

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Taxonomic History of the “*Microsorium punctatum* (L.) Copel. complex”

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



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

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

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

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

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

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

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

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

## 2.2 Methodological review

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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