

CHAPTER 6

PHYLOGENETIC STUDY

6.1 Introduction

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

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

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

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

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

6.2 Materials and Methods

Data Set

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

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

- (1) apically densely set
- 7. Scale arrangement:
 - (0) appressed
 - (1) slightly spreading
 - (2) distinctly spreading
 - (3) erect
- 8. Scales margin:
 - (0) entire
 - (1) dentate to denticulate
- 9. Presence of hyaline marginal region on rhizome scales:
 - (0) absent
 - (1) present
- 10. Scales shape (average index):
 - (0) < 2.5
 - (1) $\geq 2.5 < 3.0$
 - (2) $\geq 3.0 < 3.5$
 - (3) ≥ 3.5
- 11. Indumentum type of central region of scales:
 - (0) dark black on central region or glabrous
 - (1) bearing multiseptate hairs at least when young
 - (2) long unicellular hairs
- 12. Scales translucence:
 - (0) opaque and blackish
 - (1) translucent and brownish
- 13. Phyllopodia distinctness:
 - (0) distinct
 - (1) obscure
- 14. Frond type of dissection:
 - (0) simple
 - (1) pinnatifid
- 15. Frond color when living:
 - (0) light to dark green
 - (1) iridescent blue-green

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

- (1) all veins indistinct or secondary and smaller veins more or less immersed and vague (at least in living specimen)
- 24. Branching of included free veinlet:
 - (0) free veinlet recurrent
 - (1) free veinlet simple and once-forked
 - (2) free veinlet simple, once or twice forked
- 25. Visibility of hydathodes:
 - (0) distinct
 - (1) indistinct
- 26. Position of sori to surface of lamina:
 - (0) superficial
 - (1) slightly sunken
- 27. Sori limitation to the apical part of the lamina:
 - (0) absent or sometimes present
 - (1) always present
- 28. Sori shape:
 - (0) round
 - (1) slightly round or elongate
- 29. Sori distribution pattern:
 - (0) uniserries
 - (1) forming into 2-4 irregular rows parallel to each pair of secondary veins
 - (2) forming more than 3-10 (-15) irregular rows parallel to each pair of secondary veins
 - (3) mostly irregularly scattered on simple free or on 2 or 3 connecting veins
- 30. Presence of sori in the very marginal areoles:
 - (0) never present
 - (1) sometimes present
- 31. Presence of sori in the costal areoles or on their bordering veins:
 - (0) absent in costal areoles
 - (1) sometimes present in costal areoles
 - (2) always present in costal areoles

32. Spore surface:

- (0) cristate
- (1) plain to slightly verrucate
- (2) irregularly rugate

Phylogenetic tree construction

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

6.3 Result*Phylogenetic tree*

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

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

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

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

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

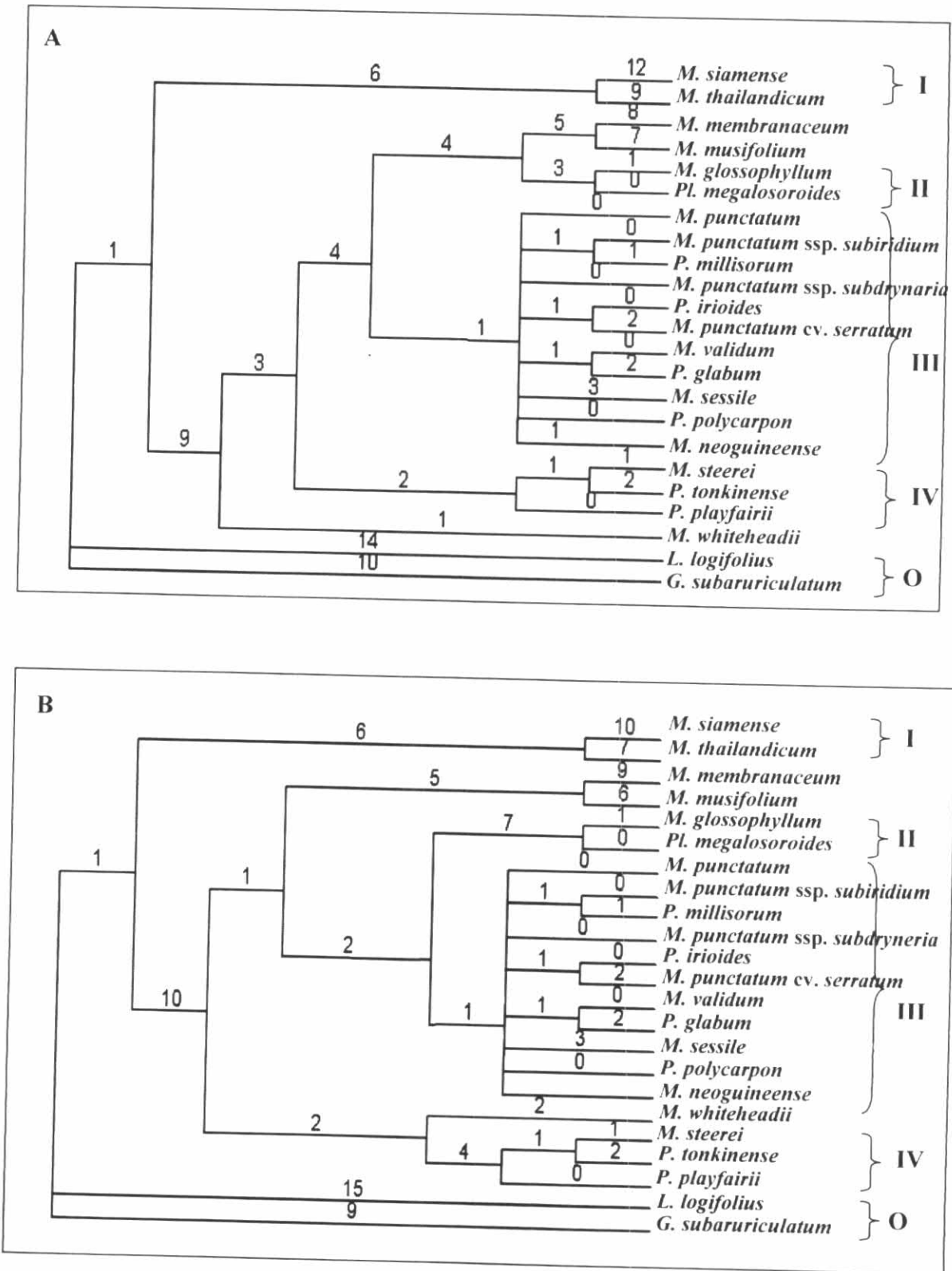


Figure 6.1 The first two topologies of the consensus trees of “the *M. punctatum* complex” base on morphological characters. The clade indicated by “O” was outgroups. I, II, III and IV were groups for discussion. Number was branch-length.

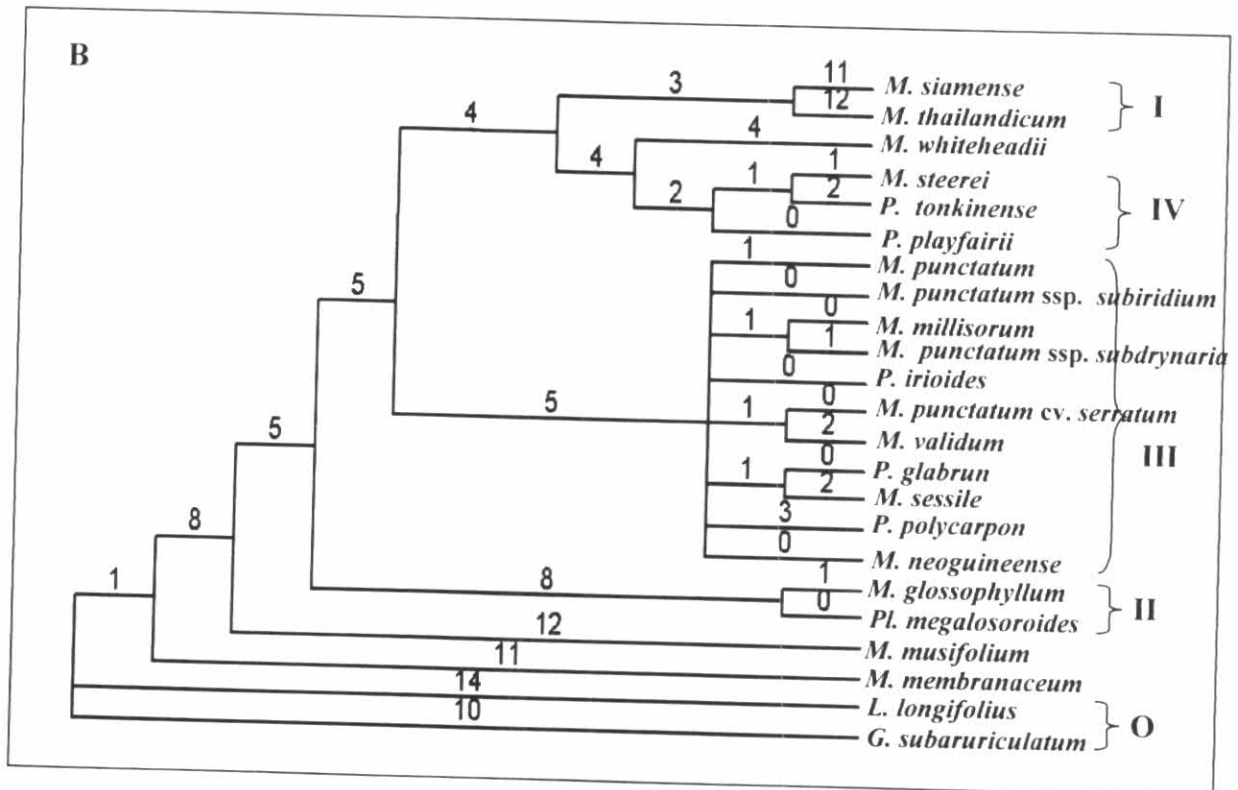
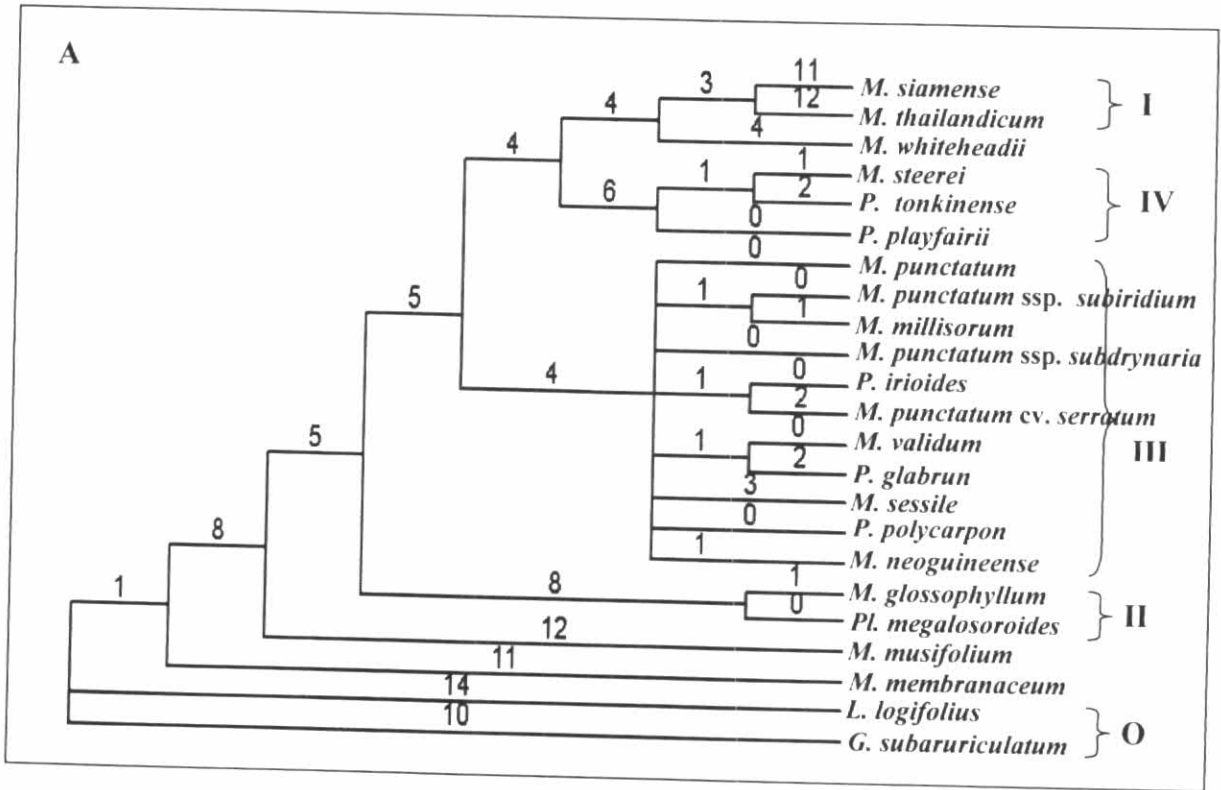


Figure 6.2 The last two topologies of the consensus trees of “the *M. punctatum* complex” based on morphological characters. The clade indicated by “O” was outgroups. I, II, III and IV were groups for discussion. Number was branch length.

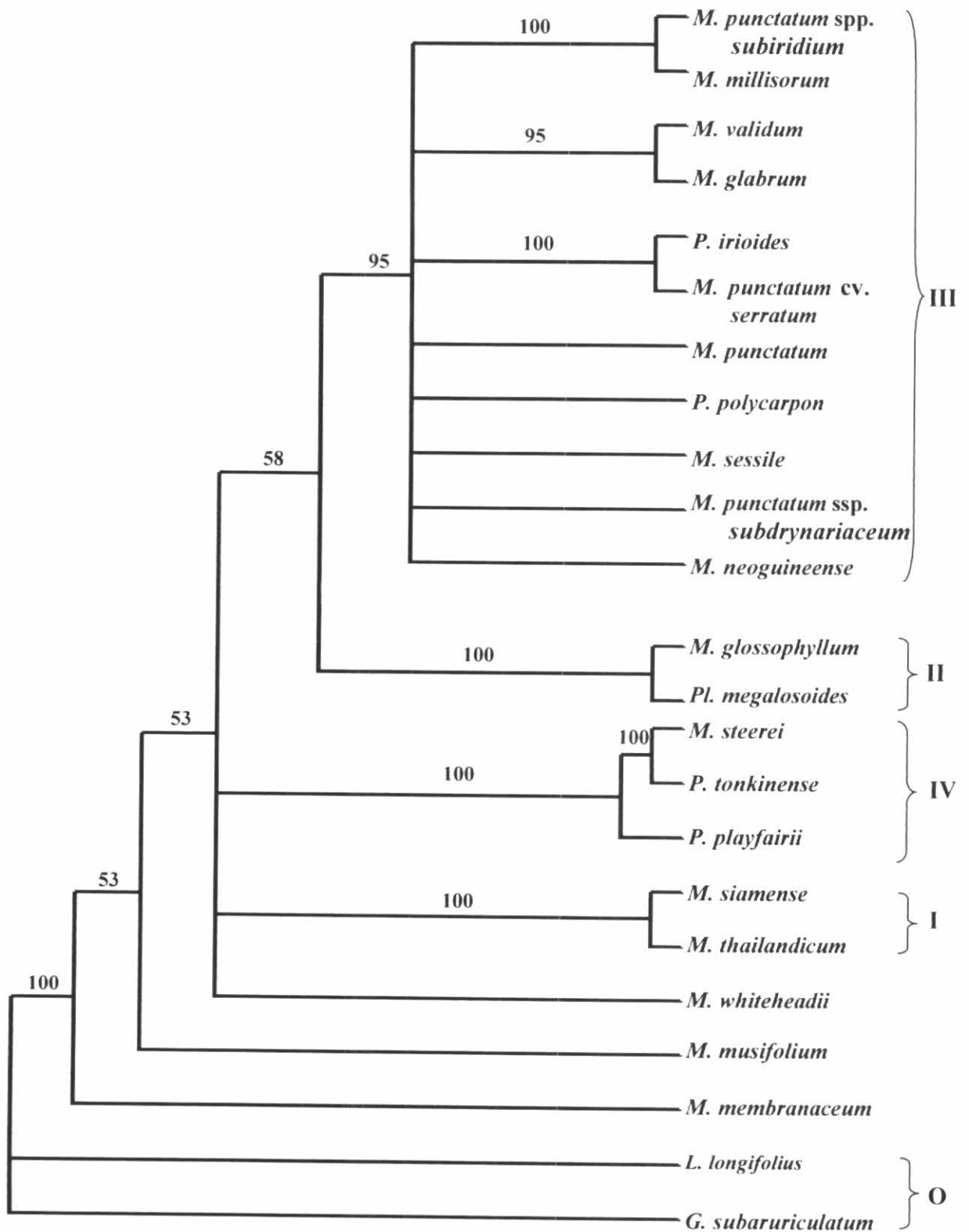


Figure 6.3 The majority rule consensus tree of “the *M. punctatum* complex” base on morphological characters. The clade indicated by “O” was outgroups. Number was majority rule 50%.

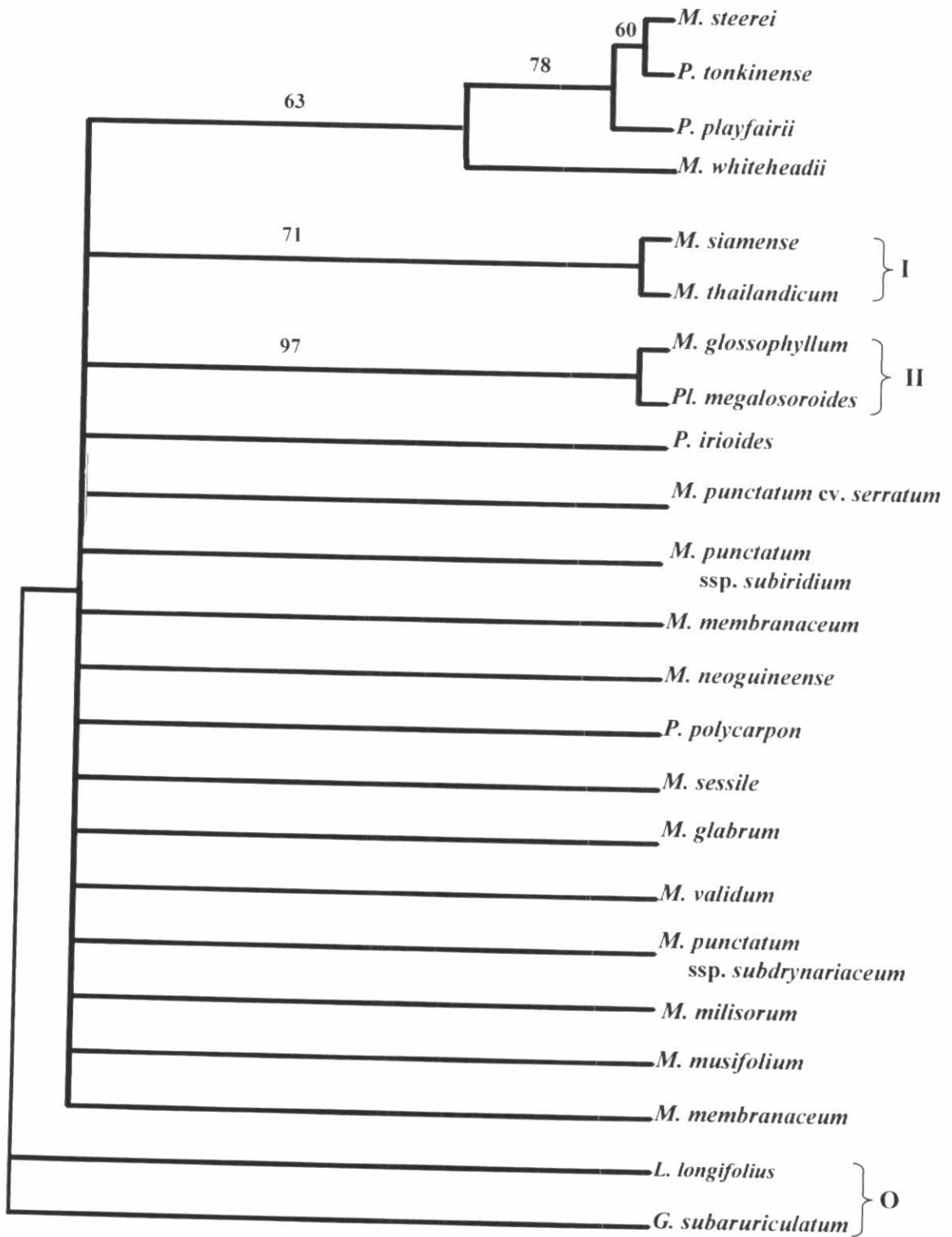


Figure 6.4 The strict consensus tree of “the *M. punctatum* complex” base on morphological characters. The clade indicated by “O” was outgroups. Number was bootstrap value from 100 replicates.

6.4 Discussion and Conclusion

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

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

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

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

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

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

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

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