UNIVERSITI PUTRA MALAYSIA

TAXONOMIC REVISION OF FERN-ALLIES LYCOPODIACEAE MIRBEL IN MALAYSIA

CLASIUS @ CLAYSIUS KONGOI

FSAS 2004 38

TABLE OF CONTENTS

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

TAXONOMIC REVISION OF FERN-ALLIES LYCOPODIACEAE MIRBEL IN MALAYSIA

By

CLASIUS @ CLAYSIUS KONGOI

March 2004

Chairman: **Associate Professor Rusea Go, Ph.D.**

Faculty: **Science and Environmental Studies**

The family Lycopodiaceae in Malaysia consists of three genera, namely, *Huperzia*, *Lycopodium* and *Lycopodiella* and comprises 32 species, including 11 varieties. Four species are reported as new records for Malaysia (*Huperzia beccarii, H. goliathensis, H. prolifera* and a species of *Lycopodium*). This family is distributed throughout Malaysia but the diversity is not as high as in the Americas, the center of distribution. Morphological studies including branching patterns, anatomy and ultrastructure were conducted as they are taxonomically significant for delimiting genera and species of Lycopodiaceae. The presence of a hair at the tip of the leaf, midvein and subpetiole are good taxonomic leaf characters in some species of Lycopodiaceae. Two basic branching patterns are observed in this study with each corresponding to a distinct genus. The genus *Huperzia* is characterized by an isotomous branching pattern while *Lycopodium* and *Lycopodiella* have anisotomous branching patterns. Additional leaf differentiation characters on the stem of Lycopodiaceae such as heterophylly, homophylly, isophylly and anisophylly were the basis for the classification of

genera and some species. The stem anatomy reveals that Lycopodiaceae has a primitive type of stele called protostele in three forms. Palynological studies reveal that spore type, shape, size, surface sculpture and aperture are taxonomically significant for delimiting or distinguishing species. Sporophyll characteristics such as margin, size, shape of leaves and apex are important for identification. Variation in the sporangial wall structure of Lycopodiaceae was found to be taxonomically significant at the genus and species levels. Based on all the characters mentioned here, the Lycopodiaceae are divided into three genera in Malaysia: *Huperzia*, *Lycopodium* and *Lycopodiella.* The current threat to Lycopodiaceae is conversion of hill forest into agriculture land, the main habitat of this family and over-collection by local people as a source of income. Conservation and legislation are needed to safeguard these untapped resources of possible future pharmaceuticals and other yet unknown benefits to mankind.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

SEMAKAN TAKSONOMI SEKUTU PAKU-PAKIS LYCOPODIACEAE MIRBEL DI MALAYSIA

Oleh

CLASIUS @ CLAYSIUS KONGOI

Mac 2004

Pengerusi: Profesor Madya Rusea Go, Ph.D.

Fakulti: Sains dan Pengajian Alam Sekitar

Famili Lycopodiaceae di Malaysia mengandungi tiga genera yang dikenali sebagai *Huperzia*, *Lycopodium* dan *Lycopodiella* yang terdiri daripada 32 spesies termasuk 11 varieti. Empat spesies dilaporkan sebagai rekod baru di Malaysia (*Huperzia beccarii*, *H. goliathensis*, *H. prolifera* dan satu spesis *Lycopodium*). Famili ini mempunyai taburan di seluruh Malaysia tetapi kepelbagaian spesis tidak seperti yang terdapat di Amerika iaitu pusat taburannya. Kajian morfologi termasuk corak percabangan, anatomi dan kajian ultrastruktur telah dijalankan yang bererti secara taksonomi bagi pembatasan genus dan spesies Lycopodiaceae. Kehadiran rambut pada hujung daun, urat tengah dan petiol adalah ciri-ciri taksonomi daun bagi beberapa spesies dalam Lycopodiaceae. Dua asas corak percabangan yang dikenalpasti daripada kajian ini dan setiap satu memberikan gambaran jelas kepada sesuatu genus. Genus *Huperzia* dicirikan sebagai corak percabangan isotomous manakala *Lycopodium* dan *Lycopodiella* mempunyai corak percabangan anisotomous. Ciri-ciri tambahan pembezaan daun pada batang Lycopodiaceae seperti heterophyll, homophyll,

isophyll dan anisophyll adalah asas bagi pengkelasan genera dan beberapa spesies. Kajian anatomi batang menunjukkan bahawa Lycopodiaceae mempunyai stel jenis primitif yang dipanggil protostel dengan tiga bentuk. Kajian palinologi menunjukkan jenis spora, bentuk, saiz, corak perhiasan dan apertur adalah bererti secara taksonomi untuk membatas atau membezakan spesies. Ciri-ciri sporofil menunjukkan bahawa tepi, saiz, bentuk dan apeks daun memainkan peranan penting dalam pengecaman. Variasi pada struktur dinding sporangium dalam Lycopodiaceae didapati bererti secara taksonomi di tahap genus dan spesies. Berdasarkan kepada ciri-ciri yang dinyatakan, Lycopodiaceae telah dibahagikan kepada tiga genera di Malaysia: *Huperzia*, *Lycopodium* dan *Lycopodiella*. Ancaman semasa bagi Lycopodiaceae adalah penukaran hutan bukit kepada tanah pertanian yang merupakan habitat utama bagi famili ini dan juga pemungutan meluas oleh penduduk tempatan sebagai sumber pendapatan. Pemuliharaan dan undang-undang diperlukan bagi melindungi sumber penting dalam bidang perubatan dan juga kepentingan lain yang belum diketahui kepada manusia.

I certify that an Examination Committee met on 31st March 2004 to conduct the final examination of Clasius @ Claysius Kongoi on his Master of Science thesis entitled "Taxonomic Revision of Fern-Allies Lycopodiaceae Mirbel in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Umi Kalsom Yusuf, Ph.D.
Associate Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

Hishamuddin Omar, Ph.D.
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Misri Kusnan, Ph.D.
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Haji Mohamed Abdul Majid, Ph.D.
Professor
Faculty of Science
Universiti Malaya
(Independent Examiner)

_____

GULAM RUSUL RAHMAT ALI, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Rusea Go, Ph.D.
Associate Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

Saberi Othman, Ph.D.
Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Faridah Qamaruz Zaman, Ph.D.
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

_____

AINI IDERIS, Ph.D.
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

CLASIUS @ CLAYSIUS KONGOI

Date: 23 MARCH 2004

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Fern and fern-allies living today belong to the division of the Plant Kingdom usually called the Pteridophyta and differ primarily from the flowering plants in that they reproduce themselves not by means of seeds but by means of spores. The ferns and fern-allies of today are the modern representatives of an ancient group of plants, which lived long ago in geological time. Paleobotanical evidence indicates a late Paleozoic origin of Lycopodiaceae (Thomas, 1992).

Fern and fern-allies' fossils are known from as far back as the Silurian period (Piggott, 1979). Fossil remains are found in rocks of the Paleozoic estimated to be at least 250 million years old (Correll, 1956). They reached their greatest proliferation and development during the Carboniferous period (Correll, 1956; Jones *et al.* 1984) in the Paleozoic era, which is around 300 million years ago (Piggott, 1979), and since then, although the species have doubtlessly increased in number, they have become a less conspicuous element in most floras (Correll, 1956).

Ferns and fern-allies, as a group, not only exhibit a tremendous range in habit but also in their habitat requirements (Correll, 1956). The club mosses proper (Lycopodiaceae) is a large and widespread group (Jermy and Camus, 1991). On the whole, most of these are terrestrial on mountains throughout the world or epiphytic in tropical areas and require abundant moisture and at least partial shade (Correll, 1956). Some Lycopodiaceae are erect shrubby plants, or they may have a trailing or creeping habit, grow on rocks in full sun and often withstand extremely adverse condition (Correll, 1956; Foster and Gifford, 1959; Jermy and Camus, 1991; Øllgaard, 1992).

The fern-allies are a polymorphic group of herbs (Wikström and Kenrick, 1999), which are closely related to true ferns but with important structural differences (Jones *et al.* 1984; Wee, 1997). They do not possess distinctive fronds as do true ferns but the leaves are small and scattered along a simple or branched stem. They contain vascular tissues and most have a woody stem structure, while a few appear closer to mosses (Correll, 1956). According to Croft (1999), the living fern-allies can be divided into five classes; *Lycopodiopsida, Selaginellopsida, Isoetopsida, Equisetopsida* and *Psilotopsida.* Lellinger (1985) and Croft (1999) provide information regarding the classification of the Lycopodiaceae (Figure 1.1).

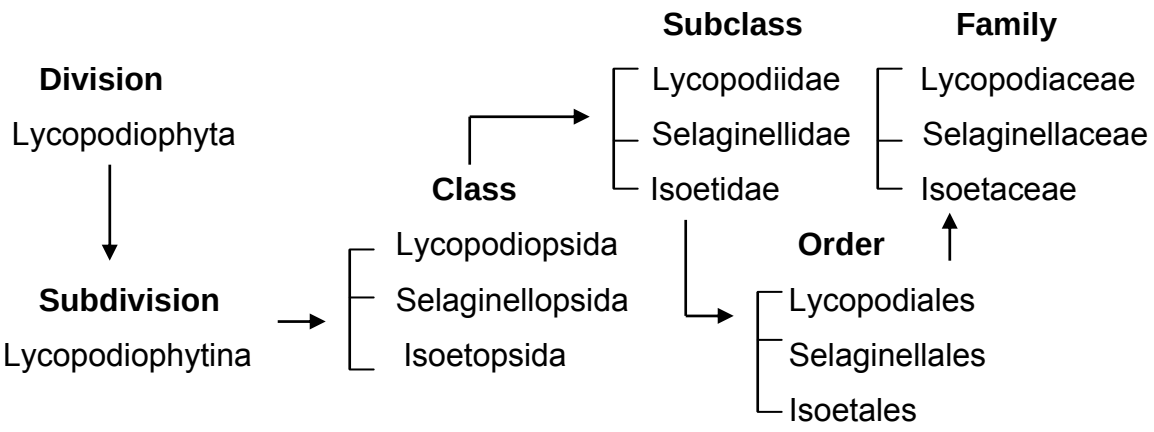

Figure 1.1: Classification of the Lycopodiaceae according to Lellinger (1985) and Croft (1999).

The club mosses are small, vascular plants, basically dichotomously branching and reproduce sexually by spores. The sporophytes are small and herbaceous (Parihar, 1973) consisting of true roots, an aerial stem and scale-like leaves called microphylls. These are small and spirally arranged on an elongated stem. Spores are all of one size and generally borne singly in the axils of specialised leaves (sporophylls) that are often aggregated into cone-like strobili similar to or different in shape and size to sterile leaves. The sporophylls may be aggregated into definite strobili or in zones on the stem (Parihar, 1973; Jermy and Camus, 1991).

The independent gametophytes in Lycopodiaceae are bisexual and are either irregularly lobed photosynthetically active masses, or subterranean, branching structures that lack chlorophyll and require a fungal symbiont. Multiflagellated sperm cells produced in an antheridium must travel through a film of water to

reach the eggs of an archegonium in order to form the zygote that may develop into the new sporophyte individual (Mohlenbrock, 1967; Lellinger, 1985).

1.2 Statement of Problems

For almost a century, no comprehensive studies were conducted on the Lycopodiaceae in Malaysia. There is no up-to-date taxonomic revision on this family in Malaysia and there is a critical need to investigate the taxonomic status of this family. The last taxonomic work in Malaysia was done by Ridley in 1919. Lack of knowledge of the individual species and their natural groupings has been responsible for the difficulties taxonomists have had in arriving at a truly acceptable concept of the family Lycopodiaceae in Malaysia. The many conflicting treatments serve as evidence of a generally poor understanding of these basic units of classification and point to the danger of attaching too much significance to any single character. There is a need to produce an up-dated taxonomic revision on this family in Malaysia and partly the Malesian region. A need to know the relationship among the species and their actual taxonomic status can only be achieved through complete taxonomic studies. Realising Lycopodiaceae are not well known in Malaysia in terms of its taxonomy, a complete, extensive and detailed study is needed to identify a set of proposed key characters to distinguish species of Lycopodiaceae. This will give a clearer picture about the taxonomic alliances of the individual taxa within genera and family.

1.3 Objective of Study

This study was initiated by a strong interest to understand the species diversity in Lycopodiaceae particularly in Malaysia. The following objectives are laid for this study:

i. To document available taxa.
ii. To solve the taxonomic problems.
iii. To investigate possible wrongly named or unnamed species.
iv. To search for key characters to be used in species identification.
v. To evaluate as many characters as possible in order to ascertain taxonomic position of the genus within Lycopodiaceae.
vi. To create awareness for conservation of species and habitat.
vii. To find out potential economic importance of the family Lycopodiaceae.

To achieve these objectives, the study focused on ascertaining the taxonomic status of the genera and species recognized in the family based on comprehensive investigation of consistent gross morphological characters, stem vascular systems as well as other microscopic evidence *via* scanning electron microscope such as spore and sporophyll ultrastructure.

Over a year, extensive collections of specimens were made in Malaysia including Sabah and Sarawak and the possibility of discovering undescribed and new taxa

is very high. Due to this, a study on these new collections is critically needed. Highland areas, the main habitat of this family, are threatened by logging, deforestation and land reclamation. Therefore, conservation is a crucial step needed to avoid the loss of the plants' genetic resource in this country.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The Lycopodiaceae *sensu lato* are an ancient and probably monophyletic family without close living relatives and have a virtually cosmopolitan distribution (Øllgaard, 1992). The estimated number of species ranges from approximately 300 to more than 400 around the world (Wikström, 2001). Worldwide the estimated number of species for both *Lycopodium* and *Lycopodiella* is about 40 (Wikström and Kenrick, 2000b) and the estimated number of species of *Huperzia* is 300 (Wikström and Kenrick, 2000a). Characters of taxonomic importance in the Lycopodiaceae are cortex and stellar anatomy, spore and sporophyll morphology, and chromosome number (Øllgaard, 1992). The taxonomic history was summarized by Wilce (1965). Rothmaler's (1944) papers stimulated new interest in the classification of this family, but also created confusion. Boivin (1950), Wilce (1965; 1972), Bruce (1976a; 1976b; 1976c) and Øllgaard (1975; 1979) provided new information and understanding of taxonomic characters and relationships. Wilce's (1972) study of spore morphology established the outline of

a classification system for the *Lycopodium*, with modifications of taxonomic ranks and circumscription.

2.1 Taxonomic Notes on Lycopodiaceae

2.2.1 History of Classification

The early classification of the Lycopodiaceae was carried out in 1842-1846 from Spring's *Monographie de la famille des Lycopodiacées*. Thirty years later, Baker's *Handbook of the Fern-Allies* came out and was published in 1887. The most recent taxonomic classification of the fern-allies, the *Index of the Lycopodiaceae* by Øllgaard, was reported in 1989.

Baker (1887) classified the Lycopodiaceae into two genera (*Phylloglossum* and *Lycopodium*), four subgenera, seven groups and 94 species occurring worldwide. According to the new classification by Øllgaard, Lycopodiaceae consists of four genera, namely *Huperzia, Lycopodiella, Lycopodium* and *Phylloglossum*. The largest genera were *Huperzia* (22 groups), followed by *Lycopodium* (nine sections), *Lycopodiella* (four sections) and *Phylloglossum* (one species).

2.2.2 Early Classification of Lycopodiaceae in Malaysia

The fern-allies Lycopodiaceae, better known as clubmosses, belong to the division Lycophyta that include both living and extinct plants (Bidin *et al.* 2002). The existence of this family in Malaysia was first described by Alderwerelt as early as 1915 and 1916 and Ridley (1919) based on morphological approaches. Alderwerelt (1915; 1916) reported that Lycopodiaceae consists of only one genus (*Lycopodium*) with two sections, *Urostachys* and *Rhopalostachys*. Each section is divided into two groups. *Selago* and *Phlegmaria* are stated as sections of *Urostachys* while *Lepidotis* and *Diphasium* belong to section *Rhopalostachys*. Forty and 12 species were recorded belonging to each of the sections, *Urostachys* and *Rhopalostachys*, respectively.

Ridley (1919), in his revision *The Fern-Allies and Characeae of the Malay Peninsula,* based on the work of Spring (1842; 1846), Baker (1887) and Alderwerelt (1915), reported the Lycopodiaceae as consisting of two genera, *Lycopodium* (thirteen species) and *Psilotum* (two species), respectively. According to Ridley (1919), no species is endemic, the terrestrial species are more widely distributed than the epiphytic ones as the area where epiphytic plants can grow is less extensive than the non-epiphytic area. Besides that, he also reported that Lycopodiaceae have two species of *Psilotum* and a species of *Lycopodium* with a very extended range. The two species of *Psilotum* are distributed over all tropical countries as far as Florida and New Zealand, with *P.*