Faculty of Resource Science and Technology

PITCHER PLANT (*NEPENTHES AMPULLARIA*) CHOICE BY FROGS OF THE *MICROHYLA NEPENTHICOLA* AND *M. BORNEENSIS* COMPLEX FOR BREEDING AT KUBAH NATIONAL PARK, SARAWAK

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This project report is submitted in partial fulfillment of the requirements for the Degree of Bachelor of Science with Honours
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DECLARATION

I hereby declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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The project entitled 'Pitcher plant (*Nepenthes ampullaria*) choice by frogs of the *Microhyla nepenthicola* and *M. borneensis* complex for breeding at Kubah National Park, Sarawak' was prepared by Mohamad Paisal Bin Wahab and submitted to the Faculty of Resource Science and Technology in partial fulfillment of the requirements for the Degree of Bachelor of Science (Honours) in Zoology.

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LIST OF ABBREVIATIONS

Cm	-	Centimetre
Cm^{-1}	-	A reciprocal centimetre unit for comparing energies when dealing with spectra
Mm	-	Millimetre
ml	-	Millilitre
M	-	Metre
Km	-	Kilometre
N	-	North
E	-	East
%	-	Percentage
IR	-	Infrared
FTIR	-	Fourier Transform Infrared
UV	-	Ultraviolet
UNIMAS	-	Universiti Malaysia Sarawak
FRST	-	Faculty of Resource Science and Technology
Hr/Hrs	-	Hour/Hours
Min	-	Minute

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ABSTRACT

This research aims to investigate the relationship between the pitcher plants of the genus *Nepenthes* with frogs of the genus *Microhyla*. Frogs of the *Microhyla nepenthicola* and *M. borneensis* complex use the pitcher plant, *Nepenthes ampullaria* to breed. The frogs deposit eggs on the sides of the pitcher. A total of 80 samples of pitcher plants *Nepenthes ampullaria* were collected in this research. Samples were divided into two groups - 40 samples containing tadpoles and 40 without tadpoles. Samples were collected in five months, from November 2011 to April 2012, at Kubah National Park, Sarawak, Malaysian Borneo. Samples were analyzed including the physical, biological and chemical parameters, such as distance of pitcher from nearest tree, size dimension, amount of liquid contained therein, the surrounding temperature, pH value, functional group of organic compounds, position of the pitcher, age condition, percentage of canopy cover and presence or absence of arthropods inside the pitcher fluid that determine factors influencing habitat choice as breeding sites. Results indicate that female frogs prefer young pitchers who grow close to trees and shaded by canopy, contain large quantities of fluid, are slightly acidic (pH value range in 4.0–4.9) and low temperature of pitchers fluid (24.0–24.9°C).

Key words: *Microhyla nepenthicola, M. borneensis, Nepenthes ampullaria*, pitchers, habitat choice, Kubah National Park, Sarawak, Borneo.

ABSTRAK

Kajian ini dilakukan bertujuan untuk menyiasat hubungan di antara periuk kera jenis Nepenthes *dengan katak* Microhyla. *Katak* Microhyla nepenthicola *dan* M. borneensis *menggunakan periuk kera* Nepenthes ampullaria *untuk membiak. Sebanyak 80 buah periuk kera telah dikumpulkan dengan 226 individual sepanjang kajian ini. Sampel telah dibahagikan kepada dua kumpulan iaitu 40 buah sampel periuk kera yang mengandungi berudu dan 40 buah tanpa berudu. Sampel telah dikumpulkan dalam tempoh lima bulan bermula daripada November 2011 sehingga April 2012 di Taman Negara Kubah, Sarawak, Malaysia Borneo. Parameter yang dikaji termasuklah fizikal, biologi dan kimia seperti jarak periuk kera daripada pokok yang paling hampir, dimensi saiz, kuantiti cecair yang terkandung di dalamnya, suhu persekitaran, nilai pH, kumpulan berfungsi bagi sebatian organic, kedudukan periuk kera, kondisi umur, peratusan perlindungan kanopi dan kehadiran atau ketiadaan serangga di dalam cecair periuk kera yang bertindak selaku faktor yang mempengaruhi katak dalam memilih habitat sebagai tempat pembiakan. Kajian telah menunjukkan bahawa katak betina telah memilih periuk kera muda yang tumbuh berdekatan dengan pokok dimana ia dilindungi oleh kanopi hutan, mempunyai jumlah cecair yang banyak di dalamnya serta sedikit berasid (nilai pH di antara 4.0–4.9) dan suhu yang rendah (24.0–24.9°C).*

Kata Kunci: Microhyla nepenthicola, M. borneensis, Nepenthes ampullaria, *periuk kera, pemilihan habitat, Kubah National Park, Sarawak, Borneo.*

1.0 Introduction

Habitat selection is usually a behavioural consequence of animals actively selecting where they live, or passively persisting at a certain habitat (Boyce and McDonald, 1999). To live in a habitat an animal must first have access to it. Once the animal has access to the habitat, it must be able to tolerate the conditions of the habitat and find resources it needs to survive.

Animals must be able to tolerate at least two kinds of factors in the habitat. Abiotic (non-biological) factors include temperature, humidity, salinity and pH value. Biotic (biological) factors include competition and predation. It is essential to understand the selection of habitat by both adults and tadpoles. Within amphibian species, tadpoles are particularly sensitive to environmental fluctuations and are influenced by various factors such as temperature, rainfall, humidity, pH, vegetation type and the presence of predators. Because of their permeable skin and unshelled eggs, amphibians are extremely sensitive to small changes in temperature and moisture (Inger and Stuebing, 2005).

Amphibians have a biphasic lifestyle and inhabit both aquatic and terrestrial systems. Their own characteristics including lack of tail, body bent over, usually plump, long hind legs while the front legs are short, eyes large and prominent, and mouth large and wide (Inger and Stuebing, 1999). *Microhyla* is a genus of microhylid frogs, which are diminutive frog-like toads. The microhylid *Microhyla nepenthicola* is one of the smallest frogs in the Old World. It can be found in the Matang range in Sarawak, Borneo (Das and Haas, 2010). Three tiny species of *Microhyla* inhabit western Sarawak. The applications of

name have been problematic and Matsui's (2011) material included only two of three tadpoles of the species, including *M. borneensis.*

The carnivorous plants of the genus *Nepenthes,* widely distributed in the Asian tropics, rely mostly on nutrients derived from arthropods trapped in their pitcher-shaped leaves and digested by their enzymatic fluid (Gaume and Forterre, 2007). As a group, the pitcher plants of Borneo are among the most famous of bizarre and unusual plants. The *Nepenthes* pitcher plants are represented by at least 100 species, most of which are found in South-east Asia (Cheek and Jebb, 2001). Pitcher plants are carnivorous which have evolved to attract, trap and digest animal for nutritional benefit (Clarke, 1997).

Nepenthes ampullaria generally grows in damp, shady forest from sea-level to 2,100 m altitude. In Borneo, it usually occur on relatively flat terrain in *kerangas* forest, peat swamp forest, and degraded swamp forest, at elevations from sea level to 1,000 m. *Nepenthes ampullaria* is a distinctive and widespread species of *Nepenthes*, present in Borneo, Sumatra, Thailand, Peninsular Malaysia and Singapore. It has largely moved away from carnivore and acquires a substantial portion of its nutrients from digesting leaf matter that falls to the forest floor and therefore partially detritivorous (Steiner, 2002).

Pitchers have waxy zones that function as traps for insects and other arthropods. *N. ampullaria* is an exception among pitcher plants. It has lower pitchers, 10 cm high and 7 cm wide grows under canopy and feeds on dead leaves that fall into the pitcher (Das and Hass, 2010). All *Nepenthes* species bear conspicuous jug-shaped pitchers on the tips of their leaves (Bauer *et al.*, 2011). The function of the pitcher, which forms from a swelling at the tip of the leaf tendril, is to attract, kill and digest invertebrates prey (Moran, 1996).

1.1 Problem Statements

According to Das and Haas (2010), the frog *Microhyla nepenthicola* breeds in the pitcher plant, *Nepenthes ampullaria.* Although we know that these frogs breed in some not all pitchers, we do not know if any specific physical, chemical or biological factors are important in the selection of the pitchers.

1.2 Objectives

1. The primary objective of this study is to investigate the relationship between an eastern tropical pitcher plant (*Nepenthes ampullaria*) and species of microhylid frogs (genus *Microhyla*) at Kubah National Park, Sarawak.

2. In particular, this study intends to determine which are the most important parameter (physical, chemical and biological) that influences the frog to choose the pitchers for breeding.

2.0 Literature Review

2.1 Pitcher plants

Pitcher plants comprise seven genera, which are distributed throughout part of Americas, Asia and Australia (Clarke, 1997). There were two main types of pitcher plants - terrestrial or ground pitchers and arboreal or upper pitchers (Steiner, 2002). Since the earliest days, these leaves have a variety of names such as jugs, little bags, pots, urns, jars, tankards, flasks, beakers, mugs, and even stomachs (Phillips and Lamb, 1996). The pitchers have four principle units which are the lid, the peristome, the upper waxy zone inside the pitcher and the lower glandular zone, also within the pitcher (Wang *et al.*, 2009; Clarke, 1997).

Nepenthes species are tropical carnivorous plants, which generally grow in areas of infertile soil, such as in *kerangas* forests, swamp forests, forests on ultra basic soils, and in limestone forests (Adam, 1997). *Nepenthes* seeds are usually wind-dispersed, and rarely travel more than a few kilometres (Clarke, 1997). Many are plants of hot humid lowland areas, but the majority is tropical plants, receiving warm days but cool to cold humid nights year round. A few are considered tropical alpine, with cool days and nights near freezing.

The ability of *Nepenthes* species to thrive on poor soils is largely attributed to their carnivorous habit of trapping prey in the pitchers, which are a modification of the leaf tip (Adam, 1997). *Nepenthes* displays carnivorous syndromes which are attract, retain, kill and digest and absorb nutrient (Mithofer, 2011). Those species which do not produce their own

digestive enzymes may rely upon bacteria or other organism to digest the prey for them (Clarke, 1997). The digestive glands on the lower part of the inner pitcher wall secrete on insect prey in the pitcher from various species in Borneo. They include insect groups such as dipteral, isopteran, coleoptera and homoptera (Adam *et al.,* 2006). Once an insect have been drown, digestion begin. The enzymes release by *Nepenthes* pitchers are powerful which small insects can be digested in a matter of hours (Clarke, 1997).

Nepenthes species certainly attract and kill their prey through active production of attractive colours, sugary nectar, and even sweet scents. These pitchers contains liquids which comprising not only enzymatic products, acids and alcohols but also products that render the interior of the pitcher walls slippery to prevent trapped insect from escaping (Steiner, 2002). They also attract and trap invertebrate prey using nectar-secreting pitchers (Moran *et al.,* 1999). Before prey can be captured and digested, visitors have to be lured to the trap. *Nepenthes* pitcher plants employ several different mechanisms to ensure prey attraction. Several trapping mechanisms have been described for *Nepenthes* pitchers, including slippery wax crystals on the inner pitcher wall, 'aquaplaning' on the fully wettable peristome, and a direction-dependent surface topography of the inner wall (Bauer *et al.*, 2011).

2.2 *Nepenthes ampullaria*

According to Clarke (1997), *Nepenthes ampullaria* is among the most attractive of all pitcher plants with the leaves are light green in colour, while the stem is usually light brown. Flowers are produce once or twice a year, though it is not known if this related to seasonal pattern and typically produced in crowded clump on the forest floor (Clarke,

1997). According to Phillips and Lamb (1996), the local people of nearby Bangka called *N. ampullaria* by '*ketakong betol'* which means the one that is used for the typing material, and not all *Nepenthes* equally good for this purpose. *N. ampullaria* is not abundant in deeper shade on wetter soil in secondary forest. They are severely threatened by continuing destruction of their habitat. *N. ampullaria* has a life span over six months (Steiner, 2002).

Nepenthes ampullaria has a cylindrical shape, 8-10 mm thick (Adam *et al.*, 2006). The stem of *N. ampullaria* is light brown in colour and may climb to 15 m in height. Leaves are light green, up to 25 cm long and 6 cm wide. According to Phillips and Lamb (1996), the pitcher can be up to 10 cm high, but are usually smaller. They are crowded into tight cluster on the ground. The pitcher is light green with dark red and brownish blotches (Steiner, 2002). Top view of the pitcher shows the bright yellow, expanded peristome and the reclined lid. The lid of the pitcher, often with bright colours and nectar glands, acts not only as an attractant for the prey animals, but also helps to prevent rainwater from diluting the pitcher content. *N. ampullaria* has smaller opening, thus cannot have any umbrella function (Steiner, 2002).

2.3 Distribution of *Nepenthes ampullaria*

The genus of *Nepenthes* is restricted to the tropical areas of the world, especially within the Malay Archipelago, with the greatest biodiversity found on Borneo and Sumatra (Pavlovic *et al*., 2009). This flask shaped pitcher plant is also present in Sri Lanka, north-eastern India, Sumatra, Thailand, Peninsular Malaysia, Singapore, Java, the Maluku Islands and New Guinea. This is common species in Sarawak; it has been recorded from Bako Park, Sungai Dua Baram, Pulau Bruit, Kayangkeran Forest Reserve, Kelapaan,

Sungai Raya, Selalang Forest Reserve, Bawang, Bau, Tebuan Hilir Kuching, Sungai Tutus and the Sebanding Forest Reserve (Adam, 2002).

Nepenthes ampullaria is a common species in Borneo, most often collected in the north (Sabah) and west (Sarawak), and occasionally in Kalimantan, but it has not so far been recorded from the central part of the island (Adam, 1991). This species usually grows below 100 m, but may also be found up to 1,000 m. It is a common plant on roadside clearings, heath forest, margin of secondary swamp vegetation, peat swamp forest and sometimes found within gap of tall canopy lowland dipterocarp forests (Adam, 2002). It also grows on the edge or in permanently or seasonally flooded habitats (Adam *et al.* 1991).

2.4 Amphibians

Approximately 650 species of amphibians are known from south-eastern Asia. Studies conducted in Borneo show approximately 180 of amphibian species, comprising six families - Bombinatoridae, Megophyridae, Bufonidae, Microhylidae, Ranidae and Rhacophoridae. Microhylinae is distributed widely in the oriental region, from India and Korea to the Greater Sunda Islands, and it comprises about 70 species in nine genera; *Calluella, Chaperina, Glyphoglossus, Kaloula, Metaphrynella, Microhyla, Micryletta, Ramanella,* and *Uperodon* (Matsui *et al.,* 2011). Five species of *Microhyla* are currently known from Borneo and all exist in primary and old secondary forest, lower than 700 metres above sea level (Inger and Stuebing, 2005).

Amphibians are the most diverse of all terrestrial vertebrates in their modes of reproduction, no doubt a reflection of various stages in their evolution towards greater terrestriality and independence from standing water (Crump, 2006). When feeding, a frog flips its tongue out at its prey, and then flips it back with prey stuck at the end. Insect and other invertebrates are the usual prey or even small snakes, small bird or mammals (Inger and Stuebing, 1997).

They typically lay eggs in puddles, ponds or lakes and their larvae (tadpole) have gills and develop in water. About half the species in South-east Asian assemblages are riparian and develop in streams, a few species developing terrestrially (Zimmerman and Simberloff, 1996). During the breeding season, many species form aggregations and call to attract potential mates (Hsu *et al.*, 2006). The advertisement call of the males contains information for species recognition. These calls are used by the females to distinguish between hetero-specific and con-specifics and further to discriminate among con-specifics to choose the fittest male for the purpose of mating (Roy, 1997).

2.5 *Microhyla nepenthicola*

Microhyla nepenthicola is a frog described from *Kerangas* forests of Borneo. It was named after pitcher plants which provide microhabitat to the developing tadpoles and breeding ground to adults. It has a narrow head, body flattened, eyes reduced, maxillary and vomerine teeth absent, reduction of Finger I, toes with reduced webbing, pupil circular, and tongue large, oval and entire. It also has an inner metatarsal tubercle under each foot. Their larvae lacking keratinized beaks, terminal mouth and laterally-positioned eyes and vent embedded medially in lower tail fin (Das and Haas, 2010).

Microhyla nepenthicola is small frog - snout vent length (SVL) is around 10.6–12.8 mm in males, 17.9 and 18.8 mm in females. Adult males have a subtriangular depressed body, teeth absent on maxilla and vomerine regions, tongue oval, smooth, rounded apically, pupil rounded, tympanic membrane and tympanic annulus not visible externally, cloacae opening at mid-level and a median subgular vocal sac (Das and Haas, 2010).

2.6 Relationship between *Nepenthes* and Animals

As well as trapping and digesting the animals, *Nepenthes* pitchers are also home to a surprisingly large number of other animals which are also referred to as infauna (Clarke, 1997). Over 150 different species (at different stages of their life cycle) have been found living in the pitcher plants (Phillips and Lamb, 1996). Some highly specialized animals may even live and develop in the pitchers (Steiner, 2002). The pitcher plant grows in the damp and wet regions which making it suitable for use by small forest floor.

According to Phillips and Lamb, (1996), ants are commonly associated with pitcher plants. If ants are dropped into the pitcher, it has no difficulty in climbing out. Other insects too live in pitchers, the communities consisting mainly of aquatic dipterans larvae (Mogi *et al.,* 1991). Mosquitoes which inhibit *Nepenthes* usually lay their eggs inside the pitchers. The eggs hatch and the larvae go through several development stages before they emerge as adult. The adult leave the pitchers, returning to lay eggs (Clarke, 1997).

There are a large number of interactions of animals with pitcher systems. From small crabs to larvae of moths and developing tadpoles, all have chosen the symbiotic life with pitcher plants (Steiner, 2002). Scientist on the Malaysian Heritage Endau-Rompin

Expedition in 1985 to 1986 recorded the small red crab, *Geosesarma malayanum* living in the pitcher of *Nepenthes ampullaria* (Phillips and Lamb, 1996). The small red crab spider, *Misumenops nepenthicola*, lives inside the lowland *Nepenthes* pitcher in Malaysia and Indonesia. It waits beneath the peristome, and ambushes the insect that crawled out from inner surface of the pitcher and also preys on larvae which live in the pitcher fluid (Clarke, 1997).

In the case of frogs, a number of species, which normally deposit their eggs in tree holes, will use *Nepenthes* pitchers for this reason (Clarke, 1997). One recent study which was conducted at Kubah National Park, Sarawak had proved that this frog has used *Nepenthes ampullaria* to lay eggs. The species is an obligate of the pitcher plant, *Nepenthes ampullaria* which breeds in senescent or mature pitchers. They remain around the pitchers clump, and adult males are heard calling at dusk, choruses peaking during the early hours of the evening (ca. 1845–2100 h) within and around patches of pitcher plants (Das and Haas, 2010). It is obvious that the carnivorous pitcher plants were not only killing and digestive properties, but also preservative and protective one (Steiner, 2002).