CHAPTER 11

CONCLUSIONS

Systematic study on West Malesian Diplazium revealed that based on morphological characters, 69 species can be recognized. Thirteen species of them are proposed to be new species, namely Diplazium asymmetricum, D. batuayauense, D. crameri, D. densisquamatum, D. halimunense, D. loerzingii, D. megasegmentum, D. megasimplicifolium, D. meijeri, D. parallelivenium, D. profluens, D. subalternisegmentum, and D. subvirescens. D. donianum is new record for Java and Sumatra.

Six species are recognized having morphological variations that led to divide the species into two or three varieties. Diplazium accedens are consisting three varieties, namely var. accedens, var. spinosum and var. ridleyi. D. cordifolium are recognized to have three varieties, namely var. cordifolium, var. integrifolium and var. pariens. D. pallidum consist two varieties, namely var. pallidum and var. montanum. D. sorzogonense have two varieties, namely var. sorzogonense and var. major. D. silvaticum are recognized to have two varieties, namely var. silvaticum and var. pinnae-ellipticum. D. vestitum are divided into two varieties, namely var. vestitum and var. borneense. D. accedens var. spinosum, D. silvaticum var. pinnae-ellipticum are new varieties proposed. Whereas D. pallidum var. montanum and D. accedens var. ridleyi are new status proposed.

Ecological study showed that most of species are terrestrial dryland ferns and found at 20 – 3400 m above sea level in the primary and secondary forest on moist humus-rich soil in light and deep shady places. Species diversity were culminated at 1000-1500 m. The different genetic load in the same species sometimes grows in the different habitat gradient.

Distributional study resulted that the total number of species for each main island are 40, 30, 29, 28 for Borneo, Java, Sumatra, and Malay Peninsula, respectively. Nineteen species of West Malesia distribute very wide. They are D. accedens, D. bantamense, D. cordifolium, D. crenatoserratum, D. dilatatum, D. donianum, D. esculentum, D. fuliginosum, D. malaccense, D. pallidum, D.
polypodioides, D. prescottianum, D. riparium, D. silvaticum, D. simplicivenium, D. sorzogonense, D. subserratum, D. tomentosum and D. xiphophyllum. Twenty three species are presumed as locally endemic: (1) Nine endemic species are occurring in Borneo (D. atrosquamosum, D. batuayauense, D. beamanii, D. crinitum, D. squarrosum, D. subalternisegmentum, D. tricholepis and D. wahauense); (2) Two endemic species in Malaya Peninsula (D. christii and D. velutinum); (3) Six species are endemic in Sumatra (D. albidosquamatum, D. betimusense, D. crameri, D. densisquamatum, D. meijerii, and D. melanolepis); and (4) Six endemic species are recorded for Java (D. asymmetricum, D. halimunense, D. megasegmentum, D. parallelivenium, D. profluens, and D. subvirescens).

Anatomical study on the transversal section of stipe showed that the leaf-trace shape of Diplazium stipe is varying among species and constant among the adult individuals in a species. The shapes are consisted of five types: (1) uninterrupted V-shaped, (2) interrupted, (3) uninterrupted U-shaped, (4) interrupted U-shaped, and (5) W-shaped. Each type seems to vary among the species. Each type may diversify into some different derivative forms that enables to determine a species among closely related species. Therefore the leaf-trace shapes are important diagnostic features which support species delimitation in Diplazium.

Cytological observations on somatic chromosomes number showed that West Malesian species showed basic chromosome number x = 41. New cytological information for science on 19 species are recorded. They are D. aequibasale (tetraploid), D. angustipinna (triploid), D. asymmetricum (triploid), D. batuayauense (tetraploid and pentaploid), D. crenatoserratum (triploid and tetraploid), D. halimunense (triploid), D. hewittii (triploid), D. profluens (tetraploid), D. loerzingii (diploid and triploid), D. pallidum (diploid), D. petiolare (diploid), D. porphyrorachis (tetraploid), D. riparium (diploid and triploid), D. spiniferum (diploid), D. subserratum (diploid, triploid, tetraploid), triploid D. subvirescens, D. tomentosum (diploid and pentaploid), D. xiphophyllum (diploid, hexaploid), and Diplazium wahauense (2n=164).
This study showed that intraspecific diversity on West Malesian *Diplazium* are high enough. Twelve species of the 31 species successfully examined are having series ploidy: *D. angustipinna* (triploid and tetraploid), *D. bantamense* (triplioid and oktoploid), *D. cordifolium* (tetraploid, pentaploid, dan hexaploid), *D. pallidum* (diploid and tetraploid), *D. silvaticum* (triploid and tetraploid), *D. tomentosum* (diploid, tetraploid, pentaploid), *D. subseratrum* (diploid, triploid, and tetraploid), *D. xiphophyllum* (diploid, tetraploid and hexaploid). Thirteen species showed only polyploidy race: *D. aequibasale* (2n=164, tetraploid), *D. profluens* (tetraploid), *D. porphyrorachis* (ca. 164/tetraploid), *D. procumbens* (triploid), *D. simplicivenium* (triploid). Whereas nine species revealed only diploid race (*D. accedens*, *D. esculentum*, *D. malaccense*, *D. petiolare*, *D. polypodioides*, *D. speciosum*, *D. spiniferum*, *D. sorzogonense*, *D. subpolypodioides*, and *D. umbrosum*).

Study on the relationships between ploidy level and morphological variation in some species, such as *D. batuayauense*, *D. crenatoserratum*, *D. loerzingii*, *D. riparium*, *D. tomentosum*, and *D. xiphophyllum*, suggested that they are autoploid. Triploid *D. silvaticum* was presumed alloplloid. However further cytological study are needed to verify the polyploidy mechanisme involved in the species mentioned above and the others.

Study on the relationship between ploidy level and habitat gradient showed that generally West Malesian *Diplazium* species are showing no any strick correlation between ploidy level and altitude, but *D. pallidum*, *D. riparium*, *D. silvaticum* and *D. xiphophyllum*. Reproductive studies revealed that many species are apogamous triploid and both the sexual and apomictic species can occur in the relatively moist habitats.

Phylogenetic study by using morphological datasets revealed that the phylogenetic relationship among species in the genus *Diplazium* was very difficult to explain due to the lack of or weak support Bootstrap value. Therefore the monophyletic species groups in this genus could not be identified confidently. The high homoplastic on morphological characters, parallel evolution and some apogamus species that suggested as originally hybrids distort the inference of phylogenetic relationship among species.
However the lack of or weak support for a phylogenetic tree does not strictly indicate that the pattern observed is incorrect but it does limit the amount of confidence that can be placed in the relationships between taxa and the conclusions can be drawn from them. This study showed that some terminal clades formed are consisting of species that presumed to be closely related species by formerly authors and congruence with topological tree generated from gene \textit{rbcL} sequences, such as the affinity of \textit{D. porphyrorachis} group and imparipinnate species group (\textit{D. hottae}, \textit{D. crameri}, \textit{D. xiphophyllym}, \textit{D. fraxinifolium}, \textit{D. subintegrum}, \textit{D. bantamense}, \textit{D. lobbianum}). This study also revealed that the classification of van Alderwereld van Rosenburgh (1908) in dividing \textit{Diplazium} into two sections (\textit{Eudiplazium} and \textit{Anisogonium}) is not natural. Moreover, this study gave indication that Kato’s classification (1977) could not be applied on West Malesian \textit{Diplazium}. The lack of phylogenetic signal in morphological datasets shows the need for other more informative data, such as molecular data, for inferring phylogenetic relationships.

Spore morphology study revealed that \textit{Diplazium} have monolete, bilateraly symmetrical, heteropolar, polar outline elliptical, perinates with with laesura concealed by its perine. Perine ornamentation varied from alate to costate alate and rugulate with variation of additional decoration such as ciliae, coralline, echinae, and fenestrae. \textit{Diplazium} species. The value of spore characters is as descriptive device and an aid to the identification of dispersed spores grains. Therefore perine ornamentations support in delimitating species concept in \textit{Diplazium}. The phylogenetic analysis using parsimony revealed that morphological variation of spore is inadequate to depict natural relationship among \textit{Diplazium} species. Superficial similarity of mature perispores would not indicate on systematic relationship among species as similar surface pattern it derived through different development pathways.

Gene \textit{rbcL} sequences data showed that this gene is very well in supporting species delimitation among species and revealing the intraspecific diversity within species of \textit{Diplazium}. This study showed that most of West Malesian species are genetically varying, mainly those having polyploidy types or seri ploidy. Therefore the division of \textit{D. pallidum} into two varieties is supported with this
gene \textit{rbcL} sequences. This study give an indication that species in which have both morphological difference and seriploidy, such \textit{D. accedens}, \textit{D. cordifolium}, \textit{D. crenatoserratum}, \textit{D. bantamens}, \textit{D. subserratum} and \textit{D. tomentosum}, should be more studied by encompassing much more sample from their range of distribution.

This study revealed that gene \textit{rbcL} is more informative in inferring phylogeny of the genus \textit{Diplazium} in West Malesia. Therefore the phylogenetic analysis of gene \textit{rbcL} sequences found that \textit{Diplazium} in West Malesia is monophyletic.

The position of \textit{D. porphyrorachis} group at the basal clade of the phylogenetic tree generated from morphological data and separated from other species is supported by the phylogenetic tree generated from molecular data (gene \textit{rbcL} sequence). This result is in accordance with the statement of Price (1983) that \textit{D. porphyrorachis} and close related species are belonging to the different lines within the genus \textit{Diplazium}. This study also showed the congruence between the clade of ‘riparium Group’ drawn by \textit{rbcL} tree and clade of ‘imparipinnate frond group’ drawn by morphological tree.

The result of this study also showed that the classification of van Alderwerelt van Rosenburgh (1908) is not natural and polyphyletic. The classification of Kato (1977) in dividing Japanese \textit{Diplazium} into six group can not be referred to West Malesian \textit{Diplazium}. Moreover the \textit{Diplazium} groups of Kato (1977) are not monophyletic.

The result of the molecular systematic study also indicates that some uncertain relationships require further analysis in the future. More data, including both more taxa that include all West Malesian species and from wider areas and more molecular data, are needed before a well resolved phylogenetic hypothesis for West Malesian \textit{Diplazium} can be offered.