



Faculty of Resource Science and Technology

**MORPHOLOGICAL AND MOLECULAR CHARACTERISATION  
OF WATER LILY (FAMILY: NYMPHEACEAE) IN  
UNIMAS LAKE EAST CAMPUS**

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**Morphological and Molecular Characterisation of Water Lily (Family:  
Nymphaeaceae) in UNIMAS Lake East Campus**

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This project is submitted in partial fulfillment of the Final Year Project (STF 3015) Course  
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(Aquatic Resource Science and Management)

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## **DECLARATION**

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree qualification of any other university or institution of higher learning.

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## Table of contents

<b>Acknowledgement.....</b>	<b>i</b>
<b>Declaration.....</b>	<b>ii</b>
<b>Table of Contents.....</b>	<b>iii</b>
<b>List of Abbreviations.....</b>	<b>v</b>
<b>List of Figures.....</b>	<b>vii</b>
<b>List of Tables.....</b>	<b>viii</b>
<b>Abstract.....</b>	<b>1</b>
<b>1.0 introduction.....</b>	<b>2</b>
<b>2.0 Literature Review.....</b>	<b>4</b>
2.1 Classification of water lily.....	4
2.2 Morphological characteristics of water lily.....	5
2.3 History of water lily.....	7
2.4 Difference between Water Lotus Family and Water Lily Family .....	8
2.5 Importance of water lily.....	9
2.6 Internal Transcribed Spacer (ITS) region.....	11
2.7 Molecular study on water lily.....	12
<b>3.0 Material and Methods.....</b>	<b>14</b>
3.1 Sampling site and sample collection.....	14
3.2 Morphological assessment.....	15
3.3 Sample collection for molecular work.....	15
3.4 laboratory work.....	16
3.4.1 Preparation of buffer solution for modified CTAB method (Doyle & Doyle, 1987).....	16

3.4.2 Total Genomic DNA extraction using modified CTAB	
Method.....	16
3.4.3 Agarose gel electrophoresis.....	18
3.4.4 Polymerase Chain Reaction (PCR).....	18
3.4.5 DNA Sequencing.....	20
3.4.6 Data Analysis.....	20
<b>4.0 Result and Discussion.....</b>	<b>21</b>
4.1 Morphological assessment.....	21
4.1.1 Meristic data.....	22
4.1.2 Morphometric data.....	25
4.1.3 Relationship between taxa.....	27
4.2 Molecular works.....	29
4.2.1 Total genomic DNA extraction of water lily.....	29
4.2.2 Polymerase Chain Reaction (PCR).....	32
4.3 Sequencing analysis.....	34
4.4 Genetic divergence analysis.....	41
4.5 Phylogenetic analysis.....	45
4.5.1 Molecular phylogeny of water lily based on ITS gene analysis....	45
<b>5.0 Conclusion.....</b>	<b>49</b>
<b>5.1 Recommendation.....</b>	<b>50</b>
<b>6.0 References.....</b>	<b>51</b>
<b>7.0 Appendices.....</b>	<b>55</b>

## LIST OF ABBREVIATIONS

<b>Abbreviation</b>	<b>Full Terms</b>
bp	Base pair
CTAB	Cethy-trimethyl Ammonium Bromide
CIA	Chlorophyll Isomyl Alcohol
ddH <sub>2</sub> O	Deionized Distilled Water
DNA	Deoxyribonucleic acid
dNTP mix	Deoxynucleotide Triphosphate Mix
EtBr	Ethidium Bromide
EtOH	Ethanol
FRST	Faculty of Resource Science and Technology
g	Gram
ITS	Internal Transcribed Spacer
MgCl <sub>2</sub>	Magnesium Chloride
mM	Millimole
ml	Milliliter
NaCl	Sodium Chloride
PCR	Polymerase Chain Reaction
s	Second
TBE	Tris-borate-EDTA
μl	Microliter

## LIST OF FIGURES

Title	Caption	Page
Figure 2.1	The diagram of classification of water lily family up to genus level	4
Figure 2.2	The diagram of Nymphaeaceae general morphology	5
Figure 2.3	The diagram of morphological anatomy between water lily and water lotus	9
Figure 2.4	The diagram of edible part of water lily in tropical and temperate zone	10
Figure 2.5	The diagram of internal transcribed spacer (ITS)	11
Figure 3.1	The diagram of location for sampling site at UNIMAS Lake, East campus	14
Figure 3.2	The diagram of PCR temperature profile for ITS gene	19
Figure 4.1	The diagram of <i>Nymphaea capensis</i> , collected from UNIMAS Lake on 18 <sup>th</sup> October 2013	22
Figure 4.2	The diagram of <i>Nymphaea lotus</i> , collected from UNIMAS Lake on 18 <sup>th</sup> October 2013	23
Figure 4.3	The diagram of <i>Nymphaea pubescens</i> , collected from UNIMAS Lake on 18 <sup>th</sup> October 2013	24
Figure 4.4	The diagram of UPGMA phylogenetic tree (morphological characters) collected from UNIMAS Lake on 18 <sup>th</sup> October 2013	28
Figure 4.5	The diagram of gel electrophoresis photograph showing total genomic DNA extraction product from water lily	29
Figure 4.6	The diagram of gel electrophoresis photograph showing Polymeras Chain Reaction from water lily	33
Figure 4.7	The diagram of BLAST result of ITS gene for WL01 and WL02 with <i>N. capensis</i> (AY707898)	36
Figure 4.8	The diagram of BLAST result of obtained sequences (WL04 and WL06) with nucleotide sequence from NCBI database.	38
Figure 4.9	The diagram of BLAST result of obtained sequences (WL08) with nucleotide sequence from NCBI database.	40



Figure 5.1	The diagram of Neighbour joining (NJ) phylogenetic tree constructed based on water lily ITS gene sequences using <i>N. pentapetala</i> as outgroup.	46
Figure 5.2	The diagram of Maximum parsimony (MP) phylogenetic tree constructed based on water lily ITS gene sequences using <i>N. pentapetala</i> as outgroup.	46
Figure 7.1	Flowchart of overall work	55
Figure 7.2	Flowchart of preparation for CTAB buffer solution	57
Figure 7.3	The diagram showing online reference from <a href="http://www.kew.org">www.kew.org</a>	58
Figure 7.4	The diagram showing online reference from <a href="http://www.plantzafrica.com">www.plantzafrica.com</a>	59
Figure 7.5	The diagram showing online reference from <a href="http://www.fao.org">www.fao.org</a>	60

## LIST OF TABLES

<b>Title</b>	<b>Caption</b>	<b>Page</b>
Table 4.1	Species identified for water lily's samples in UNIMAS Lake, East Campus.	21
Table 4.2	Morphometric data for water lily specimens, collected from UNIMAS Lake on 18 <sup>th</sup> October 2013.	25
Table 4.3	Characters and character state used in the morphology analysis of <i>Nymphaea</i> sp.	27
Table 4.4	Matrix indicating distribution of character states used in morphology-based data analysis on <i>Nympaea</i>	27
Table 4.5	Summary of mutation occurred for <i>N. capensis</i> samples	35
Table 4.6	Summary of mutation occurred for <i>N. lotus</i> samples	39
Table 4.7	Pairwise distance (Kimura two-parameter model) in percentage (%) among 5 samples in UNIMAS Lake, East Campus.	42
Table 4.8	Pairwise distance (Kimura two-parameter model) in percentage (%) between 5 samples in UNIMAS Lake, East Campus and selected sequences from GenBank	44
Table 4.9	Origin of water lily samples from Gen Bank	48
Table 7.1	Recipe of preparation for CTAB buffer solution	56
Table 7.2	Meristic data of water lily	61

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## ABSTRACT

Water lilies are aquatic angiosperms in the Family Nymphaeaceae. They are important ornamental plants that enhance the beauty of ponds and lakes. Several studies on water lilies in Sarawak had been carried out on ecological aspects, but there is limited number of molecular data on water lilies in Malaysia available in the GenBank. This study is designed to sequence the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA from the water lilies sampled from Universiti Malaysia Sarawak (UNIMAS) Lake, East Campus. A total of 11 water lilies were subjected to identification based on morphological characteristics. In addition, for molecular work, the total genomic DNA was extracted from the flowers and leaves using modified CTAB method followed by Polymerase Chain Reaction to amplify 730 bp ITS gene, DNA sequencing and data analysis. In this study, there were three species of water lily found in UNIMAS Lake identified as *Nymphaea capensis*, *N. lotus* and *N. pubescens* based on combination of morphological data and ITS gene sequence. *N. capensis*1 and *N. capensis*2 have low genetic divergent (0% - 0.9%) which suggesting intraspecific variations. *N. capensis* and *N. lotus* have 30.7 % of genetic divergent whereas *N. lotus* and *N. pubescens* have genetic divergent value of 5.5 %. *N. lotus* is monophyletic with high bootstrap value, 98% in NJ tree and 97% in MP tree. *N. capensis* has bootstrap value of 99% in NJ tree and 100% in MP tree which indicates that the species is monophyletic. *N. pubescens* is also monophyletic with 100% bootstrap value in both NJ and MP tree..

Key words: Water lily (Genus *Nymphaea*), morphological characters, ITS gene, diversity, genetic divergence.

## ABSTRAK

Teratai adalah tumbuhan berbunga akuatik yang tergolong dalam keluarga Nymphaeaceae. Teratai adalah tumbuhan hiasan penting yang meningkatkan kecantikan sesebuah kolam dan tasik. Beberapa kajian tentang teratai di Sarawak telah dijalankan, namun hanya terdapat sedikit data molekular berkenaan teratai di Malaysia di dalam GenBank. Kajian ini bertujuan untuk mendapatkan gen ITS yang terdapat di DNA ribosom daripada teratai di Tasik UNIMAS, Kampus Timur. Kesemua 11 kuntum teratai telah dikenalpasti berdasarkan ciri-ciri morfologi yang terdapat pada sampel tersebut. Kajian molekular dijalankan ke atas kesemua gen DNA yang diekstrak daripada bunga dan daun mengikut cara CTAB yang diubah suai diikuti tindakbalas berantai polimerasi untuk amplifikasi 730bp gen ITS, penjujukan DNA dan analisis data. Dalam kajian ini, sejumlah 3 spesies teratai yang ditemui di Tasik UNIMAS dikenalpasti sebagai *Nymphaea capensis*, *N. lotus* dan *N. pubescens*. *N. capensis*1 dan *N. capensis*2 mempunyai nilai percapahan genetik yang rendah (0% - 0.9%) yang mencadangkan variasi intraspesifik. *N. capensis* dan *N. lotus* mempunyai 30.7% nilai percapahan genetik manakala *N. lotus* dan *N. pubescens* mempunyai 5.5% nilai percapahan genetik. *N. lotus* adalah monofiletik dengan nilai bootstap yang tinggi, 98% dalam pokok NJ dan 97% dalam pokok MP. *N. capensis* mempunyai nilai bootstrap 99% dalam pokok NJ dan 100 % dalam pokok MP yang menunjukkan bahawa spesies ini adalah monofiletik. *N. pubescens* juga adalah monofiletik dengan nilai bootstrap 100% dalam pokok NJ dan MP.

Kata kunci: Teratai (Genus *Nymphaea*), karakter morfologi, gen ITS, kepelbagaian, percapahan genetik.

## 1.0 Introduction

Water Lily is a member of flowering plants which is included in the Class Magnoliopsida (Levine and Johnstone, 2010) and Family Nymphaeaceae (Block and Rhoads, 2011). Water lily is claimed to be the early angiosperm which then give rise to modern angiosperms (Les *et al.*, 1999). Borsch *et al.* (2007) stated that there are approximately 70 species of water lily within 6 genera namely *Barclaya*, *Euryale*, *Nuphar*, *Nymphaea*, *Ondinea* and *Victoria*. However, in Malaysia the local genera found are *Nuphar* and *Nymphaea*. Water lily or 'Bunga teratai' in Malay language is well distributed in Malaysian stagnant freshwater habitats.

Water Lily belonging to the dicotyledon group, it is a unique flowering plant since it has floating pad to enable it to float in aquatic environment (Padgett *et al.*, 1999). These perennial aquatic plants with large rhizomes regularly have few sepals (4–6) which combine into petaloid structures (Allaby, 2012). The petals is small and sometimes reduced or staminoid in structure. Uniquely, water lilies petals appear as stamen until they finally intergraded with the fertile, laminar stamens. The leaves are cordate to sagitate at the base, petioles, peduncles, and flower buds are covered in slimy mucilage. However, the structure and habit of these aquatic plants is usually confused with plants from Family Nelumbonaceae or water lotus (Simpson, 2010).

The flower of water lily is frequently blooming in the morning until noon. The colourful water lily flowers such as pink, white, yellow and purple are often sold for enhancing water garden landscape. Moreover, water lilies' flowers colour and fragrance will attract organism such as insects (Carlson and Granquist, 2007). They also provide good habitats as their leaves are used to shield animals like fishes and frogs. Besides that, Graham and Wiszinckas (2011) found that these plants are commonly used as traditional

medicine especially their rootstocks and seeds. Thus, this aquatic herb has declination in population throughout its distribution range; eastern India, China, Korea, eastern Russia and Japan (Schneider *et al.*, 2003).

Studies on water lily in Malaysia on ecology, morphology and molecular had been carried out but perhaps they are not properly documented. Therefore, very few people have knowledge on this species. Besides that, public always have confusion between Nymphaeaceae (water lily) and Nelumbonaceae (Lotus). Improper classification of plants and animals had always lead to unsustainable management of the resources. Furthermore, the water lily has been in UNIMAS Lake landscape for over 20 years but there is no proper documentation on its species diversity. The objectives of this study are:

1. To determine the species diversity of water lily in UNIMAS Lake, Sarawak using morphological characteristics assessment.
2. To sequence the internal transcribed spacer (ITS) region from water lily found in UNIMAS Lake, Sarawak in order to re-confirm their taxonomy.

## 2.0 Literature review

### 2.1 Classification of water lily

Water lily is a member of Class Magnoliopsida, Family Nymphaeaceae in Kingdom Plantae (Levine and Johnstone, 2010). Water lily is known as ‘Bunga Teratai’ for Malays, ‘Kumuda’ for Indians, and ‘Shuey lien’ for Chinese. Water lily can be defined as primitive flowering plants with large rhizomes. There are approximately 70 species and 6 genera worldwide (Borsch *et al.*, 2007). The genera are *Euryale*, *Victoria*, *Ondinea*, *Barclaya*, *Nuphar*, and *Nymphaea*. Genera *Nymphaea* and *Nuphar* are the most common in Malaysia. The classification of water lily is shown as in Figure 2.1.

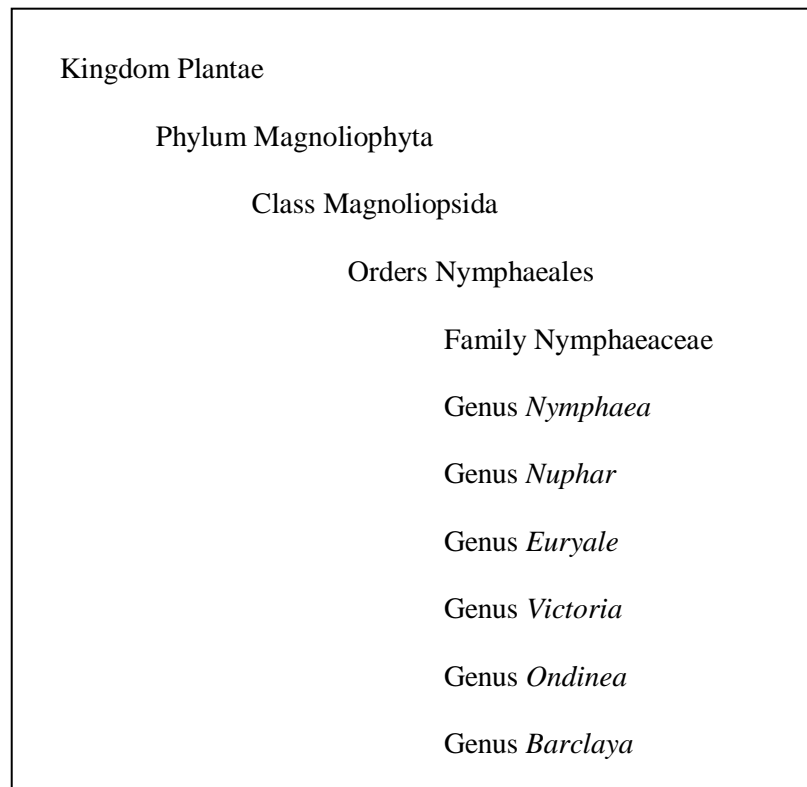


Figure 2.1: Classification of water lily family up to genus level adapted from Borsch *et al.* (2007)

## 2.2 Morphological Characteristics of water lily

The Nymphaeaceae have large, thick and black rhizome since it does not have any true stems (Kirkpatrick, 1992). The leaves grow directly from the rhizome. The structures of the leaf are large and flat, rounded or oval in shape as state by Kirkpatrick (1992). They may be also alternate, floating, pinnately veined, long-petiolate or cordate. The leaves play important role in order to maintain the buoyancy of the plant as its margin slightly rolled inwards toward the topmost side (Viljoen and Kirstenbosch, 2002). Moreover, the underneath of the leaves is constantly wet which can hold the structure against the water. Besides that, the leaf can either submerged, or floating on water surface. Nymphaeaceae has notable characters which are the presence of large flowers, numerous ovules in each carpel, the stem is not free floating and the leaves are attached with rhizome (Aona and Zappi, 2009). The meristic characters of water lily are summarized in Figure 2.2.

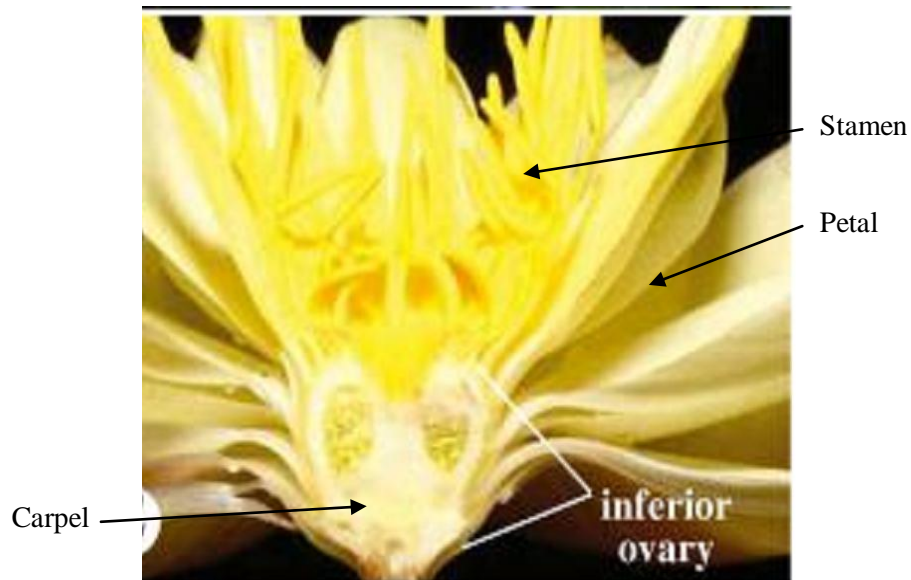


Figure 2.2: Nymphaeaceae general morphology adapted from Simpson (2006).

The flowers of water lily are large in size and may be axillary or solitary. Godfrey and Wooten (1981) stated the flowers grow on the tips of stalks with 4-5 sepals, many petals, many stamens and few pistils. The numerous laminar stamens will surround the pistils at the centre of the flower. It is bisexual plant since the flowers have both pistils and stamen in same plants. The flower bloom early in morning and close entirely in late afternoon. It will stay closed during night. The flower has a sweet fragrant which can attract insects to visit. The fruits of water lily is dicotyledon with has berry-like structure and spongy (Eppinger, 2006). The seed will either have aril or without aril.

Water lily was first discovered in the early cretaceous era in Portugal (Silvertown, 2008). Castro (2007) state that these basal angiosperms are widely distribute in tropical to the cold temperate freshwaters. Genus *Nuphar* is distributed in temperate regions of North America from Alaska to Newfoundland south to northeastern Mexico (Padgett *et al.*, 1998). *Euryale ferox* is native to Asian countries namely China, India and Japan. Genus *Nymphaea* can be found in Malaysia as its distribution is throughout North America, Africa, Europe and Asia. In this genus, *Nymphaea caerulea*, *Nymphaea alba* and *Nymphaea odorata* are native to eastern Africa and India, Europe, and United States and Canada. Genus *Victoria* can be found in Amazon region in Tropical South America, with *Victoria amazonica* is well known as the giant Amazon water lily.



### 2.3 History of water lily

The name of genus *Nymphaea* is direct translation of Greek word which is related with the early Greeks practice. Water lily seeds and tubers are consumed as food many years ago by Europeans, Asians and Africans during emergency time (Sulaiman, 2004). The Egyptians always admire and eat *Nymphaea nouchali* and *Nymphaea lotus* (Viljoen and Kirstenbosch, 2002). The rhizome, flowers, and leaves are eaten by the Egyptian while the buds are frequently portrayed on ancient monuments, furniture and murals. Water lilies are also used in religious ceremony during Egyptian civilization. Furthermore, the Egyptian royal family believes that the beautiful blooms of water lily portray purity and immortality.

Viljoen and Kirstenbosch (2002) stated that there is fossil evidence showing that genus *Nymphaea* has change slightly over the past 160 million years. In the early 18<sup>th</sup> century, the South African citizens ate the rootstock of blue water lily either raw or in curry dishes. French people always use *Nympahaea alba* in the preparation of beer while the Scottish and Irish used as dye for dyeing wool. The water lily is also symbolism for certain countries. White water lily becomes the national flower in Bangladesh and state flower in Andhra Pradesh, India while blue water lily becomes national flower in Sri Lanka. Water lily is a symbolic character for someone born in July.

## 2.4 Differences between Water Lotus Family and Water Lily Family

In Malaysia, public always confuse about water lily as lotus. Lotus family comes from Nelumbonaceae while water lily family is Nymphaeaceae. Not only in Malaysia, other countries also used both lotus and water lily for *Nelumbo* and *Nymphaea*. Ancient Egyptian refers *Nymphaea* as lotus plant whereas the true lotus plant belongs to genus *Nelumbo*. Moreover, *Nymphaea caerulea* and *Nymphaea nouchali* are known as blue Lotus and star Lotus respectively. This public confusion is because water lotus has very similar morphological characteristics found in water lily (Berman, 2009). There are some taxonomists classified *Nelumbo* under Family Nymphaeaceae (Billing and Biles, 2008). However, recent DNA works support the *Nelumbo* is categorized under Family Nelumbonaceae. Moreover, water lily and water lotus do not share the same ancestor. Thus, they are reciprocally monophyletic.

In general, morphological characteristics between Nymphaeaceae and Nelumbonaceae are different. The most distinct character that can be observed is Lotus (*Nelumbo*) has emergent leaves which means they rise above water level while water lily (*Nymphaea*) leaves is floating on the water surface. The water lotus has long tuber which has banana-like shape and peeled potato colour while water lily has rhizome as its root system. Besides that, water lily does not have split leaves while leaves of water lotus are split. The difference of water lily flowers and water lotus is illustrated in Figure 2.3.



Figure 2.3: Flowers of (A) water lily and (B) water lotus adapted from Simpson (2006) and Billing and Biles (2008) respectively.

## 2.5 Importance of water lily

Nymphaeaceae is important in both aspects of ecology and economy. Water lily family serves as good habitat for aquatic organisms such as fish, crabs, and others. The blooming flowers will attract insects to pollinate them while frogs favour to inhabit on water lily pads and wait for their prey. Besides that, the presence of water lily in the pond or lake could control the growth of algae in the pond as their leaves block the penetration of sunlight (Sigeo, 2005). These plants also become food source for other organisms (Hutchinson, 2005) as well as humans. For instance, water lily plant which is rich in carbohydrates is eaten by moose and beavers. Therefore, water lily is important to ensure the flow of energy throughout the ecosystem. Besides animals, humans also consume the carbohydrate-rich rootstalk which had been practiced by the First Nations people (Hodnett, 2010). Water lily is able to absorb nutrients from the water environment which then can keep clear and clean water. Moreover, they supply oxygen to the aquatic organism. The edible part of water lily is illustrated in Figure 2.4.

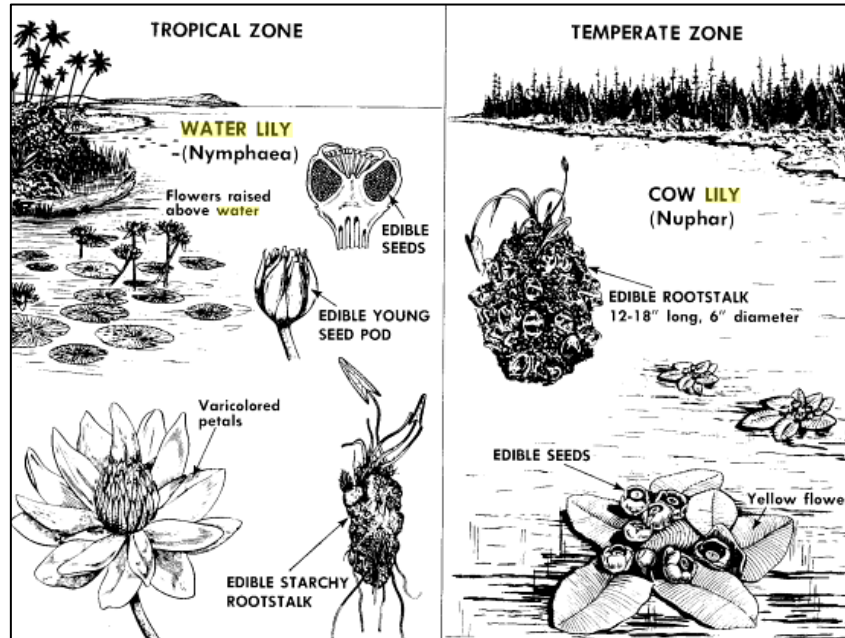


Figure 2.4: Edible part of water lily in tropical and temperate zone. (Kvasnicka, 1985)

In terms of socio economic values, water lilies are eaten by Asians and Native American especially the shoots and leaves. For instance, in Asian country, seed, fruit and rhizomes of *Euryale* sp. provide food to the locals (FAO, n.d.). This plant family can also be made as ornamentals. Since water lily is easy to grow, it has been a popular water garden plant. Moreover, the attractive colour variation of water lily enhances the beauty of the pond. Water lily could also be used as traditional medicine. For instance, the root tea from *Nymphaea odorata* is used to treat diarrhea, sores, sore throats and rash. Rayburn (2007) stated that white water lily can be used to decrease the effect of carcinogens and destroy the development of bacteria and other microorganisms.

## 2.6 Internal Transcribed Spacer (ITS) region

Internal transcribed spacers 1 (ITS 1) is located between 18S and 5.8S rRNA coding sequences and internal transcribed spacers 2 is located between the 5.8S and 25S rRNA coding sequences (Tropp, 2008). The ITS1 and ITS2 (Figure 2.5) are known as intervening internal transcribed spacer of the ribosomal cistron. This region is placed in eukaryotic nuclear ribosomal DNA genes. The spacer region of rRNA is still variable though the coding regions are conserved. Internal transcribed spacer (ITS) region has the highest probability of successful identification in molecular work as it has clearly defined barcode gap between inter- and intraspecific variation (Schoch *et al.*, 2012). Thus, ITS region is important for molecular assays although it not translated into protein (Iwen *et al.*, 2002). Moreover, ITS region have high evolution rate which is practical for phylogenetic study with related species or within species. Besides that, this region appear in all eukaryotic's nuclear genome.



Figure 2.5: The diagram of internal transcribed spacer (ITS) (Singer and Berg, 1991)

## 2.7 Molecular study on water lily

Water lily population in some Asian countries is threatened because high demand in local and international markets. Thus, the understanding of its population structure and species diversity is needed for conservation and population's management. There are several studies done based on molecular approach for past few decades. There are approximately 276 gene sequences on water lily in the GenBank, among the most common gene sequenced are chloroplast gene *rbcl*, cytochrome b, *matK* gene and nuclear ribosomal internal transcribed spacer (ITS) region.

The relationship between genera of water lilies has been studied. There was a study to determine the molecular evolutionary history of water lilies. Ten different species were sequenced providing 1183 bp segment of *rbcl* gene. Then, the data had been used to provide support for recognition of three distinct lineages of Nymphaeaceae plants (Les *et al.*, 1991). The study was done to enhance better understanding of early angiosperm evolution and improve the current classification.

*Nymphaea tetragona* is found in India (Mitra, 1990) but this species is rare and its population is threatened. The study of *N. tetragona* has been carried out including sequencing the chloroplast *trnK* intron, *matK* and *rbcl* genes for better understanding their population and distribution (Dkhar *et al.*, 2011). The *matK* gene of *N. tetragona* showed a high number of non-synonymous substitutions. *N. tetragona* could also be found in Japan, China and Russia. However, the population is restricted to a particular region in India. People claimed that *N. tetragona* in China and India is related to each other due to their similarity in morphological characteristics. Thus, molecular work had been carried out. The genetic data in agreement that *N. tetragona* has migrated from China to India. There is a

possibility changes occur in the DNA sequence and protein sequence during the migratory process.

Padgett *et al.* (1999) investigated the phylogenetic relationship in *Nuphar* by analyzing the sequence data of chloroplast gene *matK* and internal transcribed spacer (ITS) region. The study was conducted as there are not enough phylogenetic hypotheses related to the genus *Nuphar*. Five hundred bp of nuclear ribosomal DNA sequences with addition of 2100 bp chloroplast DNA and 17 morphological characters had provided a more robust phylogenetic tree. In their study, phylogenetic tree constructed using *matK* data sequences showed that *N. variegata* is closely related to *N. sagittifolia*. However, the ITS sequences is unable to determine the relationship between these two species.

### 3.0 Materials and methods

#### 3.1 Sampling site and sample collection

The sampling of water lily plants was carried out in UNIMAS Lake, Sarawak (Figure 3.1). A total of 11 samples of water lily plants were collected.

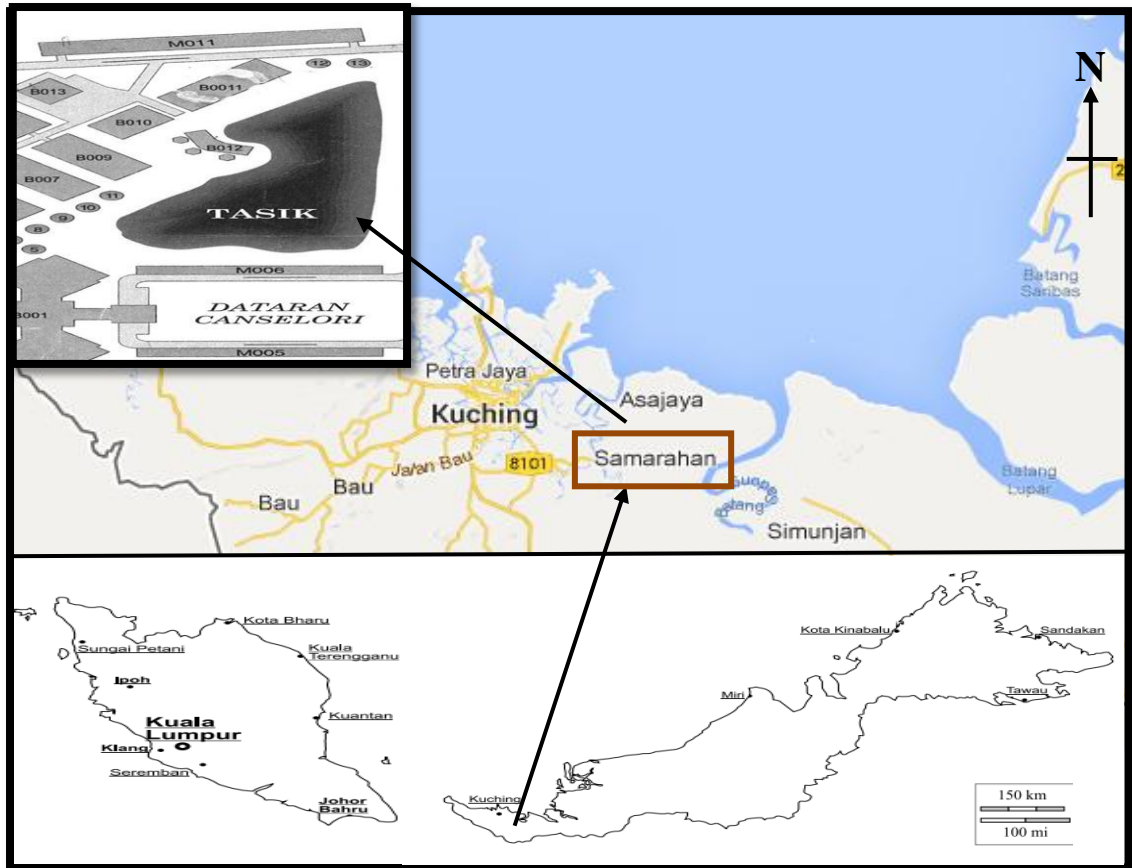


Figure 3.1: Location of sampling site at Unimas Lake East Campus, Kota Samarahan.  
(Source: Google maps)