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Phenetic study of the *Microsorum punctatum* complex (Polypodiaceae)

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ABSTRACT: *Microsorum punctatum* (L.) Copel. is a widespread species of ferns. It is distributed in the tropics and subtropics of Asia and Africa from sea level to about 2800 m elevation. At present, the taxonomic status and boundary of this species is still uncertain due to the great variation in frond form, size, and venation patterns. These variations depart from previous systematic treatments, so this species group was proposed as a species complex worth investigating. Cluster analysis and canonical discriminant analysis were performed on 694 herbarium specimens deposited at BCU, BKF, BM, K, B, L, and P and consisting of 20 taxa of *Microsorum punctatum* complex. Twenty-five quantitative and 31 qualitative characters were employed. Cluster analysis separated these 20 taxa into seven groups, viz. *M. thailandicum*, *M. membranaceum*, *M. musifolium*, *M. glossophyllum*, *M. siamense*, *M. steerei*, and *M. punctatum*. The seven cluster groups are discussed. From a canonical discriminant analysis using the 20 taxa as priori groups, it can be concluded that *M. siamense*, *M. thailandicum*, *M. membranaceum*, and *M. musifolium* are obviously distinct taxa, while characteristics of *M. glossophyllum* and *Pleopeltis megalosoides* are mixed and should be proposed as the same species. Likewise, specimens of *M. steerei*, *P. tonkinense*, and *P. playfairii* should be recognized as *M. steerei*. Moreover, the specimens of the species previously recognized as the other 10 synonyms of *M. punctatum* are not distinct from *M. punctatum* and are treated here as members of *M. punctatum*. The four most important characters that have separated the seven species are stipe length, density of sori, number of sori rows between adjacent secondary veins, and diameter of sori. These quantitative characters, together with some qualitative characters were coded into a database using Descriptive Language for Taxonomic Analysis (DELTA) software. An identification key to species of the *Microsorum punctatum* complex was then created using the associated program INTKEY. The differentiation between the taxa studied is discussed.

KEYWORDS: systematics, fern, numerical taxonomy, morphometric analysis

INTRODUCTION

Microsorum punctatum (L.) Copel. (Polypodiaceae) is a common species of fern in Africa and Asia and occurs naturally in various forest types of tropics and subtropics from sea level up to 2800 m elevation[1,2]. The most recent treatment of *Microsorum* treated several species as synonyms of this species[3]. For example, *M. musifolium* Copel. and *M. glossophyllum* Copel. were previously recognized as distinct species[2]. Holttum[4] pointed out that dried herbarium specimens of *M. punctatum* are very similar to the narrow-frond specimens of *M. musifolium* and it is not easy to distinguish them. Moreover, Nooteboom[1] noted that *M. musifolium* and *M. glossophyllum* are connected with *M. punctatum* by many intermediates. He also pointed out that *M. membranaceum* and *M. steerei* are very close to *M. punctatum* and that they might be conspecific or in part varieties.

Another related species, *M. polycarpon* (Cav.) Tardieu has different morphological characters of their fronds from *M. punctatum*; but currently was considered as a synonym of *M. punctatum* by some pteridologists[1,4]. Likes *Polypodium punctatum* L. subsp. *subirideum* Christ and *P. punctatum* subsp. *subdrynariaceum* Christ were firstly proposed in 1906 and were finally treated as synonyms of *M. punctatum* in 1998[2,3].

Recently, *M. thailandicum* Boonkerd & Noot., and *M. siamense* Boonkerd were discovered from limestone mountains in southern Thailand[5,6]. These two species were noted that they resemble to the widespread polymorphic *M. punctatum* and *M. steerei*.

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. 'Serratum'. These variants are not included in the previous recognized systematic treatments.

These examples show that *M. punctatum* and related species are not clearly circumscribed or delimited. It seems that the taxonomic status of *M. punctatum* and its related taxa is still unclear. Nooteboom placed these collective taxa, thus as a species complex and suggested that they are worth investigating (personal communication, 9 September 2005).

It can be seen that members of this species complex have a history of circumscriptional uncertainty, suggesting the need for further taxonomic evaluation. The objectives of this work were as follows: (1) to investigate the morphometric relationship and determine the suitability of the species circumscriptions of *M. punctatum* and of other related taxa, and (2) to determine the importance of some macro- and microscopic characters that can be used to distinguish these taxa. With these objectives in mind, both cluster analysis and discriminant analysis were performed based on both qualitative and quantitative characters (i.e., 56 characters) examined from 694 herbarium specimens.

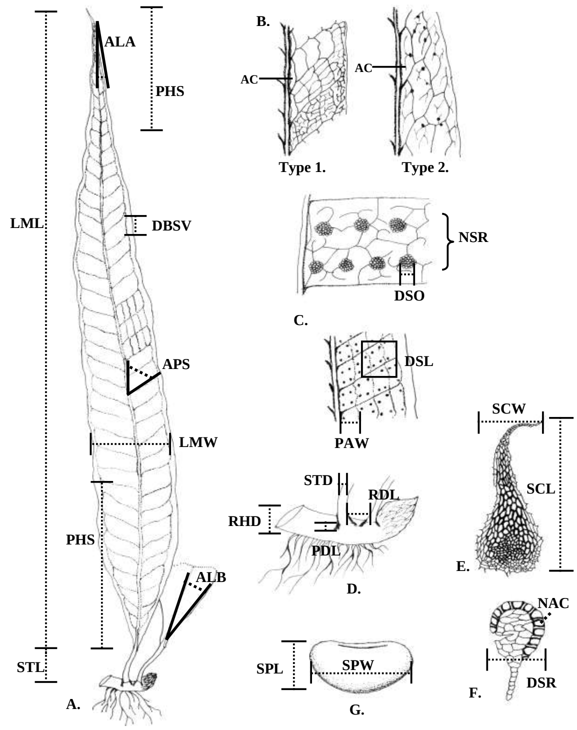

Fig. 1 Measurment of rhizome, scale and frond part. A. plant, B. venation, C. sori distribution, D. rhizome, E. scale, F. sporangium, and G. spore. Noted: The alphabet AC = Costal areole.

MATERIALS AND METHODS

Plant materials

In this study, we examined about 1500 herbarium specimens kept in B, BCU, BKF, BM, K, L, and P (herbarium abbreviations according to Ref. 7). Six hundred and ninety four complete specimens of 20 taxa were used (Table 1), considering each specimen as an operational taxonomic unit (OTU).

Data analysis

Twenty-five quantitative characters (Fig. 1 and Table 2) and thirty-one qualitative characters (Table 3) were subjected to cluster and canonical discriminant analysis (data matrices are available from the corresponding author). Cluster analysis was carried out based on both quantitative and qualitative characters using the Gower similarity coefficient[8] 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 weighed.

A subset of characters that maximized differences among the groups determined by cluster analysis was selected by stepwise discriminant analysis[9]. 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[10]. In comparison, the groupings consisting of the 20 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[10].

For the construction of a key to species, qualitative characters of the segregated taxa were tabulated and compared with the results from morphometric analysis for their importance in discriminating the 20 taxa. Subsequently, the best characters for separating the segregate genera as suggested by DELTA[11] were used.

Table 1 List of the 20 taxa of the *Microsorum punctatum* (L.) Copel. complex and their present status.

No.	Taxon	Taxon according to Nooteboom (1997), Boonkerd and Nooteboom (2001), and Boonkerd (2006)	Taxon according to the present study
1.	*M. siamense* Boonkerd	*M. siamense* Boonkerd	*M. siamense* Boonkerd
2.	*M. thailandicum* Boonkerd & Noot.	*M. thailandicum* Boonkerd & Noot.	*M. thailandicum* Boonkerd & Noot.
3.	*M. membranaceum* (D. Don) Ching	*M. membranaceum* (D. Don) Ching	*M. membranaceum* (D. Don) Ching
4.	*M. glossophyllum* (Copel.) Copel.	*M. punctatum* (L.) Copel.	*M. glossophyllum* (Copel.) Copel.
5.	*Pleopeltis megalosoides* Alderw.	*M. punctatum* (L.) Copel.	*M. glossophyllum* (Copel.) Copel.
6.	*M. musifolium* (Blume) Copel.	*M. punctatum* (L.) Copel.	*M. musifolium* (Blume) Copel.
7.	*M. steerei* (Harr.) Ching	*M. steerei* (Harr.) Ching	*M. steerei* (Harr.) Ching
8.	*P. tonkinense* Baker	*M. steerei* (Harr.) Ching	*M. steerei* (Harr.) Ching
9.	*P. playfairii* Baker	*M. steerei* (Harr.) Ching	*M. steerei* (Harr.) Ching
10.	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
11.	*M. punctatum* ssp. *subirideum* Christ	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
12.	*M. punctatum* ssp. *subdrynariaceum* Christ	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
13.	*Polypodium irioides* Poir.	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
14.	*M. validum* (Copel.) Ching	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
15.	*P. glabrum* Wall.	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
16.	*P. millisorum* Baker	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
17.	*M. sessile* Fée	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
18.	*P. polycarpon* Cav.	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
19.	*M. punctatum* (L.) Copel. 'Serratum'	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.
20.	*M. neoquineense* Copel.	*M. punctatum* (L.) Copel.	*M. punctatum* (L.) Copel.

Table 2 Twenty five 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) 7 clustering groupings and (II) 20 taxa groupings.

No.	Abbrev.	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)	-	-	−0.01	−0.09	250.10	0.00	−0.06	−0.18
2.	LML[a,b]	Lamina length (mm)	-	-	−0.03	0.05	-	-	−0.03	0.04
3.	STL	Stipe length (mm)	4682.59	0.00	**0.87**[c]	0.00	1491.98	0.00	**0.86**	0.02
4.	STD	Stipe diameter (mm)	249.03	0.00	−0.02	−0.05	63.67	0.00	−0.03	−0.06
5.	DMP	Diameter of phyllopodia (mm)	237.82	0.00	−0.07	0.03	57.78	0.00	−0.08	0.02
6.	PDL	Phyllopodia length (mm)	161.23	0.00	−0.08	0.09	32.27	0.00	−0.09	0.09
7.	RHD[a,b]	Rhizome diameter (mm)	-	-	−0.04	−0.04	-	-	−0.05	−0.04
8.	RDL	Distance between closest phyllopodia (mm)	359.81	0.00	−0.13	0.20	101.46	0.00	−0.13	0.20
9.	SCL	Scale length (mm)	140.01	0.00	0.00	−0.19	29.23	0.00	0.00	−0.18
10.	SCW[b]	Scale width (mm)	133.98	0.00	0.00	−0.12	-	-	0.03	−0.08
11.	ALA	Angle of frond apex	169.30	0.00	−0.10	−0.13	34.05	0.00	−0.10	−0.14
12.	ALB	Angle of frond base	731.54	0.00	−0.15	0.03	165.90	0.00	−0.15	0.02
13.	DSO	Diameter of sori (mm)	278.22	0.00	−0.01	**−0.37**	52.30	0.00	−0.01	**−0.36**
14.	DSR[a,b]	Diameter of sporangium (mm)	-	-	0.00	−0.05	-	-	−0.01	−0.05
15.	NAC	Number of annulus cell	423.82	0.00	−0.03	−0.02	126.09	0.00	−0.03	−0.01
16.	SPL	Spore length (mm)	219.42	0.00	0.02	−0.19	44.13	0.00	0.02	−0.19
17.	SPW[b]	Spore width (mm)	146.42	0.00	0.01	**−0.16**	-	-	0.00	−0.07
18.	DSL	Density of sori per cm^2	203.27	0.00	0.00	**0.43**	41.00	0.00	0.00	**0.42**
19.	PLS	Distance between lowest sori and frond base (mm)	178.92	0.00	−0.05	0.12	47.79	0.00	−0.06	0.12
20.	PHS	Distance between highest sori and frond apex (mm)	128.51	0.00	−0.02	−0.06	30.72	0.00	−0.02	−0.07
21.	NSR	Number of sori rows between closest secondary vein	1325.89	0.00	0.02	**0.57**	439.81	0.00	0.02	**0.58**
22.	DBSV	Distance between closest secondary vein (mm)	190.00	0.00	0.02	0.22	38.34	0.00	0.02	0.23
23.	APS	Angle between primary and secondary vein	529.00	0.00	−0.02	0.29	85.67	0.00	−0.02	0.32
24.	SAW	Secondary areole width in mm	153.54	0.00	0.01	−0.01	36.12	0.00	0.01	−0.03
25.	PAW	Primary areole width (mm)	314.46	0.00	−0.05	−0.38	72.90	0.00	−0.05	−0.37

[a,b] 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.
[c] Numbers in bold are the important variables associated with each axis.

Table 3 Comparison of 31 qualitative characters of the seven clustering groupings.

No.	Character	Taxa						
		I	II	III	IV	V	VI	VII
1.	Shape of rhizome in transverse section: (1) approximately cylindrical, (2) dorso-ventrally slightly flattened or flattened	1	1,2	1,2	1,2	1	1,2	1,2
2.	Rhizome surface: (1) not waxy, (2) at least sometimes waxy under the scales or often waxy	1	1	1	2	1	2	2
3.	Differentiation of vascular bundle sheaths: (1) vascular bundle sheaths be parenchymatous, (2) vascular bundle sheaths be collenchymatous	1	1	2	2	1	1	1
4.	Attachment of scales: (1) pseudopeltate, (2) peltate	1	1	2	1	2	1	1
5.	Density of scales: (1) densely set, (2) apically densely set or moderately densely set	1	2	2	1	1	2	2
6.	Spreading of scales: (1) distinctly or slightly spreading, (2) appressed	1	1	2	1	1	1	1
7.	Scales shape: (1) narrowly ovate to ovate, (2) triangular	1,2	1,2	1	1	1,2	1,2	1,2
8.	Scales margin: (1) entire, (2) dentate to denticulate	2	1	1	2	2	2	2
9.	Scales apex: (1) acute (2) acuminate to slightly caudate	2	1,2	2	2	2	1,2	2
10.	Presence of hyaline margin on rhizome scales: (1) absent, (2) present	1	2	2	1	1	1	1
11.	Indumenta type of central region of scales: (1) dark black on central region or glabrous, (2) bearing multiseptate hairs at least when young	1	2	1	1	2	1	1
12.	Scales translucence: (1) opaque and blackish, (2) translucent and brownish	2	2	2	1	2	2	2
13.	Phyllopodia distinctness: (1) distinct, (2) obscure	1	1	2	1	1	1	1
14.	Lamina texture: (1) membranaceous, (2) firm herbaceous, (3) subcoriaceous-coriaceous	3	1	2,3	2	3	3	2,3
15.	Frond colour when living: (1) light to dark green, (2) iridescent blue-green	2	1	1	1	2	1	1
16.	Lamina shape: (1) linear, (2) (narrowly) elliptic, (3) (narrowly) ovate, (4) narrowly obovate to oblanceolate	1	1–3	4	4	2	4	1–4
17.	Lamina base: (1) cuneate, (2) angustate, (3) truncate to obtuse, (4) attenuate, (5) abruptly narrowed	5	2	3	1	4	2	2
18.	Margins of lamina: (1) margin entire, (2) margin entire, undulate	1	1	1	1	2	1	1,2
19.	Presence of indumentum on fronds: (1) with only scales, (2) with a few scales and short glandular hairs, (3) with only short glandular hairs	3	3	2	1	3	3	2
20.	Presence of stipe: (1) present, (2) absent or obscure	1	1	2	1	1	2	1
21.	Midrib character: (1) slightly raised or raised on both surface, (2) raised on lower surface, grooved on upper surface	2	1	1	1	1	1	1
22.	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	2	1	1	1	1	1	1
23.	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	1	1	1	2	2	2
24.	Branching of included free veinlet venation: (1) free veinlet simple and once-forked, (2) free veinlet simple, once or twice forked,	2	2	1	1	1	2	1
25.	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 (2-) 3–10 (-15) irregular rows parallel to each pair of secondary veins	1	2	3	3	1	3	3
26.	Visibility of hydathodes: (1) distinct, (2) Indistinct	2	2	2	2	1	2	2
27.	Sori position on lamina surface: (1) superficial, (2) slightly immersed	2	1	1	1	1	1,2	1
28.	Sori distribution: (1) on the whole surface of the lamina or restricted up to the distal 1/2, (2) absent from the basal parts for 1/5–4/5 of total length of lamina	2	1,2	1,2	2	2	2	1
29.	Spores shape: (1) plano-convex, (2) concavo-convex	2	2	2	1,2	2	2	1
30.	Spores colour: (1) hyaline, (2) yellowish hyaline, (3) yellow	2	3	1	1	3	1	1,2
31.	Spore surface: (1) plain to slightly verrucate, (2) irregularly rugate	1	2	1	1	1	1	1

I = *Microsorum thailandicum*, II = *M. membranaceum*, III = *M. musifolium*, IV = *M. glossophyllum*, V = *M. siamense*, VI = *M. steerei*, and VII = *M. punctatum*.

QUANTITATIVE CHARACTERS

Cluster analysis

Firstly, 31 qualitative and 25 quantitative characters were used in this analysis and as a result the dendrogram showed the segregation of 694 specimens into seven groups at the 0.90 phenon level of Gower similarity coefficient (Fig. 2). Specimens classified as group I to group III consisted of *M. thailandicum* (1), *M. membranaceum* (2), and *M. musifolium* (3), respectively. All specimens of *M. glossophyllum* (4) and *Pleopeltis megalosoroides* (5) were placed in group IV. Group V included all specimens of *M. siamense* (6), while those of *M. steerei* (7) and its synonyms, i.e., *Polypodium tonkinense* (8), and *P. playfairii* (9) were placed in group VI. The last group, group VII,

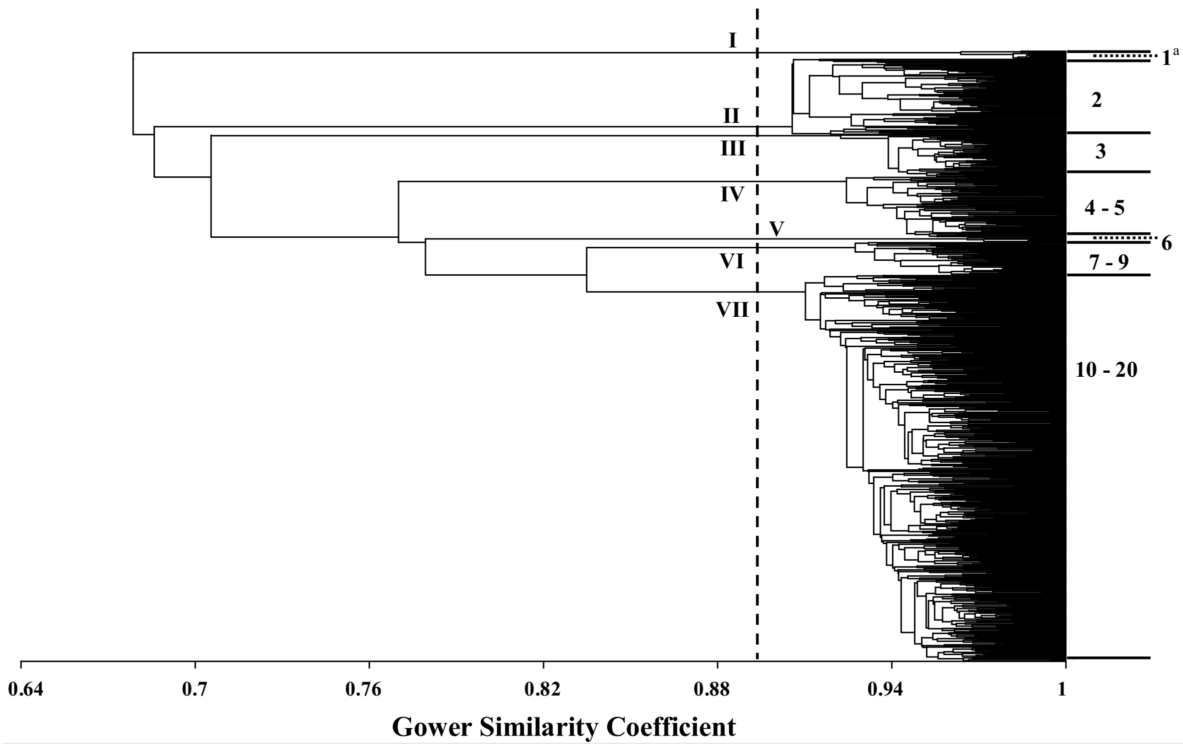

Fig. 2 UPGMA clustering of 694 OTUs based on Gower Similarity Coefficient calculated between means of 31 qualitative and 25 quantitative characters of the *M. punctatum* (L.) Copel. complex (a = numbers correspond to those in Table 1, I = *Microsorum thailandicum*, II = *M. membranaceum*, III = *M. musifolium*, IV = *M. glossophyllum*, V = *M. siamense*, VI = *M. steerei*, and VII = *M. punctatum*).

consisted of specimens of *M. punctatum* (10) and its eleven synonyms (11–20).

Likewise, the results obtained from only 25 quantitative characters showed segregated groups of 694 specimens at a high phenon level of Gower similarity coefficient (Fig. 3). Specimens of *M. membranaceum* (Group II in both of 31 qualitative and 25 quantitative characters analysis), *M. musifolium* (III), *M. glossophyllum* and *Pleopeltis megalosoides* (IV), *M. thailandicum* (I), and *M. punctatum* and its eleven synonyms (VII) were classified as group A, B, C, D, and F, respectively. Group E, on the other hand, was consisted of 2 subgroups: *M. siamense* (V) and *M. steerei* (VI). First subgroup is solely *M. siamense* (V). The second subgroup is composed of all specimens of *M. steerei* and its synonyms (VI). Specimens of both subgroups, however, are separated from each other.

From both results, it can be seen that the specimens of *M. thailandicum*, *M. membranaceum*, and *M. musifolium* are distinct species. The specimens of *Pleopeltis megalosoides*, which was previously treated as a synonym of *M. glossophyllum* by Bosman[3], and the specimens of *M. glossophyllum* were placed together in both analyses (Fig. 2 and Fig. 3). Then, the group of these specimens should be considered a distinct species, i.e., *M. glossophyllum*. Likewise, *M. steerei* and its two synonyms, *Polypodium tonkinense* and *P. playfairii*[1,3], were placed in the same group in both results and should also constitute distinct species. The largest group, *M. punctatum* and its previously treated synonyms, is also recognized here as a distinct species from the result of both cluster analysis. Theses cluster groupings were supported by previous taxonomic work[3,5,6,12].

The results from the second cluster analysis indicated that *M. siamense* and *M. steerei* are closely related species (Fig. 3) since some quantitative characters of these two species overlapped. However, they do separate when both qualitative and quantitative characters were employed (Fig. 2) or only qualitative characters, such as rhizome surface (2), attachment of scales (4), frond colour when living (15), lamina base (17), margins of lamina (18), the presence of distinct hydathodes (26), spores colour (30) were in consideration (Table 3). Our findings clearly emphasize

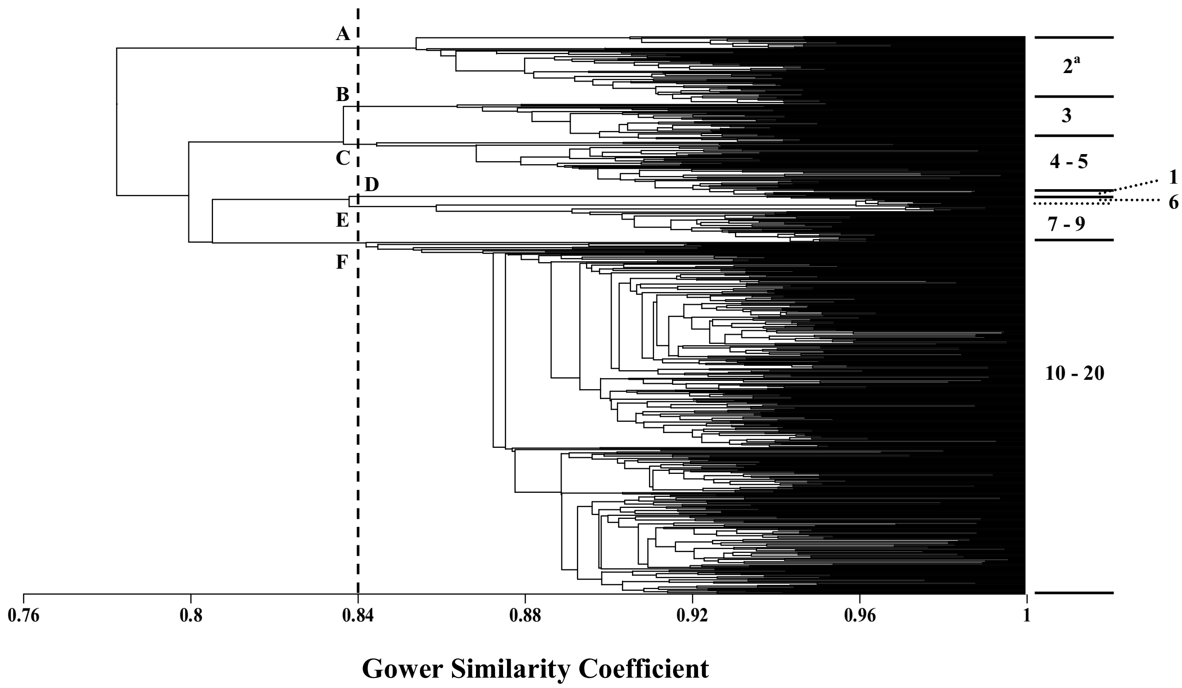

Fig. 3 UPGMA clustering of 694 OTUs based on Gower Similarity Coefficient calculated between means of 25 quantitative characters of the *M. punctatum* (L.) Copel. complex (a = numbers correspond to those in Table 1, A = *M. membranaceum*, B = *M. musifolium*, C = *M. glossophyllum*, D = *Microsorum thailandicum*, E = *M. siamense*, *M. steerei*, and F = *M. punctatum*).

the importance of both qualitative and quantitative characters in plant classification and identification.

Canonical discriminant analysis

The seven-cluster groupings: When using the results of the groupings from cluster analysis as a priori groups, specimens of the 20 taxa can be separated into 7 groups (Fig. 4a) on canonical axis 1. They, however, were not clearly distinct on canonical axis 2. The nature of the group differences is demonstrated by the pooled within a canonical structure (Table 2). Canonical variable 1 is 99.1% correlated with 21 characters and the variance explained is 79.0% (data matrix available on request from the corresponding author). It is most highly associated with stipe length (3). Canonical variable 2 explains 11.6% of the total variance. This axis is most highly associated with DSO (13), SPW (17), DSL (18), as well as NSR (21). The four variables LMW (1), LML (2), RHD (7), and DSR (14) were not selected by stepwise discriminant analysis to be used in further canonical discriminant analysis (Table 2).

The canonical plot on two canonical axes (Fig. 4a) shows the division of specimens into 7 groups on canonical axis 1, but was not clearly distinct on canonical axis 2. It is evident from both cluster analysis (Fig. 2) and canonical discriminant analysis (Fig. 4a) that the 694 specimens collectively grouped in to 7 distinct species, namely *M. thailandicum* (I), *M. membranaceum* (II), *M. musifolium* (III), *M. glossophyllum* (IV), *M. siamense* (V), *M. steerei* (VI), and *M. punctatum* (VII).

Regarding *M. membranaceum*, this species clearly separated from *M. punctatum* in both cluster analysis (Fig. 2) and canonical discriminant analysis (Fig. 4a). Although Nooteboom[1] suggested that *M. membranaceum* might be a variety of *M. punctatum*. They also clearly differ in at least 8 qualitative characters, namely rhizome surface (2), scale margin (8), presence of hyaline margin on rhizome scales (10), lamina texture (14), visibility of veins (23), spore shape (29), spore colour (30) and spore surface (31). As regard to their habitats *M. membranaceum* usually occurs above 600 m altitude whereas *M. punctatum* has a much wider vertical distribution from sea level to 2800 m altitude[1]. These two species also differ in life-span of fronds. *M. membranaceum* usually sheds its fronds annually while *M. punctatum* has perennial fronds[1].

In previous treatment of the microsoroid ferns, Nooteboom[1] recognized *M. musifolium* as a synonym of *M. punctatum* and noted that this species is a

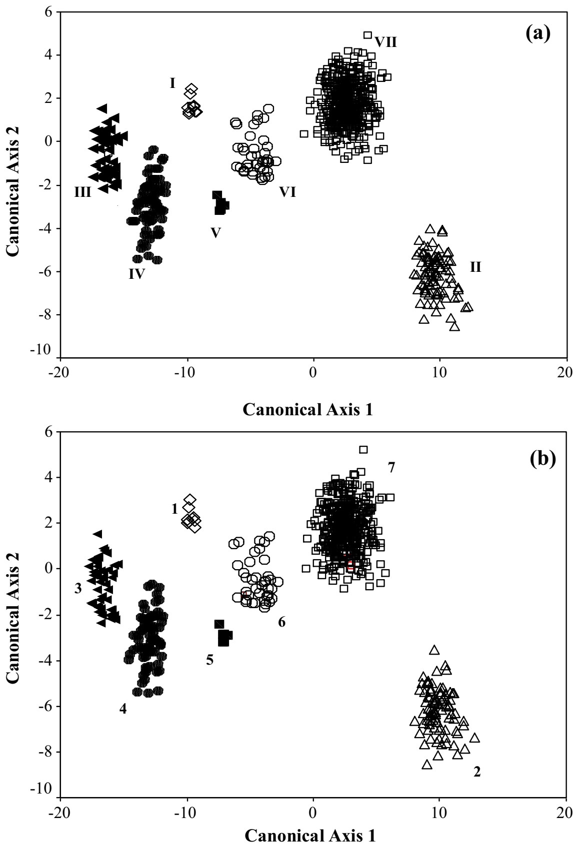

Fig. 4 The ordination of 694 herbarium specimens of the *M. punctatum* (L.) Copel. complex **A** 7 clustering groupings, **B** 20 taxa groupings :- 1 = *Microsorum thailandicum* (◊); 2 = *M. membranaceum* (△); 3 = *M. musifolium* (◀); 4 = *M. glossophyllum* (●); 5 = *M. siamense* (■); 6 = *M. steerei* (○); and 7 = *M. punctatum* (□).

form with broader leaves and more connecting veins, connected with *M. punctatum* with many intermediates. The results of cluster analysis (Fig. 2 and Fig. 3) and canonical discriminant analysis (Fig. 4) clearly indicated the distinction between these two taxa. This finding also corresponds with the difference in qualitative characters of at least 8 characters, namely, rhizome surface (2), differentiation of vascular bundle sheaths (3), attachment of scales (4), spreading of scales (6), scale margin (8), lamina base (17), visibility of veins (23), and spore shape (29). Hence *M. musifolium* should be recognized as its own species.

Microsorum glossophyllum is another species that was previously treated as a synonym of *M. punctatum*[1,3]. In contrast, our results from cluster analysis (Fig. 2 and Fig. 3) and canonical discriminant analysis (Fig. 4) strongly supported the segregation of this species from *M. punctatum*. The two species also differ in at least 7 qualitative characters (Table 3), namely differentiation of vascular bundle sheaths (3), density of scales (5), scale translucence (12), lamina base (17), presence of indumentum (19), visibility of veins (23), and sori distribution (28). Thus it is thus clear that *M. glossophyllum* constitute a distinct species, as Bosman has suggested[3].

Likewise, the dendrogram of cluster analysis using only 25 quantitative characters (Fig. 3) showed the separation of *M. siamense* and *M. steerei* into two subgroups. The result of canonical discriminant analysis, also, using the same set of data (Fig. 4a) showed a distinction between *M. siamense* (V) and *M. steerei* (VI). It can be seen from Fig. 4a that the phenetic gaps between groups are larger than those within groups. This finding agrees with the results of morphological studies based on qualitative characters (Table 3) that the two taxa differ in at least 7 characters, i.e., rhizome surface (2), attachment of scales (4), frond colour when living (15), lamina base (17), margins of lamina (18), the presence of distinct hydathodes (26), and spores colour (30).

It was earlier suggested that *M. steerei* might be a reduced form or a variety of *M. punctatum*[1]. Our results based solely on qualitative characters (Table 3) and cluster analysis using both qualitative and quantitative characters (Fig. 2) agreed well with this suggestion. However, cluster analysis using only quantitative characters (Fig. 3) and canonical discriminant analysis using the same data set (Fig. 4a) showed distinction between these two species. This discrepancy needs further investigation, especially on molecular data, which is beyond the scope of this study.

The 20 taxa groupings: Twenty taxa (Table 1) were used as a priori group. Twenty-five quantitative characters were used in this analysis (Table 2). The canonical variable 1 is 99.1% correlated with 20 characters and the variance explained by it is 76.6%. It is most highly associated with stipe length (3). Canonical variable 2 accounted for 11.3% of the total variance which is the axis most highly associated with DSO (13), DSL (18) and NSR (21). According to the stepwise discriminant analysis five variables, viz. LML (2), RHD (7), SCW (10), DSR (14), and SPW (17) were not selected for further use in canonical discriminant analysis (Table 2).

The canonical plot on two canonical axes (Fig. 4b) also shows the separation of 694 herbarium specimens of the 20 taxa (Table 1) into 7 groups on canonical axis 1, but is not clearly distinct on canonical axis 2. Specimens of *M. thailandicum*, *M. membranaceum*, *M. musifolium* and *M. siamense* were included to group 1(◊), 2(Δ), 3(◀) and 5(■), respectively, whereas group 4(●) is comprised of all specimens of *M. glossophyllum* and its previous treated synonyms[3]. All specimens of *M. steerei*, *Polypodium tonkinense* and *P. playfairii* were mixed in group 6(**o**). Likewise, members of the 11 previously treated synonyms of *M. punctatum*[1–3] were pooled into the largest group, i.e., group 7(□).

Based on the results of cluster analysis together with canonical discriminant analysis, it is reasonable to divide the 20 taxa (Table 1) of the *M. punctatum* complex into seven distinct taxa, viz. *M. thailandicum*, *M. membranaceum*, *M. musifolium*, *M. siamense*, *M. glossophyllum*, *M. steerei*, and *M. punctatum*, respectively.

Boxplots of the four most important characters, namely stipe length (STL), density of sori/cm^2 (DSL), number of sori rows between closest secondary vein (NSR), and diameter of sori (DSO) that collectively segregated these seven taxa are presented in Fig. 5.

Classificatory discriminant analysis

The stepwise discriminant analysis suggested that twenty-one of the twenty-five quantitative characters were essential for the division of the seven taxa (Table 2).

The linear discriminant function classification result appears to be 100.0% correct. For this reason, the linear discriminant function (Table 4) can be used to identify unnamed specimens in the *M. punctatum* complex. To employ the discriminant function in Table 4 for identification, it is necessary to multiply each character score by its coefficient in each column. Then the total in each column is calculated, 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.

QUALITATIVE CHARACTERS

Table 3 shows the summary of thirty-one qualitative characters of the seven recognized taxa. Rhizome scales character (the presence of hyaline marginal region) is useful in separating the *Microsorum punctatum* complex into 2 groups. Group I is composed of 2 species, namely *M. membranaceum* and *M. musifolium*. Scale attachment, shape and texture of lamina, and spore surface are valuable characters to segregate each species. Group II included all specimens of *M. punctatum*, *M. glossophyllum*, *M. steerei*, *M. thailandicum* and *M. siamense*, the last two species can be separated from the other species by the presence of iridescent blue-green fronds. *M. glossophyllum* can also be separated from *M. punctatum* by using character of lamina indumenta and sori distribution pattern. It seems likely that only quantitative characters are not clearly placed the seven taxa into distinct taxa.

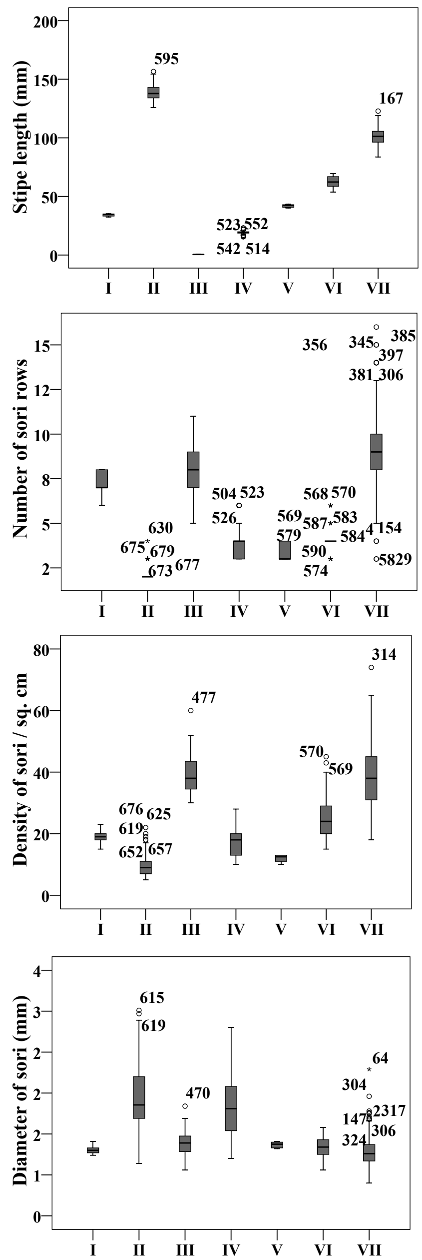

Fig. 5 Boxplots of the four most important characters of the *M. punctatum* (L.) Copel. (I = *Microsorum thailandicum*, II = *M. membranaceum*, III = *M. musifolium*, IV = *M. glossophyllum*, V = *M. siamense*, VI = *M. steerei*, and VII = *M. punctatum*).

Table 4 Classification Function Coefficients of seven clustering groups obtained from cluster analysis based on 21 quantitative characters.

Character	Categories						
	I	II	III	IV	V	VI	VII
STL	0.39	3.90	−0.53	−0.01	0.85	1.43	2.70
STD	1.21	7.83	2.91	0.42	2.57	−4.57	3.04
DMP	1.51	−3.62	−2.21	1.90	−2.48	4.36	−0.91
PDL	0.31	−5.84	2.85	1.55	0.87	−0.75	−2.91
RDL	0.40	0.76	2.00	2.39	1.06	0.94	1.75
SCL	2.54	2.01	3.61	4.33	0.64	4.40	1.27
SCW	3.51	0.31	7.12	−0.53	10.06	−1.81	−0.89
ALA	−0.11	0.34	1.01	0.79	1.08	0.81	0.28
ALB	0.29	1.22	2.55	0.61	0.29	0.37	1.14
DSO	34.49	46.71	37.64	47.31	35.85	36.55	35.08
NAC	22.50	13.95	15.33	15.60	15.91	16.56	14.76
SPL	1.21	1.70	1.49	2.17	1.56	1.47	1.63
SPW	1.70	1.57	1.46	1.04	1.52	1.51	1.21
DSL	0.60	0.71	1.05	0.77	0.58	0.89	0.98
PLS	−0.01	−0.02	0.01	0.01	−0.02	−0.01	0.00
PHS	0.38	0.61	0.90	0.71	0.57	0.72	0.61
NSR	1.94	−0.31	0.66	−0.14	0.57	0.44	1.69
DBSV	2.87	−0.54	1.51	0.52	0.45	0.69	0.33
APS	1.95	1.13	1.23	1.32	1.23	1.91	1.68
SAW	−1.06	3.16	0.11	0.90	1.11	1.66	2.84
PAW	−2.98	−1.54	−0.88	−2.40	−2.76	−2.38	−4.26
(Constant)	−497.09	−569.17	−363.41	−359.65	−325.02	−394.29	−445.24

I = *Microsorum thailandicum*, II = *M. membranaceum*, III = *M. musifolium*, IV = *M. glossophyllum*, V = *M. siamense*, VI = *M. steerei*, and VII = *M. punctatum*.

Table 5 Means and standard deviation of 25 quantitative characters of the seven clustering groupings of the *M. punctatum* (L.) Copel. complex.

Character	I		II		III		IV		V		VI		VII	
	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE
LMW	16.97	1.48	72.64	26.81	96.53	16.45	77.66	10.77	37.63	3.33	39.33	10.58	57.84	18.38
LML	304.54	52.37	536.25	184.31	831.55	156.73	958.36	134.75	230.62	3.11	317.22	66.39	735.91	437.00
STL	34.19	1.05	138.77	6.47	0.48	0.11	19.28	1.65	42.01	1.27	62.34	4.61	101.38	6.96
STD	2.92	0.18	4.75	0.97	5.75	1.04	5.37	1.21	2.49	0.11	1.54	0.36	4.53	0.92
DMP	2.84	0.29	3.46	1.05	5.56	1.33	6.19	1.43	1.79	0.11	3.01	0.55	4.39	1.06
PDL	3.41	0.32	2.67	0.88	6.13	1.25	5.87	1.45	3.19	0.39	3.28	1.15	4.40	1.27
RHD	4.93	0.25	5.56	1.47	6.98	1.40	7.51	1.51	3.63	0.32	4.99	0.88	5.74	1.25
RDL	1.63	0.19	2.83	1.32	12.45	2.99	11.60	2.70	4.00	0.20	3.48	1.64	8.20	1.88
SCL	3.02	0.20	4.42	1.34	3.64	0.40	4.01	0.64	2.60	0.27	4.03	1.03	3.30	0.62
SCW	1.15	0.08	1.46	0.36	1.42	0.21	1.25	0.18	1.24	0.06	1.04	0.19	1.18	0.24
ALA	7.44	1.24	14.25	4.36	23.16	5.17	19.32	3.56	19.33	0.82	16.71	5.53	12.96	3.03
ALB	1.78	0.83	2.02	1.09	18.93	5.98	6.04	1.52	2.50	0.55	1.50	0.66	4.20	2.52
DSO	1.31	0.06	1.93	0.39	1.39	0.15	1.85	0.41	1.37	0.04	1.33	0.13	1.28	0.17
DSR	0.18	0.01	0.19	0.02	0.16	0.01	0.19	0.02	0.18	0.01	0.16	0.03	0.17	0.15
NAC	28.33	1.80	19.41	1.76	18.90	0.75	19.78	0.97	20.83	0.75	21.56	1.37	19.26	1.00
SPL	45.22	1.89	58.75	8.14	47.71	4.89	57.97	4.68	51.25	1.37	50.64	4.16	51.70	4.11
SPW	31.94	3.70	36.60	5.57	33.33	3.62	32.53	3.94	33.75	1.37	33.27	3.98	31.39	3.11
DSL	19.11	2.37	9.87	3.78	39.50	6.59	17.34	4.63	12.00	1.26	25.97	7.92	38.42	9.97
PLS	138.91	17.19	196.78	113.64	421.81	112.53	517.69	144.79	59.44	2.81	156.98	43.55	381.50	120.39
PHS	5.01	1.75	16.79	6.07	20.05	4.38	17.66	7.57	11.22	1.15	16.90	7.13	14.38	6.62
NSR	7.22	0.67	2.24	0.46	8.08	1.47	4.01	0.87	3.33	0.52	4.10	0.88	9.35	1.96
DBSV	16.22	0.63	10.03	2.36	13.27	1.95	8.66	1.68	7.10	0.64	9.05	1.87	13.27	2.63
APS	49.78	1.72	21.51	3.57	27.74	3.60	29.10	4.84	29.50	1.52	44.73	6.04	34.02	5.43
SAW	1.80	0.13	6.80	1.58	7.28	1.40	6.94	1.43	4.29	0.27	3.96	0.78	6.64	1.95
PAW	1.09	0.11	4.14	1.97	4.24	1.33	3.11	1.73	1.46	0.13	2.16	0.59	1.10	0.56

I = *Microsorum thailandicum*, II = *M. membranaceum*, III = *M. musifolium*, IV = *M. glossophyllum*, V = *M. siamense*, VI = *M. steerei*, and VII = *M. punctatum*.

Qualitative and quantitative characters were therefore, collectively used to construct a key to these seven segregated taxa.

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[4], should be treated as two distinct species as suggested by the results from our study. This study supports the recognition of *M. steerei* and its previous treated synonyms, i.e., *Polypodium tonkinense*, and *P. playfairii*. Finally, we also found that the other synonymous species and infraspecific taxa related to *M. punctatum* (Table 1) should be placed in *M. punctatum*.

To sum up, the results from our study provide justification for the recognition of seven species, namely *M. thailandicum*, *M. membranaceum*, *M. musifolium*, *M. siamense*, *M. glossophyllum*, *M. steerei*, and *M. punctatum* within the *M. punctatum* complex.

IDENTIFICATION LIST

Only specimens with an identifiable collector and collection number are mentioned. Numbers after the colon refer to the species numbers as given in the following list. (T) denotes a type specimen.

1. *Microsorum steerei*
2. *M. siamense*
3. *M. thailandicum*
4. *M. membranaceum*
5. *M. glossophyllum*
6. *M. musifolium*
7. *M. punctatum*

Abeysiri 55: 4 — Abraham 666: 7 — d'Alleizette herb 460 T: 1 — Allen 7, 173: 7 — Andrews 108: 7 — Antun-Cupffert 337: 7 — Ashton 19 060: 7 — Avon 370: 7.

B200091734: 1 — B 200 099 607, 200 099 652, 200 099 749: 7 — J.M. Baker 84: 7 — Bakhuizen van den Brink 5739: 7 — Bakshi 207: 7 — Balansa 45, 70, 107 (T), 148 (T), 198: 1; 1990: 4 — van Balgooy 2329, 4628: 7 — Balker 339: 4 — Balslev 342: 7 — Banerji et al 1313, 2604, 26 957: 4 — Barrett (&Dorr) 201: 7 — Beckett 648: 7 — Beddome 48/341: 7 (T); 67, 101, 159, 177, 339: 4; 1911: 6; 1991: 7 — Beer 7768: 7 — Ben 438: 7 — Benson 106, 1293: 7 — Bernhardi 234: 6 — van Beusekom et al 258, 683: 7 — Bhargava et al 2347, 2836, 6356: 7— Bidgood 4775: 7 — Blackwood 188: 5 — Bliss 41, 51, 189: 4 — Bloembergen 18, 3424: 7 — Blume (n.v.): 6 (T)— Bon 200, 1274: 1; 2119: 7 — Boonkerd 1442: 3 (T) — van Borssum Waalkes 3053, 3228: 7 — Bos 4106, 4218: 7 — Botavae 74: 7 — Bourdy 306: 7 (T) — Braker 4136: 7 — Braithwaite 2306, 2570, 4136, 4370: 7; 4721, 4866: 5 — Brass 535, 673, 2756: 7; 11 319, 23 055: 5; 24 220: 7; 24 483: 5; 25 458, 29 373: 7; 27 212, 29 549, 29 786, 30 498, 31 569, 32 403: 5 — Brooks 357: 6; 416: 7 — Brownlie 1304, 1384, 8454, 16 074: 7 — Bryan 1114, 1167: 7 — Bulmer 103 837: 5 — Bunk 384: 7 — Bünnemeijer 1848, 3519, 4315, 12 427: 7 — Buwalda 4159, 6052, 6336, 6978: 7.

Cadet 3824: 7 — Cadière 30: 1; 98: 7 — Carr 7660: 5; 12 148: 7; 13 015, 13 340: 5 — Caruallo 2279, 4235: 7 — Castro 5910: 7 — Cavalerie (& Fortunat) 2635:7; 3390: 4; 4012: 7 — Chapman 581, 3132: 7 — Chase 5220: 7 — Chevalier 21 088: 7 — Christ 1940: 1 — Christensen 529: 7; 1339, 3418: 1 — Chusan 1847: 7 — Clarke 8739, 21 388, 27 186, 33 720: 4; 40 735, 43 399: 7 — Clemens & Clemens 7133: 5 — Combes 4097: 7 — Conn 152: 5; (& Kairo) 157: 5 — Coode 6251: 7 — Cooks 1909: 7 — Copeland 275: 7; 388: 5 (T); 1535: 7; 1537: 6; 1776, 15 356: 7 — Corner 30 247: 6 — Coveny 6900: 7 — Croft 151:5; 199: 4; 203, 451, 533, 568:5; 1129: 7; 1728:5; 61 266, 61 160, 61 578, 65 453: 7; 65 719: 5 — Cusclah 17 347: 7.

Darbyshire 624: 7 — Darnaedi 71, 2107: 7 — Dawkins 389: 7 — Decary 17 754: 7 — Deighton 6056: 7 — Deplanche 23, 198: 7 — Dickason 7637, 7986: 7; 8118, 9029: 4 — Dümmer 472: 7.

EBL 1537: 6 — Edelfelt 220: 5 — Edward 38, 2411: 1 — Elbert 913: 7 — Elmer 5873: 4; 5884 (T), 7854, 7991, 8263: 7 ; 8367: 4; 9946: 7; 10 500: 6; 10 920, 13 813, 13 598, 16 863: 7; 20 871: 6; 22 330: 7 — Endert 1889, 2358: 7; 4022: 6; 15 062: 7 (T)— Ernst 11 045: 7 — Esquirol 2245: 4; 3601: 7 — Eyma 3254: 7.

Faden 69–946: 7— Flenley 2084: 5 — Floto 7237: 7 — Floyd et al 5682: 7; 5974, 6820: 5 — Forster 10 852: 7 — Fosberg 14 149: 7 — Foxworthy 42 135: 7 — Franck 11 448: 7 — Fris et al 7120: 7 — Fung 20 053: 7.

Gamble 884, 1925, 4000, 4847, 6366, 6367, 9699, 14 409, 14 870: 4; 16 350: 7 — Gardner 1145, 1298: 4 — H.B.G. Garrett 59, 59b: 4; 288: 7; 391: 4 — Gay 132: 6; 405, 1031: 7; 1806: 5 — Germain 40: 7 — Giesenhagen 1910: 1 — Glover 263: 7 — Gough 3243, 6055, 8289, 16 350: 7 — Gutierrez 117 367: 7 — Gutzwiller 1305: 7.

Hafashimane 26, 357: 7 — Haines 5379: 4 — Hancook 342, 357, 1892: 6 — Hartley 11 536: 7 — Haniff 21 028: 6 — Henderson 19 704, 19 708: 6 —

Table 6 Key to species: The following is a simplified key to identify taxa in the *M. punctatum* (L.) Copel. complex based on the output obtained from DELTA [12].

1a.	Fronds linear to narrowly elliptic, lamina base abruptly narrowed, living fronds iridescent blue-green; sori mostly irregularly scattered on simple free or on 2 or 3 connecting veins	2
1b.	Fronds linear to ovate or narrowly obovate to narrowly oblanceolate, living fronds light to dark green; sori forming irregular rows parallel to each pair of secondary veins	3
2a.	Lamina up to 4 cm width; scale peltate; hydathodes present	*M. siamense*
2b.	Lamina less than 2 cm width; scale pseudopeltate; hydathodes absent	*M. thailandicum*
3a.	Rhizome surface not waxy	4
3b.	Rhizome surface at least sometimes waxy under the scales or often waxy	5
4a.	Scales pseudopeltate; stipe present	*M. membranaceum*
4b.	Scales peltate; stipe absent or obscure	*M. musifolium*
5a.	Frond up to 100 cm long; stipe present, more than 2.0 mm diam	6
5b.	Frond less than 40 cm long; stipe absent or obscure, less than 1.5 mm diam	*M. steerei*
6a.	Distance between lowest sori and frond base up to 50 cm; all veins distinct; scales opaque and blackish	*M. glossophyllum*
6b.	Distance between lowest sori and frond base less than 35 cm; all veins or secondary and smaller veins more or less sunken and obscure (at least in living specimen); scales translucent and brownish	*M. punctatum*

Hennipman 3065, 5112, 5462, 5981: 7 — Henry 339: 4; 1895: 1, 1951: 1; 10 899: 7 — Hepper 8682: 7 — Heward 183: 7 — den Hoed 909: 7 — Holstvoogd 772: 7 — Holttum 15 702: 7 — Hoogland et al 4497, 6877: 5; 10 588: 7 — Hooker 526: 7; 1145, 1298 : 4; 1803, 1820, 1867, 3799: 7 — E.G. Hose & G. Hose 1822, 1827, 1894: 6; 4823: 7 — Humblot 666: 7 — J. Hutchison 1139: 7.

Inaeteay 173: 7 — Inder 4022: 6 — Ismail 2744: 7 — Iwatsuki et al 3252: 6; 9600: 4; 10 900: 7; 15 642: 4.

Jackson U123: 7; 51: 5 — Jacobson 10: 7 — Jacquemont 600: 4 — Jacques-Fe’lix 864: 7 — Jalconer 68: 4 — Jaman 4036: 6 — Jarrett 68, 766, 784: 7 — Jati 10: 4; 875: 7 — Jeffrey 208: 7 — Jermy (& Rankin) 3573, 3628, 8220: 7 — J.D.H. 750, 2223: 7 — J.J. 6061: 4 — Johns 7995: 5 — de Joncheere 1113, 1325: 7 — Jones & Onochie FHI 16952: 7.

Kampu 1, 2, 3, 4, 5: 1 — Kandau 62 458: 7 — Katendo 1187: 7 — Kato et al B3252: 6 — Keke 902: 4 — Khasya 1867: 4 — Khwaunju 1259: 4 — G. King’s collector (=Kunstler) 192: 6; 5069: 7 — Køie & Sandermann Olsen 1149: 7 — Konar 56: 4 — Korthals 33, 113, 148, 165, 185, 196, 273, 527, 684, 973: 7 — Kostermans 59: 7 — Kulong 11 582: 7.

Lace 4894: 4 — Lam 1108: 7 (T); 1365: 5; 3717: 7 — Larsen et al 2314: 4; 2597, 3096, 3374, 5078: 7 — Laumonier 876: 4; 1796: 6; 6150, 9085, 10 720, 26 980: 7 — Lauterbach 567: 6 — Lecerber 2042, 2617: 7 — Ledermann 7695, 8549, 8743: 6 — van Leeuwenberg 1785, 2542, 5032, 6651: 7; 9853: 5; 10 647, 13 492: 7 — Leland et al 65 641: 5 — Leonard 1618: 7 — Linder 759: 7 — Littke 394: 7; 469: 6 — Loher 867: 1 — Louis et. al. 950, 1417, 1932: 7 — Lucas et al 230: 7 — Lungchow 83: 7 — Lütjeharms 4750, 4990, 4999, 5019, 5151, 5251, 5293: 7.

Maddine 1867: 4 — Madhusoodanan CU 29683: 4 — Maitban 281: 7 — Manickam 31 220: 7; 31 442: 4 — Marie 10 775: 7 — Matthew 2, 4, 1907, 1928: 7; 1967: 4 — McDonald 3829: 5 — Melsetter 46 915: 7 — Melville et al 2023, 3669: 7 — Mense 343: 4 —Merrill 7331: 7; 11 691: 4 — van Mettenius 276: 5 — Meyer 7997: 7 — Mitchell 378: 7 — Mildbraed 198, 4426, 6524: 7 — Miller 1364: 7 — Mitchell 132, 154: 7 — Mooney 128: 4; 2383: 7 — Mooze 30: 7 — Moseley 1874, 3412 (T), 6447: 7 — Mousset 20, 7166: 7 — Murata et al 16 387, 17 674: 7.

Nakaike 408: 5 — N.C. Nair (BSI-series) 51 452: 4 — Narasimtan 165 111: 4 — de Néré 332, 1412: 7 — Nooteboom 5915: 7 — Noumea 29, 199: 7 — Nuttall 1867: 7.

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