MORPHOLOGICAL VARIATION WITHIN *Salvinia molesta* D.S.MITCHELL (SALVINIACEAE) POPULATIONS IN THE KINABATANGAN, SABAH.

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DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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ABSTRACT

MORPHOLOGICAL VARIATION WITHIN *Salvinia molesta* D.S.MITCHELL (SALVINIACEAE) POPULATIONS IN KINABATANGAN, SABAH.

This study aimed to understand the principle of variation within the same species of plant, though it grew in a narrow environmental gradient area, such as the tropic. An aquatic fern species and has the potential to become a weed in the Sabah freshwater ecosystem was chose for this study: *Salvinia molesta* D.S Mitchell. Taxonomical and ecological informations of this species in Sabah were still at the initial stage and very limited. The Lower Kinabatangan River is the place when it was first recorded and became the focus area in this study. Samples of *S. molesta* from twelve stations along the Lower Kinabatangan River and three comparison stations from the Sandakan inland were analyzed to determine morphological differences between: quadrates (within the same population), between populations (from different stations), and between two types of water bodies (open and closed water bodies). By considering the maximum level of individual's maturity stage, the statistical analysis of One-way ANOVA and t-test were used to compare the means of morphometric measurements and meristic counts on six quantitative characters of this species. The quantitative characters were from the vegetative structure (floating leaf length and width, submerged leaf length, rhizome diameter) and reproductive structure (number of fertile axis, number of sporocarps). All the samples analyzed showed that there were morphological differences within the *S. molesta* populations in the study area. Variation between quadrates was significant ($\rho<0.001$) within the *S. molesta* populations with the size of more than five meter square. Variation between populations along the Kinabatangan River resulted with a highly significant ($\rho<0.001$) morphological differences from one stations to another. Morphological differences for samples from the two type of water bodies (open and closed water bodies) were also showed a highly significant ($\rho<0.001$) different; samples from the open water body were bigger in sizes of leaves and rhizome. Based on regression and correlation analysis, relationship between the quantitative characters were positive and significant ($\rho<0.01$). However, regression between the characters and the stations positioned showed that the plants were bigger at the upper stream area. The cluster analysis of Ward's method showed samples from the Sg. Koyah was a distinctive group among others. No consistent morphological characters can be used to distinguish between intraspecific populations of this species. The data obtained from this present study agrees with the hypothesis that there was an intraspecific variation occurred within the *S. molesta* populations in the Kinabatangan and rejected the null hypothesis. This study also added evidence on *S. molesta* as a single species of a highly morphological variability even in a narrow environmental gradient area, such as the tropic.

KEYWORDS: Intraspecific variation, *Salvinia molesta*, weed species, aquatic fern

ABSTRAK

Kajian ini bertujuan untuk memahami prinsip variasi di dalam satu spesies tumbuhan yang sama, walau pun tumbuhan tersebut berada di kawasan yang mempunyai gradien persekitaran yang sempit seperti Tropika. Sejenis paku-pakis akuatik dan berpotensi hidup sebagai lalang di ekosistem air tawar di Sabah telah dipilih bagi kajian ini: Salvinia molesta D.S. Mitchell. Maklumat taksonomi dan ekologi spesies ini di Sabah masih di peringkat pengenalan dan sangat terhad. Hilir Sungai Kinabatangan merupakan kawasan yang paling awal merekodkan kehadiran spesies ini dan dijadikan kawasan utama dalam kajian ini. Sampel S. molesta dari 12 stesen di sepanjang Hilir Sungai Kinabatangan dan tiga stesen perbandingan dari daratan Sandakan telah dianalisa bagi mengenalpasti perbezaan morfologi yang wujud: antara kuadrat (dalam satu populasi yang sama), antara populasi (pada stesen yang berbeza) dan antara dua jenis badan air (terbuka dan tertutup). Dengan mengambilkira tahap pertumbuhan yang maksimum bagi individu matang spesies ini, analisis statistik One-Way ANOVA dan t-test telah digunakan untuk membandingkan purata ukuran morfometrik dan meristik terhadap enam ciri kuantitatif S. molesta. Ciri-ciri kuantitatif tersebut dipilih daripada struktur vegetatif (panjang dan lebar daun terapung, panjang daun separa-tenggelam, diameter rizom) dan struktur reproduktif (bilangan paksi fertil, bilangan sporokap). Kesemua sampel yang dianalisa telah menunjukkan bahawa wujudnya variasi morfologi di antara populasi S. molesta yang terdapat di kawasan kajian. Variasi antara kuadrat sangat signifikan ($p<0.001$) pada populasi S. molesta yang bersaiz lebih dari lima meter persegi. Manakala analisis variasi antara populasi pula menunjukkan berlakunya perbezaan morfologi yang sangat signifikan ($p<0.001$) antara satu stesen dengan stesen lain yang terdapat di sepanjang Sungai Kinabatangan. Variasi yang sangat signifikan ($p<0.001$) juga dikesan wujud pada populasi S. molesta yang dikumpul daripada dua jenis badan air yang berbeza (badan air terbuka dan badan air tertutup); sampel daripada badan air terbuka menunjukkan saiz daun dan rizom yang lebih besar. Berdasarkan analisis korelasi dan regresi, hubungan di antara ciri-ciri kuantitatif yang dipilih adalah positif dan signifikan ($p<0.01$). Manakala hubungan di antara ciri-ciri spesies ini dengan kedudukan stesen kajian dari kawasan tengah ke hilir Sungai Kinabatangan menunjukkan bahawa sampel di kawasan tengah mempunyai saiz daun yang lebih besar. Analisis pengelompokan (Cluster Analysis) menggunakan kaedah Ward's pula menunjukkan sampel dari Sungai Koyah sebagai suatu kumpulan yang agak berbeza berbanding dengan populasi lain. Walau bagaimanapun, tidak terdapat sebarang ciri morfologi yang boleh digunakan secara konsisten untuk membezakan antara populasi intraspesifik. Data yang diperolehi dari kajian ini menyokong hipotesis kajian bahawa terdapat variasi intraspesifik bagi populasi S. molesta di kawasan Kinabatangan dan menolak hipotesis sifar. Kajian ini turut menambah bukti tentang S. molesta sebagai suatu spesies yang menunjukkan variasi morfologi yang tinggi walaupun berada pada kawasan yang mempunyai gradien persekitaran yang sempit seperti di kawasan tropika.

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ABBREVIATIONS

%	percentage
ANOVA	Analysis of Variance
AS	Anak Sungai
cm	centimeter
D.	Danau
DK	Danau Kecil
DO	Dissolved Oxygen
ed.	edited
eds.	edited
gpos	geographical position
Kg.	Kampung
km	kilometer
m	meter
ms^{-1}	meter per second
N	sample size
-na-	not available
No.	Number
p	probability
ppt	part per thousand
Q	Quadrate
r	correlation coefficient
r^2	r-square; for regression
Sal.	Salinity
SD	Standard Deviation
Sg.	Sungai
Tg.	Tanjung
-im-	immature plants
Velo.	Velocity
W.temp	Water temperature

CHAPTER 1

INTRODUCTION

1.1 Plant variation

Variation within a species has been the subject of studies during the last several decades. Henry (2005) has considered the diversity within a population of plants of the same species as a primary level of variation. As plants vary in different ways, our level of knowledge to understand the differences also varies. The vascular plants are better known than the lower plants, or plants of the temperate regions than those of the tropics, or about plants of great economic value than about those of little commercial interest (Panggabean *et al.*, 1971). It should be realized that for the great majority of taxa, particularly lower plants and tropical plants, the taxonomic study on morphological variation is still at the exploratory phase. The study includes the description of the variation of organisms, the investigation of the causes and consequences of this variation, and the manipulation of the data obtained to produce a system of classification (Stace, 1989).

Variation occurs at any level in the taxonomic classification. Focusing at a lower taxonomic level, the process of speciation and its genetic basis imply that not only an organism varies in their genetic make-up between species but they also vary within the same species (Lawrence, 1973). This variability was commonly known as "intraspecific variation". The intraspecific variability can be demonstrated to be of genetic origin and is not randomly dispersed throughout the species range, but is distributed in such a way that neighboring plants tend to resemble one another (Davis and Heywood, 1967). In

spite of the heredity, some of this variation may be due to the environment (Solomon *et al.*, 2002). The inherent variability of species has been accepted as a phenomenon which requires detailed study and appropriate treatment (Davis and Heywood, 1967).

Individuals within a population which sexually reproduce, show variation in many recognizable traits, namely morphologically, physiologically or behaviorally (Campbell and Reece, 2002; Starr and Taggart, 2004). For those that reproduce asexually, they are genetically identical and most of the time appear rather uniform (Starr and Taggart, 2004). The uniformity appearance can be commonly demonstrated by plants which reproduce by the vegetative mode or through clonally growth.

There are numerous studies on intraspecific variation within plants, but these rarely involve genetically identical populations of a species. Latest studies on this subject include those conducted by Barney *et al.* (2005), and Kobayashi *et al.* (2001). These kinds of study were so important to illustrate the principle of morphological variation occurring at the specific level and gives clues to the way in which a plant has adapted to their environment (Stuessy, 1990). As more study on this matter is needed for plants in the tropics, an aquatic species was chosen to show its potential of persisting in a variety of habitats. One of the species observed is the *Salvinia molesta* D.S. Mitchell.

1.2 *Salvinia molesta*

Salvinia molesta or known locally as 'kiapok kecil' is a floating aquatic fern species which has never been scientifically studied in Sabah. It is a cosmopolitan species (Holm *et al.*, 1977) and one of the genetically homogenous plants which can be found in the tropics (Room, 1986). Within a localized population, it is genetically

uniform since it reproduces vegetatively (Thomas and Room, 1986; Room, 1990). Information on this species is rather lacking, especially when it comes to taxonomic aspects. In Sabah, prior to this study, there was no preserved specimen found in herbaria. Said (2005) is the first to record this species in Sabah. In terms of its introduction, there is still no record on when and how exactly *S. molesta* started to invade Sabah water bodies (Kaw, 2005). However, Vaz (1998) and Azmi (1998) have recorded the occurrence of *S. molesta* in Kinabatangan River which is said to occur since 1993. In the early of year 2005, the species was reported to bloom in some oxbow lakes in this river (Kaw, 2005).

This aquatic plant species is commonly found in stagnant water, ponds, slow moving river, rice cultivation and various water systems (Holm *et al.*, 1977; Thomas and Room, 1986). The significance of *S. molesta* (also commonly known as salvinia) is its role as one of the invasive weed species in the South East-Asian region (Soerjani, 1976) and the world (Holm *et al.*, 1977). Salvinia shows strong vegetative propagation with a very high growth rate and tolerant to wide range of environmental conditions (Thomas and Room, 1986; Room, 1990). It is able to form thick, spread floating mats up to one meter, and complete coverage of the water surface in relatively short time period (Thomas and Room, 1986; Room, 1990). Most studies relating *S. molesta* are directly related to control measures (Room and Gill, 1985; Julien, 1987; Room, 1990; Room and Fernando, 1992). Taxonomic and ecological aspects are rather badly neglected, especially when it occurs in tropical countries (Panggabean *et al.*, 1971).

1.3 Aim and objectives of study

This study is aimed at illustrating the principle of morphological variation within a genotypically homogenous species such as *Salvinia molesta* which occurs in a narrow environmental gradient within the tropics.

Pattern of the variation was observed based on selected morphological characters in natural populations and analyzed morphometrically. Significant seasonal, interpopulational and intrapopulational variation within the species were to be described in detail. Furthermore, morphological variation between samples which occur in the open-ended water bodies and close-ended water bodies were also compared. As to support understanding on the variation occurs, interaction among the selected characters and with the position of the populations were also been analyzed. From these data, we would be able to understand habit of the character itself and how does it react towards the environment.

At the same time, information on taxonomic and ecological aspects of *S. molesta* is also emphasized. Distribution of the species within the study area will contribute to demonstrate its infestation in the east part of Sabah area.

In short, objectives of this present work are:-

i) to determine significant morphological variation within the *S. molesta* populations at various level : seasonal, interpopulational and intrapopulational ;

ii) to compare morphological variation of *S. molesta* occurs in an open water bodies and close water bodies; and

iii) to demonstrate the relationship between characters of *S. molesta*.

Hypothesis of this study:-

H_1: Intraspecific variation does occur within *S. molesta* populations along the Kinabatangan River in Sabah

H_0 : Intraspecific variation does not occur within *S. molesta* populations along the Kinabatangan River in Sabah

CHAPTER 2

LITERATURE REVIEW

2.1 Intraspecific variation in plants

Variation exists in almost all living organisms in the world. No matter what causes it and where it will lead us, variation is definitely an important part in the context of a taxonomic study. Taxonomy is a study aimed at producing a system of classification of organisms which best reflects the totality of their similarities and differences (Cronquist, 1988). Similarities and differences among the members of the same species are commonly known as intraspecific variation (Campbell and Reece, 2002).

2.1.1 Basis of variation

Not all the variations observed in a population are heritable. They may be expressed genotypically or phenotypically. The genotype is the sum total of all the genes present in the individual (Starr and Taggart, 2004). The genotypes of the offspring will differ from each other to a greater or lesser degree depends on the degree of heterozygosity of one or both parents. The phenotype is the cumulative product of an inherited genotype and a multitude of environmental influences (Campbell and Reece, 2002). It represents the result of external factors (such as growing conditions) on its genotype (Starr and Taggart, 2004).

There are three basic factors that can cause variation between the individual members of a population (Davis & Heywood, 1967): (a) external environmental factors, (b) mutation, and (c) genetic recombination. The mutation and genetic recombination will not be discussed further in this thesis as the Salvinia species are not known to reproduce sexually (Holm *et al.*, 1977; Room,

1990). Both factors are related to the changes at the DNA base pair sequence level and largely determined by the sexually breeding system (Davis and Heywood, 1967). The only area of concern to be discussed in further details in this thesis is the external environmental factors that cause variation which is better known as the "phenotypic plasticity".

2.1.2 Phenotypic plasticity

Phenotypic plasticity is the ability of an individual organism to alter its physiology or morphology in response to variation in environmental conditions (Schlichting, 1986). Stace (1989) defined the term phenotypic plasticity as the ability of an organism to express a genotype as different phenotypes according to external condition. Features which are under environmental regulation are said to be 'plastic', rather than 'variable' which are due to genetic differences (Stuessy, 1990). Plants certainly are 'plastic', and the limits of this plasticity are important to be understood before making final taxonomic decisions based on selected characters and states (Stuessy, 1990). Much of the phenotypic variation encountered by the taxonomist is the result of plastic response of the individual to the factors of the environment . The problems it poses are most acute in certain groups such as aquatic and marsh plants and in certain regions such as the tropics (Davis and Heywood, 1967).

Phenotypic plasticity in response to environmental factors has clearly contributed to the differences between individuals in many populations. This has been numerously studied on higher plants and the bryophytes (Stace, 1989). Some examples of species recently studied in this context include: *Chamelaucium uncinatum* (Egerton *et al.*, 1998), *Licuala glabra* (Loo *et al.*, 2001), *Plantago asiatica* (Kobayashi *et al.*, 2001), *Syntrichia laevipila* complex (Teresa *et al.*, 2005), *Arundo donax* (Spencer *et al.*, 2005), and many others. Most of the

studies focus at the variation occurring in certain contrasting environments such as shady versus exposed, alpine versus lowland, and wet versus dry conditions. Naturally, such contrasting conditions would lead to extreme ecophene in a wide range of species. More data are needed to understand the plastic responses by plants at the tropics, especially when it deals with a narrow environmental gradient within a given area.

2.2 *Salvinia molesta* : An aquatic fern

In the wet tropics, ferns are the most abundant and most diversified plants (Holttum, 1988). It can be found in all kind of habitats: dry, wet, watery, shady, rocky and even arid areas. In Malaysia, there are about 1136 species of ferns (Parris & Latiff, 1997). In Sabah there are about 804 species of ferns from 148 genera, and 34 families (Said, 2005).

Based on their growth in shallow fresh water which is either still or moving slowly, Jones (1987) and Brummit (1992) considered four families as proper aquatic ferns: Azollaceae (genus *Azolla*), Parkeriaceae (genus *Ceratopteris*), Marsileaceae (genera *Marsilea*, *Pillularia*, and *Regnellidium*) and Salviniaceae (genus *Salvinia*). These aquatic ferns are frequently found in low-lying areas which are periodically flooded, and here they may grow as annuals (Jones, 1987). Chin (1997) argued that a watery environment is not the best habitat for ferns. Only few fern species are adapted to survive under such conditions.

In Malaysia, Bidin (1986) and Piggott (1988) recorded only four genera of aquatic ferns: *Azolla* (*A. pinnata*), *Ceratopteris (C. thalictroides)*, *Marsilea (M. crenata)* and *Salvinia* (*S. cucullata*, and *S. molesta)*. Said (2005) listed three genera of aquatic ferns found in Sabah: *Salvinia*, *Marsilea* and

Ceratopteris. Somehow, Said (2005) did not discover *Azolla* in the wetland areas in Sabah, although this genus might occur in Sabah.

2.2.1 Taxonomy and classification

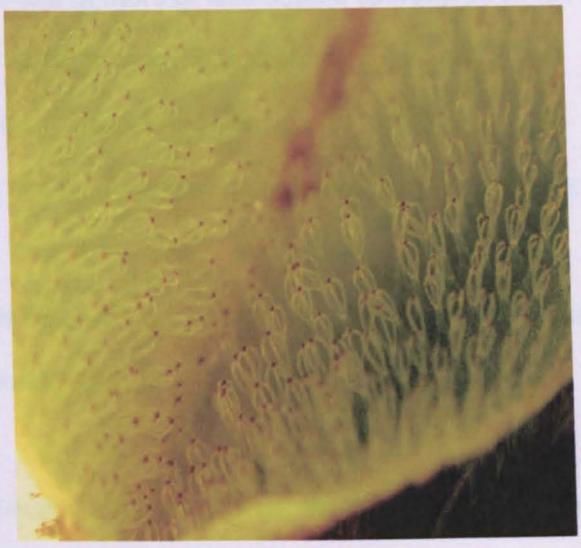
Schneller (1990) and Rajan (2000) classified the *Salvinia* plant as follow:

Division:	Pteridophytes
Subdivision:	Pteropsida
Class:	Leptosporangiate
Subclass:	Salviniidae
Order:	Salviniales
Families:	Salviniaceae

The Salviniales include all the aquatic fern families or commonly referred to as *'water ferns'*. There are two families, Salviniaceae and Azollaceae, which are very close since they are from the same combined group known from the Cretaceous Era (Mabberley, 1997). Salviniaceae is a genotypic family represented by a single genus, *Salvinia* (Brummit, 1992). Under the genus *Salvinia*, there are some 10 species (Schneller, 1990; Gifford and Foster, 1996) or 12 species (Holm *et al.*, 1977). One of them is *Salvinia molesta*.

Salvinia molesta is grouped together with *Salvinia auriculata* Aublet, *S. biloba* Raddi and *S. herzogii* de la Sota under what is known as the S. *auriculata* complex (Mitchell and Thomas, 1972). They have been grouped on the basis of closed divided hairs on the upper leaf surface each of which form an 'egg-beater like' structure (Forno, 1983). These hairs which can be clearly seen with a hand lens give a velvety appearance and repel water (Figures 2.1a and 2.1b).

(a)



(b)

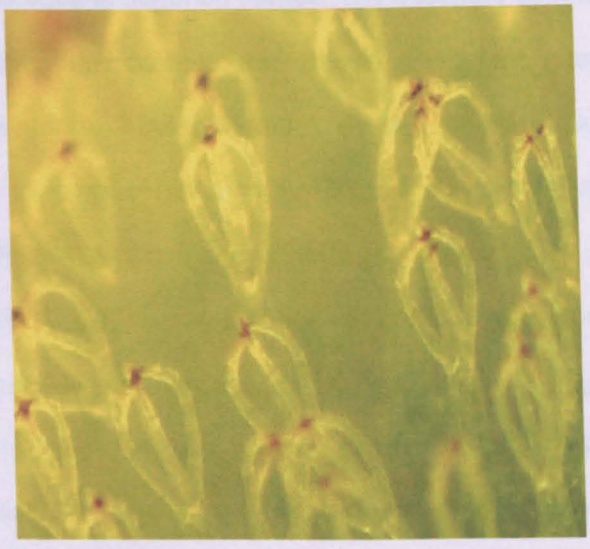


Figure 2.1 (a) 'Egg-beater like' structure on upper leaf surface of S.*auriculata* complex species (25x)
(b) A detailed structure of the 'egg-beater like' structure (40x)

Based on Forno (1983), *S. molesta* can be distinguished from other species in the *S. auriculata* complex by the following characters:-

(i) the arrangement of sporocarps, when the fertile axis present - fertile axis bears a large number of more or less sessile sporocarps which are arranged dorsiventrally in a cymose system,

(ii) the pattern of primary areolation, when fertile axis is absence – there are five to ten primary areoles between the keel and the margin of a tertiary leaf,

(iii) the chromosome number - the chromosome number for *S. molesta* is 45 (Loyal and Grewal, 1966).

Salvinia molesta has a synonym which is *S.cucullata* Roxb. ex Bory (Piggott, 1988) as well as several common names. The accepted common names were salvinia (which is the same as the genus name), water fern, giant salvinia, water spangles, floating fern (United States), Kariba weed (Lake Kariba, Rhodesia), African Pyle or Payal (Africa), giant Azolla or Australian Azolla in the Philippines (www.invasiveplants.net), butterfly fern as referred to its look-like a butterfly at rest (Fisher, 2000), aquarium watermoss and eared watermoss refering to its size and use in freshwater aquaria (tncweeds.ucdavis.edu). It is also known as Paku Apong in Malay (www.ooi'sfloramalaysiana). E. de la Sota (1995) has proposed to replace *S. molesta* with *S. adnata*, but Moran and Smith (1999) had shown that *S. adnata* was a name of uncertain application (www.ars-grin.gov).

2.2.2 Description

The basic individual unit of *Salvinia molesta* is called a 'ramet' and colonies of ramets held together by horizontal, branching rhizomes are known as 'genet' (Room, 1990). Each ramet comprises an internode, a node, a pair of floating

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