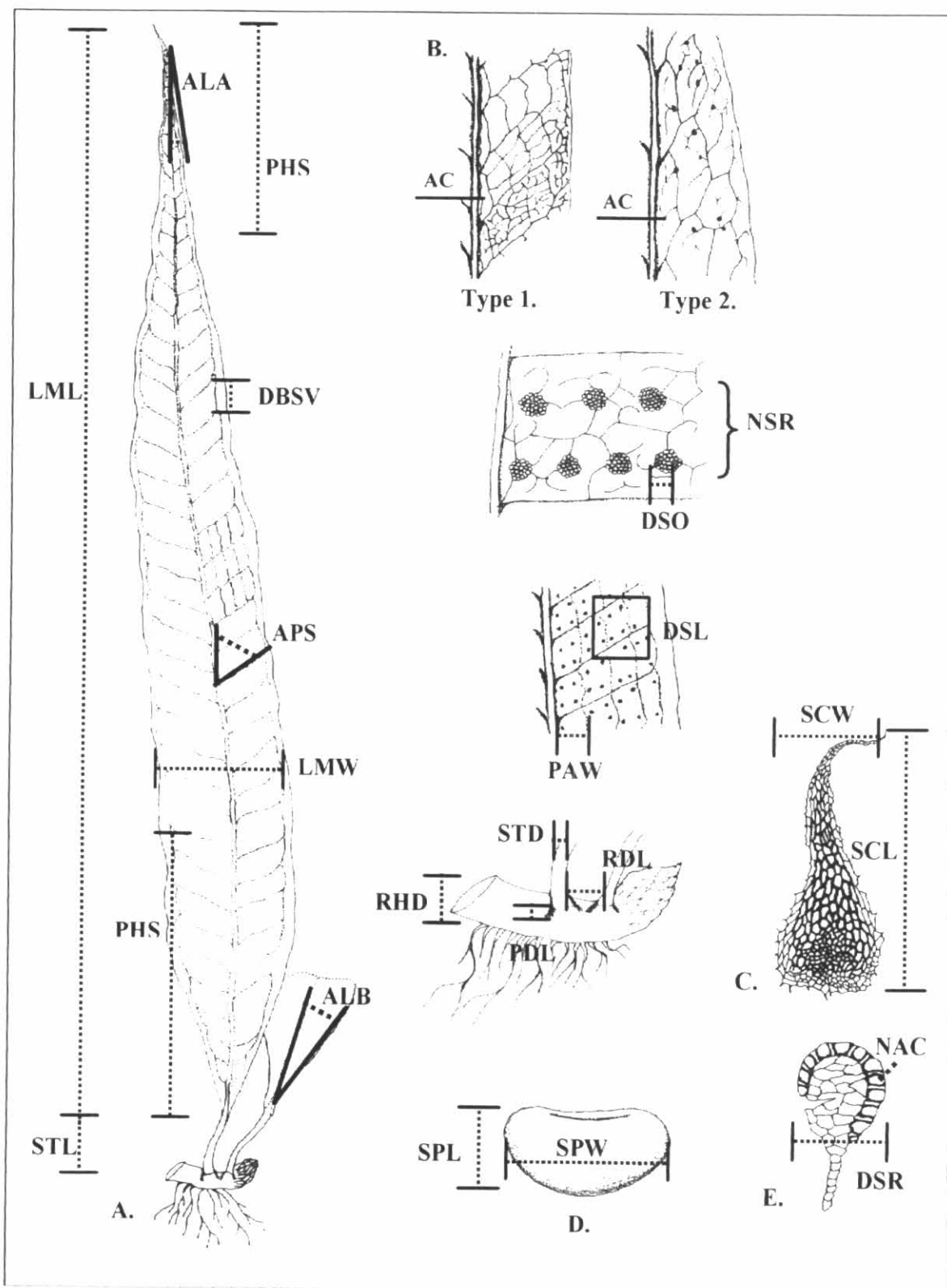


Figure 4.1 Measurement of rhizome, scale and frond part. --- A plant; B. venation; C. scale; D. spore; and E. sporangium.

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).

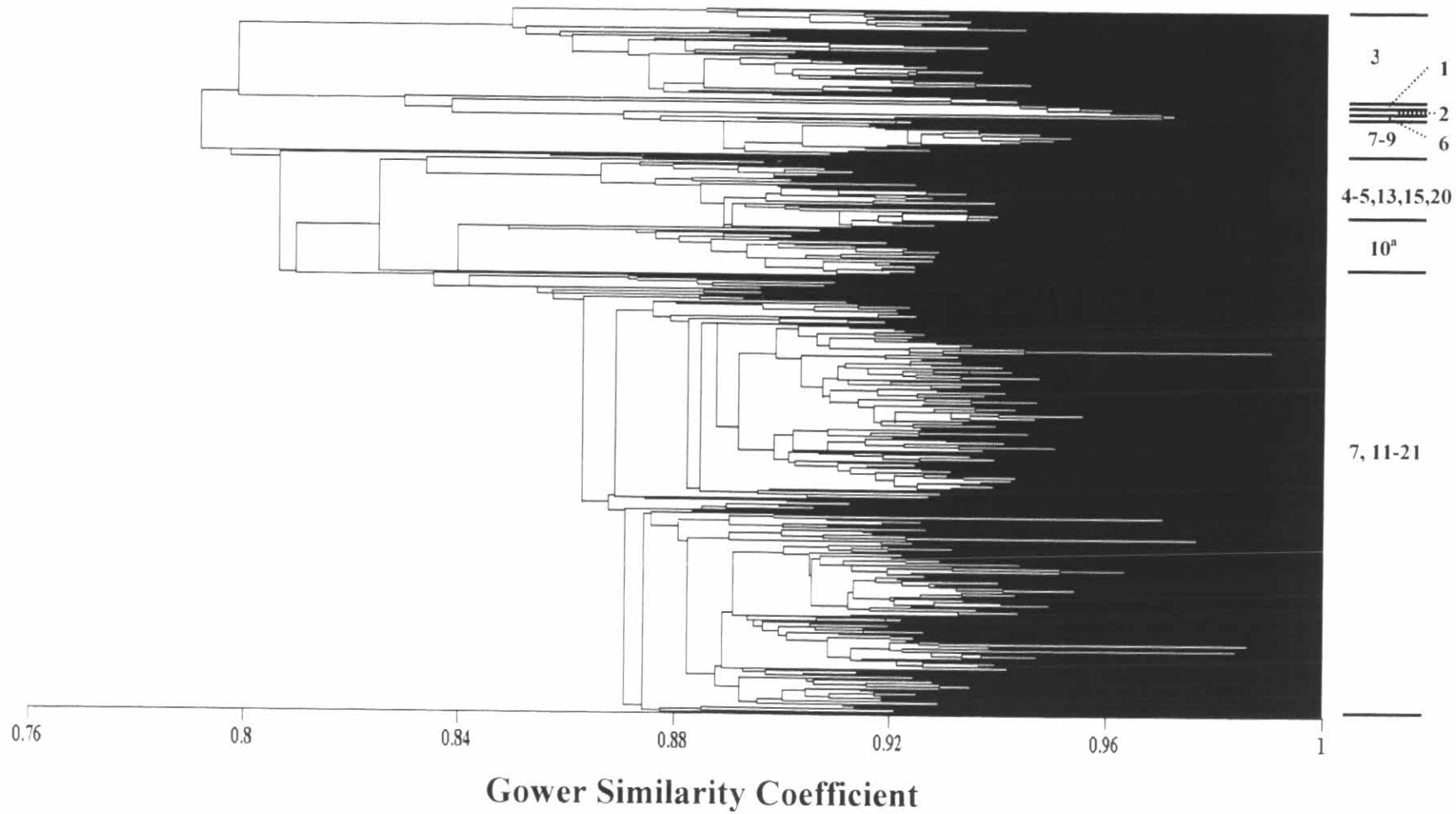


Figure 4.2 UPGMA clustering of 707 OTUs based on Gower Similarity Coefficient calculated between means only of 23 qualitative characters of the *M. punctatum* (L.) Copel. complex (a. numbers correspond to those in Table 4.1).

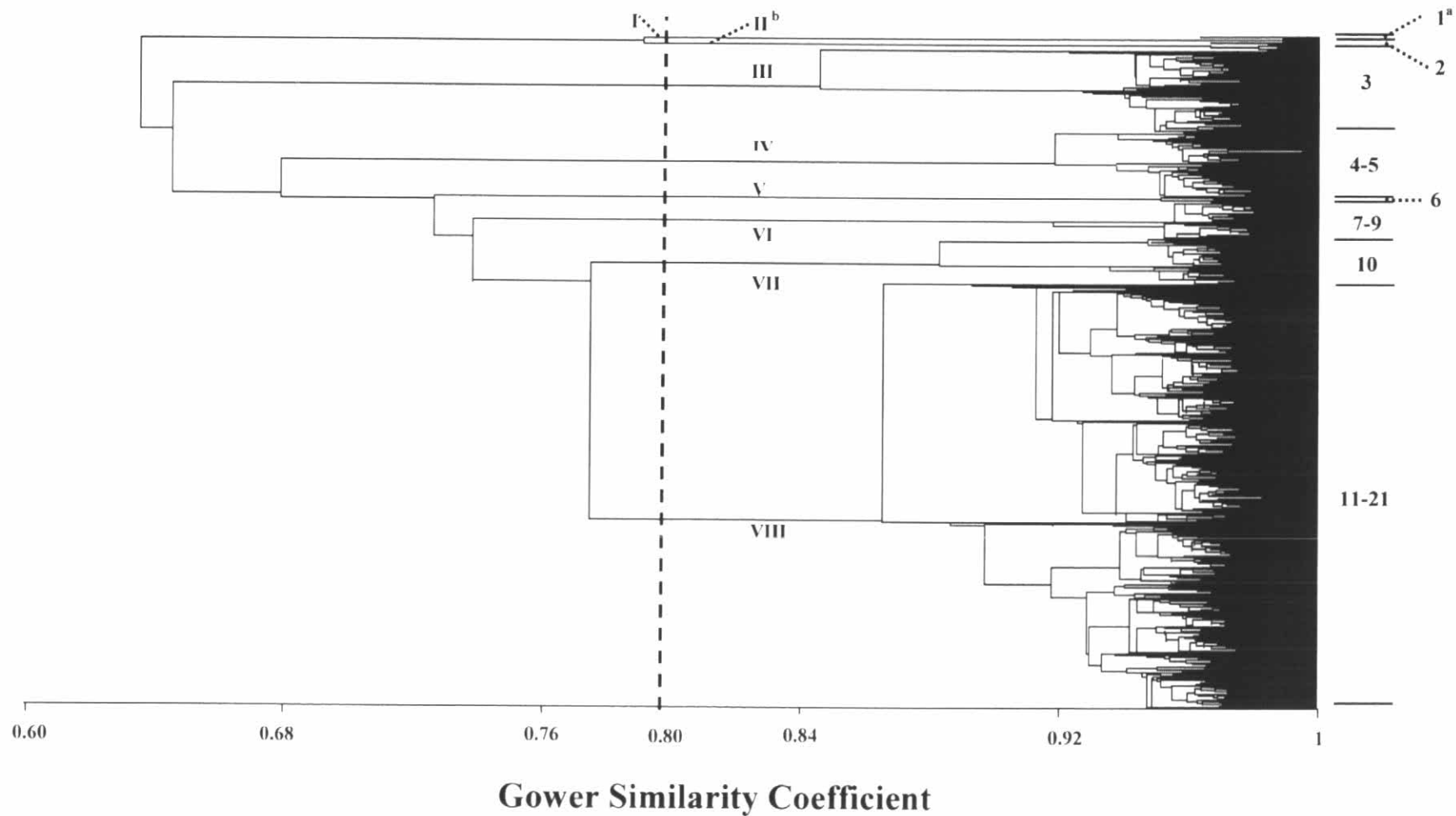


Figure 4.3 UPGMA clustering of 707 OTUs based on Gower Similarity Coefficient calculated between means of 23 qualitative and 35 quantitative characters of the *M. punctatum* (L.) Copel. complex (a. numbers correspond to those in Table 4.1; b. numbers correspond to those in Table 4.3).

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; Nootboom, 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 & Nootboom, 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; Nootboom, 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 Nootboom (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; Nootboom, 1997). Likewise group 7, the largest group, is composed of members of 11 previous treated synonyms of *M. punctatum* (Nootboom, 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.

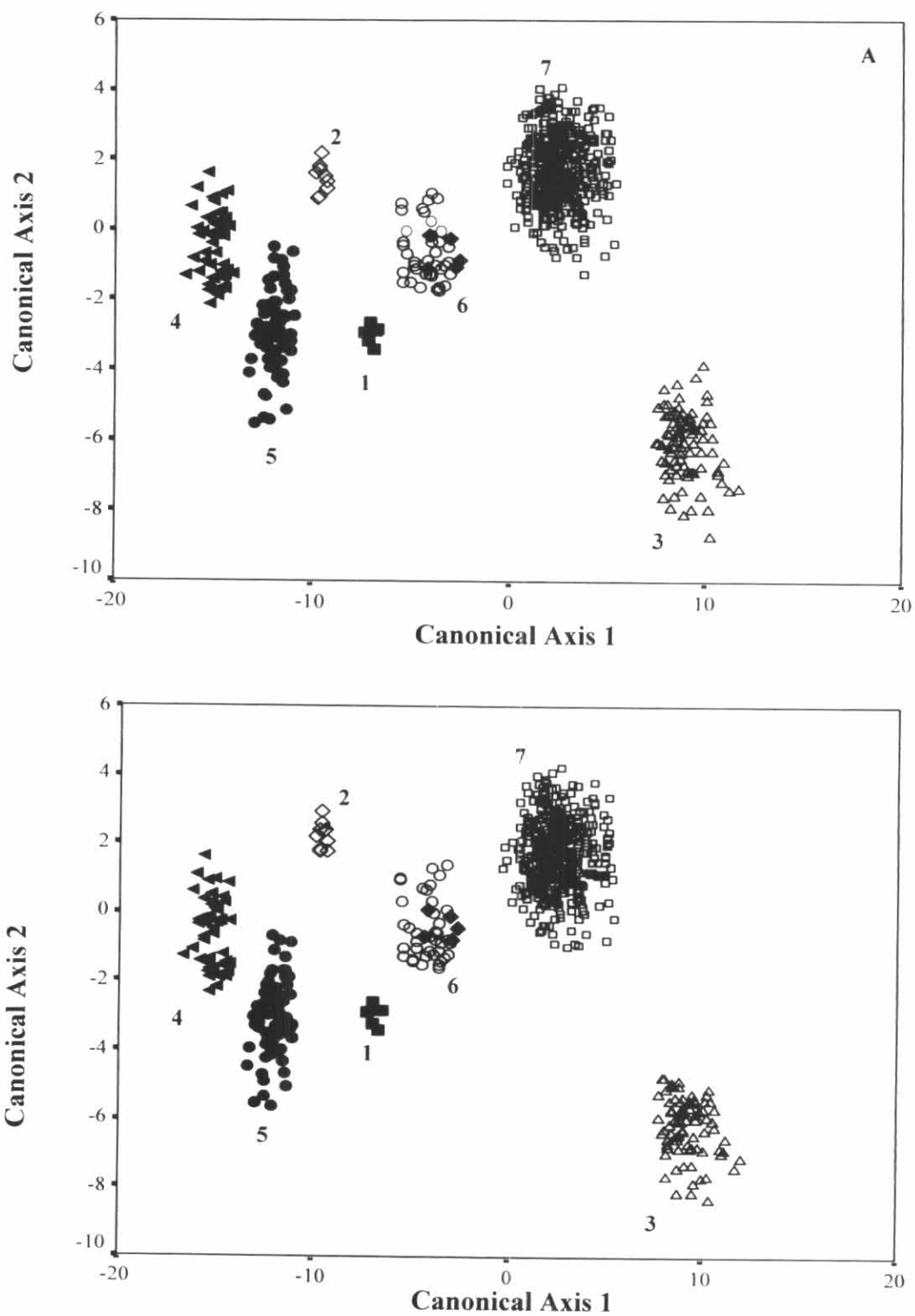


Figure 4.4 The ordination of 707 herbarium specimens of the *M. punctatum* (L.) Copel. complex
 --- A. 8 clustering groupings; B. 21 taxa groupings (1. *M. siamense* (■); 2. *M. thailandicum* (◇);
 3. *M. membranaceum* (△); 4. *M. musifolium* (◄); 5. *M. glossophyllum* (●); 6. *M. steerei* var.
whiteheadii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

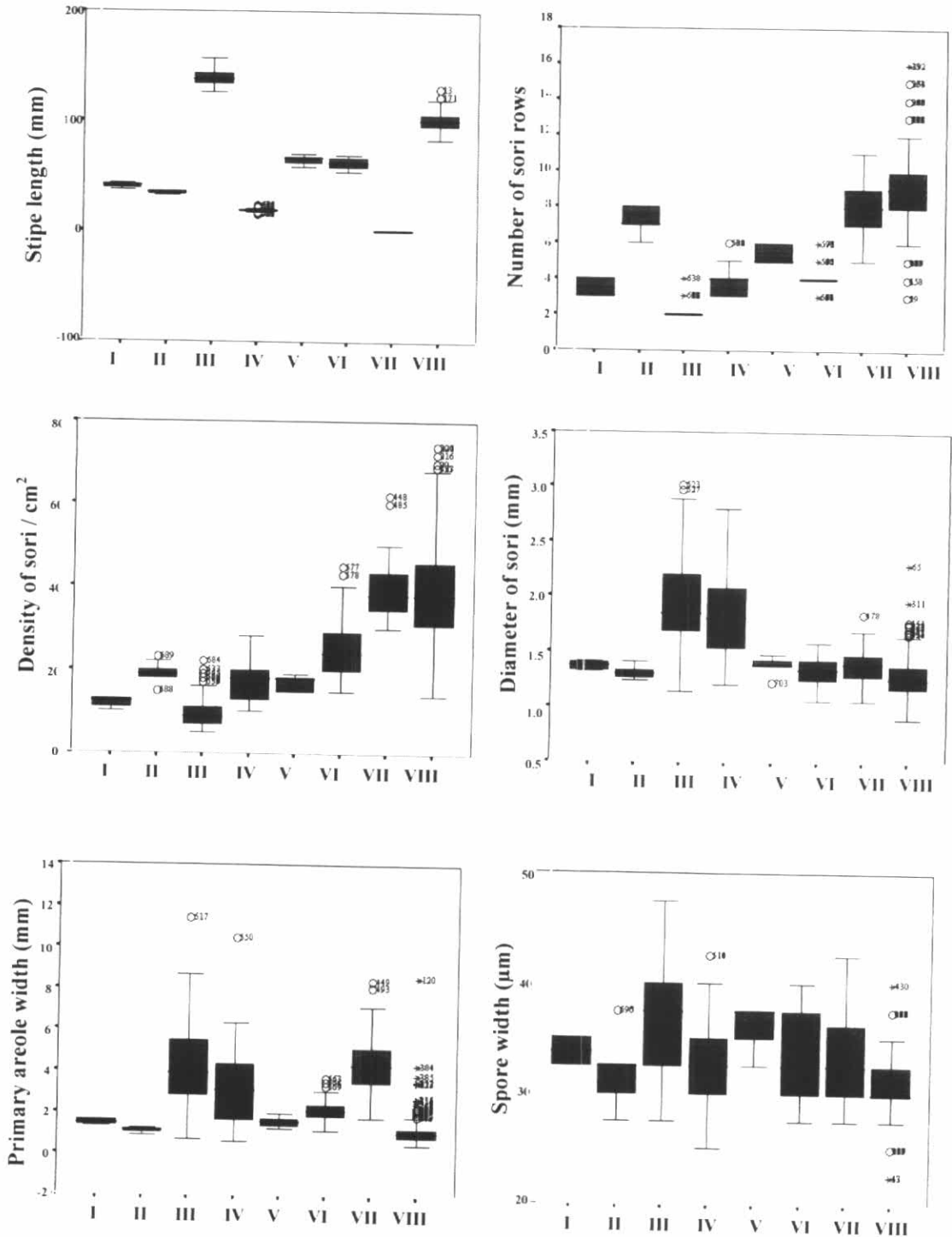


Figure 4.5 Boxplots of the six most important characters of the *M. punctatum* (L.) Copel — I. *Microsorium siamense*; II. *M. thailandicum*; III. *M. membranaceum*; IV. *M. glossophyllum*; V. *M. steerei* var. *whiteheadii*; VI. *M. steerei* var. *steerei*; VII. *M. musifolium*; and VIII. *M. punctatum*.

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*.