CHAPTER 1

GENERAL INTRODUCTION

1.1. Taxonomical Aspects of Diplazium and Its Systematic Problems

Diplazium was established by Swartz (1801) and typified by Asplenium plantaginifolium L. (Diplazium plantaginifolium Sw.). Etymologically Diplazium is formed from the Greek diplazios which means double, because indusia lie on both sides of the vein.

Diplazium is a large genus consisting of about 400 species occur mainly in the tropics (Ching 1964a; Copeland 1947; Tagawa & Iwatsuki, 1988), sparingly in the sub tropic and only locally extending into temperate (Kramer et al. 1990). This genus member has diagnostic characters as follow: Groove of frond axis open to admit the groove of axis of lower order; frond axes U-shaped with a flat base in most species; acroscopic basal pinnules equal or smaller, laminar margin not cartilaginous; sori linear, double (diplazioid) or single, the single ones opening toward the main veins or the central veins of the ultimate segments, the double ones in opposite directions (van Alderwerelt van Rosenburgh 1908; Holttum 1966; Kato 1977; Tagawa & Iwatsuki 1988; Kramer et al. 1990).

a. Species Delimitation.

Taxonomically, Diplazium is very difficult and quite insufficiently known. It is in great need of monographic study. The young plants may be fertile and difficult to assign to a species (Kramer et al., 1990). Many taxa are considerably morphologically diversified. Their morphological variations are continuous through apparently intermediate forms, which are commonly regarded as putative hybrids (Takamiya et al. 1999).
b. Polyploidy and Polymorphic Species Complex

Many cytological complexities including polyploidy from 4x to 8x, hybridity, apomixis, have been reported in ca. 20% of cytological investigation of Diplazium taxa (Lovis 1977). Recent studies on Japanese Diplazium revealed that one of the reason for the taxonomic complexity of the Diplazium species group with bi- to tripinnate leaves is apomictic reproduction and most of the group’s members have been found to be triploid apomics (2n=3x=123). Many polymorphic Diplazium taxa having large evergreen bi-to tripinnate leaves occur under evergreen broad-leaved forest from the Ryukyus Islands to southwestern Japan. Several putative hybrids are also known within Diplazium, thus it is increasing the taxonomical dispute (Takamiya et al 1999). Cytological observation of Diplazium from Java (Praptosuwiryo & Darnaedi 1994, 2004) and Lesser Sunda Island (Praptosuwiryo 2003) also showed many polyploid types in which several species were apomix. Therefore, polyploidy, apomixis and hybrid in diplazioid ferns creates difficulty in species delimitation.

c. Subdivision of Diplazium.

Natural subdivision of the genus has not been assigned (Kramer et al 1990). Van Alderwerelt van Rosenburgh (1908) divided this genus artificially into two sections based on its venation, viz. Eudiplazium and Anisogonium. The first section includes species with free veins such as Diplazium bantamense, D. crenatoserratum and D. porphyrorachis; while the second section includes species with anastomosing veins, such as D. cordifolium, D. esculentum, and D. fraxinifolium. Copeland (1908), in his revision on the Philippine species of Athyrium, merged Diplazium into Athyrium and recognized 13 non formal group of Athyrium, namely drynarioid species (A. hyalostegium, A. loheri), A. japonicum group (A. japonicum, A. grammitoides, A. acrotis), A. filix-femina group (A. drepanopteron), A. macrocarpum group (A. halconense, A. anisopteron), A. nigripes group (Diplazium aristulatum, D. aristulatum var. sphanicolum, Athyrium philippinense, A. brevipinnulum, A. nigripes var. mearnsianum, A. elmeri, A. stramineum, A. platyphyllum), A. cyatheafolium group (A. cyatheafolium=Diplazium ebenum, A. atratum=D. atratum, A. oligosorum=D. oligosorum), A. silvaticum group (A. silvaticum, A. blumei= D. polypodioides, A.

Based on the characters such as scales, stipe, lamina and venations, Kato (1977) recognized genus Diplazium that consisting of five groups, namely: (1) Diplazium dilatatum group that includes member with groove generally U-shaped with a flat base, acroscopic basal pinnules or segments equal to or smaller than the basiscopic or subsequent ones, scales entire or toothed, but not clathrate. The representative species are D. dilatatum, D. esculentum, D. donianum, D. mettenianum, D. subsinuatum, D. hachijoense, D. nipponicum, D. sibiricum, D. squamigerum, D. tomitaroanum, D. pullingeri, D. lobatum, D. yaoshanense, and D. kawakami; (2) Diplazium wichurae group that includes species member with groove U-shaped, acroscopic base of pinna auricled, adaxial surface of lamina concave along veins, scales entire, sometimes subclathrate. The representatives are D. wichurae, D. okudairae, and D. pin-faense; (3) Diplazium mesosorum group that having characters groove U-shaped with a flat base, acroscopic basal pinnules or segments equal to or slightly larger than the basiscopic or subsequent ones, scales entire and subclathrate and only represented by D. mesosorum; (4) Diplazium javanicum group in which includes species with groove V-shaped, frond pinnate or imparipinnate, laminar margin entire or undulate, veins sagenoid-reticulate and scales entire. This group represented by D. heterophlebium, D. javanicum, D. cavalerianum, and D. marginatum; and (5) Diplazium longicarpun group. This group includes species with frond pinnate, acroscopic base of pinna
truncate, basiscopic cuneate, adaxial surface of lamina not concave along veins, scales entire. This group represented only by one species, *D. longicarpum*.

d. The status of *Diplazium* a separates genus.

The delimitation of *Diplazium* is still in doubt. Some taxonomist merged *Diplazium* into *Athyrium*, while others maintained them as separate genera. Beddome (1883) separated *Diplazium* from *Athyrium* based on the difference of sorus shape. *Athyrium* species have special reniform or round sori, meanwhile *Diplazium* has elongated sori. Copeland (1908) transferred *Diplazium* in Philippine into *Athyrium*. In accord with his previous papers, Copeland (1929) has described a considerable number of diplazioid ferns as species of *Athyrium*, merged the whole species of *Diplazium*. Furthermore Copeland (1947) united *Diplazium* into *Athyrium* because he believed that *Athyrium s.str.* (*Euathyrium*) and *Diplazium* as a whole is almost surely a phyletic entity. He was unable to find any line where the genus can be divided to produce distinguishable natural groups.

Ching (1964a, 1964b) splits *Diplazium* into several genera, e.g., *Allantoidea*, *Callipteris*, *Diplaziopsis*, *Monomelangium*, and *Diplazium sensu stricto*. Ching (1964a) delimited *Diplazium Swartz* as represented by *D. plantaginifolium* (Linn) Urban of Tropical America, *D. bantamense* Blume of Java and *D. donianum* (Mett.) Tard.-Blot of the Orient which characterized by imparipinnate (sometimes simple or trifoliolate) fronds of firm texture with large similar pinnae (2-6 pairs or rarely more), in similar way the simple frond, the upper side of pinna-costa has a very shallow or even obsolete longitudinal canal provided with low rounded edges on each side and not open to the rachis-groove at point of insertion and by very long, linear and more often double sori extending from the costa to near the leaf margin. *Diplazium sensu stricto is a pantropical genus of medium-size* (Ching 1964a). On the other hand *Allantoidia sensu Ching* (1964a) included the bulk of the species of *Diplazium* of Christensen’s Index Filicum as represented by *Diplazium dilatatum* Blume, *D. polypodioides* Blume, and their allies. Their short-linear sori and indusia being asplenioid or sometimes diplazioid but not allantoid. Ching (1964a) delimited *Allantoidea* R. Brown as follow: fronds vary from simply to 2-3-pinnate with lateral pinnae gradually becoming shorter upwards and finally merged into a deltoid, acuminate pinnatifid
apical part, with the pinna-rachis or costa or costule of pinnules (in the compound-leaved species) deeply grooved above and raised (becoming flat upon drying) knife-edge margins on each side, which are decurrent along the rachis or costa of pinnae or costule of pinnule, and the grooves of rachis. Pinna-rachis or of costa and costule are open to each other at the point of insertion, the leaf-texture is herbaceous or rarely chartaceous and the sori are thick, short-linear or ovoid-oblong only with the anterior basal one usually diplazioid. It is a large genus of about 350 species mostly in tropical and subtropical Asia with a few extending to the temperate region in the Northern Hemisphere.

However, recent molecular phylogenetic study on the ferns species included in Athyriaceae sensu Ching (1964a) by using evidence from chloroplast TrnL-F region sequences revealed that Diplaziopsis C.Chr. is included in the Diplazium Sw. clade (Wang et al 2003). Wang et al (2003) also gave evidence that Allantoidea R. Br. and Callipteris Bory should be included in Diplazium Sw.

Christensen & Holttum (1934) separated Diplazium from Athyrium because they thought that merging the whole mass of species of Diplazium in Athyrium would result too unnatural grouping. Holttum (1940) originally showed that in Malaya Diplazium and Athyrium are quite distinct, so that he recognized the genus Diplazium. Holttum pointed out that Diplazium had pinnae and pinnules of pinnate frond with subequal base, venation never anadromous, sori elongated along the veins with lateral indusium, the diplazioid sori usually with two quite separate indusia, near grading into horse-shoe shape, with indusium continuous all around, while Athyrium had pinnules often with equal bases, an anadromous venation and division, and sori are always short and broad, the diplazioid ones often of a horse-shoe shape, the two sides of the sorus being connected. In contrast to his 1940 treatment, Holttum (1947, 1955) recognized the close relationship of Diplazium with Athyrium and hence associated the two genera because the distinction between them were no longer distinct to justify their separation. Inconsistently, Holttum (1966) without doubt stated that genus Diplazium should be maintained because cytologically the two groups were constantly different. According to Holttum (1966) Athyrium has x = 40 and Diplazium x = 41. Despite Holttum (1966) others taxonomist such as, Ching

Kato (1977) differentiated between the two genera as follows. *Athyrium* had stipe bases on ascending to erect rhizomes swollen with pneumatophores, frond axes V-shaped in transaction, acroscopic basal pinnules larger than others, laminar margin cartilaginous or not, spines present adaxially at the junction of costules or not, sori horse-shoe- or J-shaped, or linear, scales entire. Meanwhile *Diplazium* had stipe bases neither swollen nor bearing pneumatophores, frond axes U-shaped with a flat base in most species, acroscopic basal pinnules equal or smaller, laminar margin not cartilaginous, spines absent, sori linear, scales toothed or entire. Furthermore, Kato (1977) merged *Callipteris* Bory, *Allantoidea* R. Brown, *Hemidictyum* Presl, *Diplaziopsis* C. Christensen, *Monomelangium* Hayata, *Dictyodroma* Ching and *Rhachidosorus* Ching into *Diplazium*. In accord with Kato (1977), recent molecular phylogenetic studies included *Monomelangium* in the monophyletic *Diplazium* clade; *Rhachidosorus* is separated from monophyletic *Diplazium* clade and not closely related to either *Athyrium* and *Diplazium* (Sano et al 2000a, 2000b).

e. Classification of *Diplazium*.

1.2. The Diversity of *Diplazium* in Malesia

Malesia is one of the center megadiversity of tropical plants. With ca. 40,000 species of vascular plants, the Malesian region is among the most species-rich areas world-wide (Roos 1993).

It is firstly recognized by Swiss botanist, Heinrich Zolliger, in 1858, that Malesian region is different from the Asia and Australia due to a vast diversity of vascular plants. It is predicted that not less than 40,000 species of vascular plants occur in the region (from John 1995). This region extends from Malay Peninsula to New Guinea and cover more than 3,000,000 km² at 0-5000 a.s.l. The region included seven countries, namely Brunai Darussalam, Indonesia, Malaysia, Timor Leste, Papua New Guinea, Philippines and Singapore. Phytogeographically, this region is divided into three subregion, viz. West Malesia, Central Malesia and East Malesia. West Malesia, known as Sunda Shelf, cover Malay Peninsula, Sumatra, Borneo, Palawan, Jawa and Bali. Central Malesia is subregion covering Philippina, Sulawesi, Moluccas and Lesser Sunda. East Malesia, recognized as Sahul Shelf or the Papuasia, is a subregion included Irian Jaya and Papua New Guinea (John 1995).

Malesia is also a species-rich region for Pteridopyta. It is estimated that 4,400 fern species of ca. 12,000 fern species known in the world are distributed in this region (Roos 1993). New Guinea is the world’s most species-rich island subcontinent with ca. 2,000 species, Borneo and the Philippines each have 1,000 species, and Java, sumatra and Celebes each have 500 species. Seram Island, a small island of 18,000 km² in the Moluccas, is quite rich and is known to have about 700 species (Kato 1992). Parris *et al* (1992) reported that more than 600 fern species occur in Mt. Kinabalu (400 km²). Because of the species richness, Malesia ferns are very suitable for studying their biodiversity and evolution.
Malesian region is the center of *Diplazium* diversity. It is predicted that 75% of species in the world (ca. 300 species) are existing in this region (Roos, 1995). However, the revision of *Diplazium* for the Malesian region has not yet been done. However, some short studies based on local areas have been conducted for *Diplazium*. Holttum (1940) described 25 species of *Diplazium* from Malay Peninsula and recognized 29 species on his monumental book ‘Ferns Flora of Malaya (Holttum 1966).


1.3. The Biological Aspects on Systematics Study of Ferns

1.3.1 Morphological and Anatomical Evidence in Taxonomy

Morphological characters are very important in systematics, although many biological approaches are applied in constructing classification system, such as anatomy, palinology, cytology, and molecular analysis. The morphological data are used for grouping, identifying, studying the relationship of plants (Davis & Heywood 1963).

Fern systematists have employed various species concept. Some systematists employed morphological species concept, using morphology as primary criterion in recognizing species and formulating initial hypotheses about new lineages. In addition, modern floristic surveys are also based primarily on morphology and provide the first clues to identify certain individuals or populations as potentially as potentially unique, and deserving of further scrutiny (Haufler *et al* 2000).

The comparative study of plant structure, morphology and anatomy, has always been the backbone of plant systematics to elucidate plant diversity,
phylogeny and evolution. The second half of the 20th century has been a fascinating period in which systematics and structural studies greatly profited from new techniques and methods. The advancement of new techniques and methods such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), sinematografi, cladistics, evolutionary paleobotany, and molecular systematics and molecular developmental genetics are just exactly supporting morphological data (Endress et al. 2000).

Although morphological data series has revealed more homoplasy than those from molecular, however combination of morphology and molecular data might produced more robust phylogeny. Thus, in reality the two data are mutually complementary. Even, there is an amazing fact that the success of molecular phylogenetic studies are predicted based on previous the morphological data (Endress et al, 2000).

1.3.2. The Role of Palynology in Systematics: Spore Morphology Evidence in Pteridopytes

Palynological characters have now been used in plant systematics for almost two centuries. Studies of a various taxa employing transmission electron microscopy (TEM) and or SEM (Scanning Electron Microscopy) have elucidated the complex nature of both exorspore and perispore development (e.g. Mitsui 1986; Tryon 1986). These investigations suggest that fine-scaled comparisons of spores among taxa may be necessary to distinguish superficial and gross similarity from true synapomorphy.

Measurement of spores serves as useful probes for establishing hypothesis of evolutionary relationships within polyploid complexes. Spore size of polyploid is dependent upon two factors: size of cells in diploid progenitors and ploidy level. Both factors may be used to predict cell size of missing members of polyploid complexes from cell-size means of the known members, as long as environmental variation does not compromise the analysis (Barrington et al 1986)
1.3.3. Cytological Evidence in revealing taxonomic problems on *Diplazium* and Its Closely Related Genera

Once a species has been diagnosed, it represents a working hypothesis that can be tested through the application of new evidence or analytical methods (Haufler *et al.* 2000). The first auxiliary tool that had a major impact on species concept on ferns was the development of techniques for determining chromosomes numbers and studying meiotic behaviour (Manton 1950). The new perspectives that were obtained through chromosomal studied led to a revolution in he recognition of species boundaries in many groups. Polyploids discovery in taxa that had been considered simply as ‘polymorphic assemblages’, Manton demonstrated that evolutionary mechanism in ferns were more complex than previously appreciated and that hybridization between distinct species was an important component of the history of fern lineages (Manton 1950). This new approach results a new perception of fern species. Manton (1950) and her followers analysed meiosis in artificial hybrids to characterize the limits of fern lineages as reproductively isolated units, and to identify genetic similarities between genomes. These studies showed that interactions among ‘primary’ species involving hybridization and polyploidy generated ‘secondary’ species (terminology *sensu* Grant 1981), and resulted in reticulate species complexes whose intricacies challenged subsequent systematists.

As reported by Löve *et al.* (1977) cytological research on *Diplazium* was firstly conducted by Manton (1953) for Ceylon species. From 1953 until 1977, only about 15.5% of ca. 400 species were recognized for its chromosome number.

Intensive studies on cytology of *Diplazium* in Japan (Ohta & Takamiya 1999; Takamiya *et al.* 1999, 2000, 2001; Sano *et al.* 2000) revealed that cytological approach through chromosomal information, is very helpful in analysing polymorphic species. However, few cytological investigations on Malesian *Diplazium* have been conducted. Manton (1954) initiated cytological examination on this genus for Malayan species, moreover Holtum & Roy (1965) conducted similar study for New Guinean species.

Preliminary cytological observation on *Diplazium* in Java was reported by Darnaedi (1992) for tetraploid *D. cordifolium* and diploid *D. esculentum*. In
addition, Praptosuwiryo and Darnaedi (1994) reported cytological research on six species of Diplazium from Gede-Pangrango National Park, West Java (one species of its, viz. D. opacum, have been treated as Cornopteris opaca)

Praptosuwiryo (2003) and Praptosuwiryo & Darnaedi (2004) reported recent cytological information of Diplazium from Malesian region. The first covered two species of Diplazium from Lombok Island, namely D. malaccense and D. pallidum, while the second report 43 collection numbers from 10 localities of Java included 10 species.

1.3.4. The Utility of Molecular Techniques for phylogenetic studies of pteridophytes: Gene rbcl Sequences

The use of morphology in reconstructing phylogeny of ferns is often complicated due to the lack of phylogenetically informative characters (Haufler & Rangker 1995). For example, the simplicity of foliar morphology of Ophioglossaceae has limited number of characters available for reconstructing classifications and understanding relationships (Hauk et al 2003).

The lack of informative morphological characters in ferns led to the search for new sources of characters in molecular data, including restriction site and nucleotide sequence data, to infer phylogenetic relationships (Eastwood et al 2004).

Nucleic acid sequencing is a relatively new approach in plant systematic, however the power of the techniques and the data generated have made it become one of the most utilized for inferring phylogenetic history. DNA sequence data are the most informative tool in molecular systematics. Comparative analysis of DNA sequences is becoming increasingly important and so valuable in plant systematics. The major reasons of its valuable area: (1) the characters (nucleotides) are the basic units of information encoded in organism and (2) the potential sizes of informative data sets are immense. Systematic informative variation is essentially inexhaustible and different genes or parts of the genome might evolve at different rates. Therefore, questions at different taxonomic level can be addressed using different genes or different region of a gene (Soltis & Soltis 1998)
Molecular systematic studies of the pteridophytes have generated robust phylogenies at all taxonomic scales. In many cases, molecular phylogeny are highly congruent with morphological-based hypothesis (Wolf & Conant 1994). The use of molecular data to infer phylogeny has yielded valuable insight into the relationships and evolution of ferns, some with taxonomic implications (Hasebe et al 1995, Gastony & Ungerer 1997, Murakami et al 1999).

1.4. Objectives

The objectives of the research are:

1. To obtain delimitation of the genus and species concepts
2. To provide better data on distribution of species in the West Malesia region
3. To provide an identification keys to the species and infra species.
4. To collect data on species diversity.
5. To understand the anatomical and palinological data in supporting the species delimitation.
6. To recognize ecological aspect of species.
7. To provide cytological map of species in its distribution site.
8. To unravel species which is having taxonomical problems.
9. To obtain phylogenetic hypotheses for Diplazium based on morphological data and gene rbcL sequence.