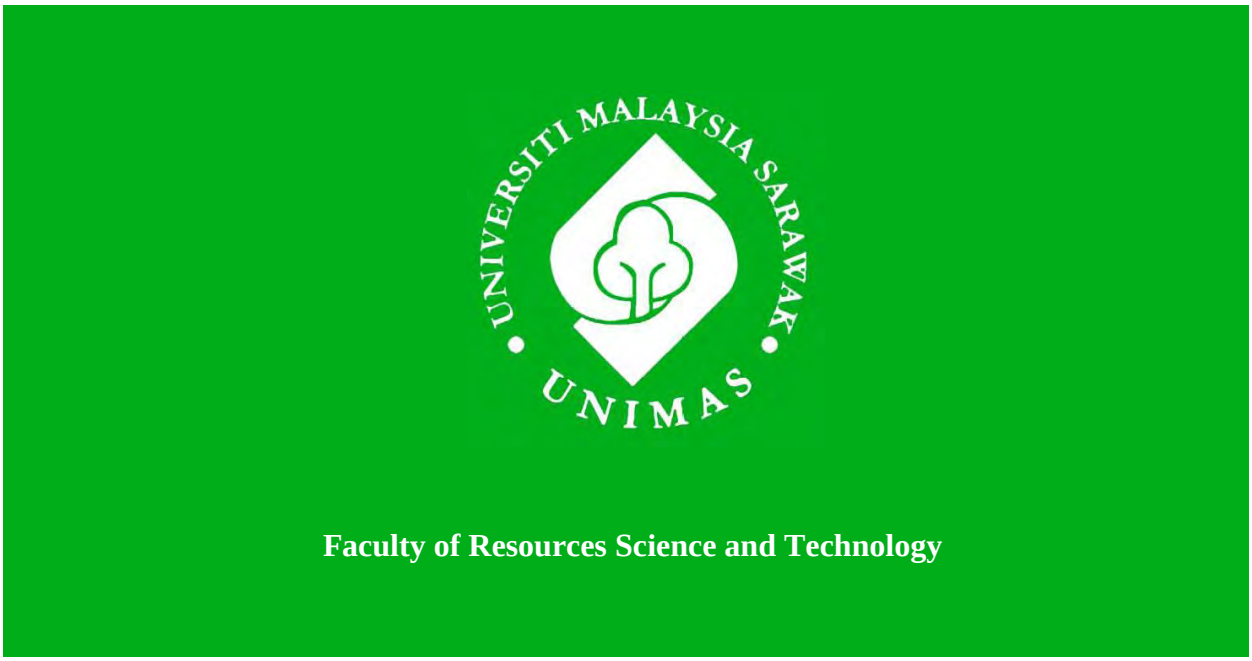

Faculty of Resources Science and Technology

STAGING OF LATE LARVAL DEVELOPMENTAL STAGES OF THE MICROHYLID FROG, *MICROHYLA NEPENTHICOLA* (ANURA: MICROHYLIDAE)

Adi Shabrani bin Mohammad Ridzuan

Bachelor of Science with Honours
(Animal Resources Science and Management)
2013

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ADI SHABRANI BIN MOHAMMAD RIDZUAN

This project report is submitted in partial fulfillment of the requirements for the Degree of Bachelor of Science with Honours
(Animal Resource Science and Management)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2013

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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ADI SHABRANI BIN MOHAMMAD RIDZUAN

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The project entitled 'Staging of Late Larval Developmental Stages of the Microhylid Frog, *Microhyla nepenthicola* (Anura: Microhylidae)' was prepared by Adi Shabrani bin Mohammad Ridzuan 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
IBEC	– Institute of Biodiversity and Environmental Conservation
UNIMAS	– Universiti Malaysia Sarawak
DSLR	– Digital Single-lens Reflex
GIMP	– GNU Image Manipulating Programme
mm	– millimetre

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ABSTRACT

Frog larval form requires separate treatment from its adult form because it has different morphology, diet and breathing mode. Staging table is essential in the study of frog larval development. A newly described species *Microhyla nepenthicola* Das & Haas 2010 is staged for its larval development. Colour photographs in different aspects are included in documenting the larval development. The late development staging table is recorded starting from Stage 33 until complete metamorphosis. Information of the larval development include time taken for metamorphosis, with changes in limb bud and tail regression are recorded. The metamorphosis duration is fast in this Microhylid frog, which are contributed by its body size. Metamorphosis of *Microhyla nepenthicola* tadpoles is documented by referring to Gosner (1960), which provided standardised details on each stage of larvae. This will provides a guideline for the larvae stages in *Microhyla nepenthicola*.

Key words: *Microhyla nepenthicola*, staging table, larvae, metamorphosis, Borneo.

ABSTRAK

Bentuk larva katak memerlukan layanan berlainan daripada bentuk dewasa kerana ia mempunyai morfologi, pemakanan dan mod pernafasan yang berbeza. Jadual peringkat adalah penting dalam kajian pembentukan larva katak. Spesies Microhyla nepenthicola *Das & Haas 2010 yang baru di jumpai telah di bekalkan dengan jadual peringkat untuk pertumbuhan larva. Fotografi berwarna dari aspek berbeza disertakan dalam dokumentasi pertumbuhan larva. Pertumbuhan akhir direkodkan bermula dari Peringkat 33 sehingga metamorfosis lengkap. Maklumat mengenai pertumbuhan larva termasuk tempoh masa yang di ambil untuk metamorfosis, dengan perubahan pada putik sendi dan pengurangan ekor di rekodkan. Kadar metamorfosis adalah cepat dalam katak Microhylid ini kerana saiz badannya. Metamorfosis berudu* Microhyla nepenthicola *telah di dokumentasi dengan merujuk jadual peringkat oleh Gosner (1960) yang mengandungi perincian piawai untuk setiap peringkat larva. Ini telah memberikan garis panduan untuk setiap peringkat larva* Microhyla nepenthicola.

Kata kunci: Microhyla nepenthicola, , *jadual peringkat, larva, metamorphosis, Borneo.*

CHAPTER 1

INTRODUCTION

Information on tadpoles for Bornean frogs is inadequate (Inger, 1985; Das & Haas, 2005). Tadpoles are less studied in the past, relative to adults, due to lack of acknowledgement of their taxonomic important (Inger, 1985). Amphibian larvae, especially frogs, are morphologically distinct from their parents. Compare to adult form, frog tadpoles have lidless eyes, possess dorsal and ventral fins, thus they have similar resemblance to a fish (McDiarmid & Altig, 1999). This could be problematic especially in determining the related larvae with its interspecific parents. In addition, the tadpoles could also have different morphology in different developmental stages. Information on tadpole developmental stages provides a basis for identification to the parent species.

The genus *Microhyla* Tschudi, 1838 is widely distributed in Asia, from Ryukyu Archipelago of Japan, China, west to Pakistan, India, Sri Lanka and throughout South-east Asia (Frost, 2011). In Borneo, five species has been described: *Microhyla berdmorei* (Blyth 1856); *M. borneensis* Parker 1928; *M. maculifera* Inger 1989; *M. perparva* Inger & Frogner 1979 and *M. petrigena* Inger & Frogner 1979 (Frost, 2011; Haas & Das, 2012), with two new species has been described in recent years: *Microhyla nepenthicola* Das & Haas 2010 (Haas & Das, 2012) and *M. malang* Matsui 2011 (Matsui, 2011). Some of the Bornean *Microhyla* larval development had been documented (e.g. Leong & Chou, 1999; Leong, 2004).

M. nepenthicola are highly associated with pitcher plant *Nepenthes ampullaria.* This specific microhabitat requirement restricted the distribution of the microhylid frog only in the availability of the plant. It is found usually within a clump of *N.ampullaria* pitcher and male frog will form a group within and around it to call (Das & Haas, 2010). It is still largely unknown why *Nepenthes ampullaria* is chosen as its habitat. It is suggested *Nepenthes ampullaria* are selected due to physical, chemical and biological features of pitcher plant, which includes pH of the liquid, position of the pitcher, age of the pitcher and size (Paisal, 2012).

Morphological description of tadpoles of *Microhyla nepenthicola* based on Stage 36 had been reported by Das & Haas (2010), however a table of larval development stage in *M. nepenthicola* remains unavailable. Staging the development of frog larva can contributes in the information on this new species. Gosner (1960) had produced a standard staging table, where there are 46 different stages in an anuran larval development, before metamorphosis is completed. By utilizing staging tables, information on larval life history can be better understood.

1.1 Problem statement

A staging table for tadpole development is important in understanding the biology and life cycle of frog. The table also contributes to information on the morphological features of the tadpoles that change in stages. However, because of less attention on tadpole studies, many frog species lacks information on its tadpoles, in addition, its larval development are less understood. By providing staging table, development process in *Microhyla nepenthicola* and its biology can be better studied.

1.2 Objectives:

The purpose of producing staging table is:

i. To record morphological characteristics in each stage of *Microhyla nepenthicola* larvae, based on a generalized staging table by Gosner (1960).

ii. To understand development process in microhylid frog, *Microhyla nepenthicola.*

CHAPTER 2

LITERATURE REVIEW

2.1 Frogs in Borneo

Frogs are grouped under the order Anura, derived from ancient Greek word *an-* (without) and *oura* (tail). Frogs possess no tail, and they have specialized hind leg for leaping locomotion. Data from Amphibiaweb.org suggest that there are 6, 211 different species of frogs in the world (as on October, 2012).

Both of the adult and tadpole of frogs are very sensitive to changes in their surrounding environment. They lay unshelled eggs, thus it has no protection against physical and chemical stress. In addition, unshelled eggs lose fluid more easily to the environment (Duellman & Trueb, 1994). Therefore, frog often lays their eggs in water or produce protective foam nest to provide moisture to the

eggs (Inger & Stuebing, 2005). Apart from humidity and temperature, tadpoles also sensitive to physical stress. Relyea & Hoverman (2003) reported the presence of predators and competition among tadpoles will affect the development in their tail and body size. Adult frogs are also dependent to moisture as their skin needs to be moist to allow gaseous exchange for respiration and heat regulation (Duellman & Trueb, 1994). Thus, their distribution is often restricted to location that has enough moisture and water for them to survive and reproduce. For this reason, frogs are found in greater diversity in tropical rainforest compared to other places (Inger & Stuebing, 2005).

The annually constant rainfall on Borneo produced the largest area of tropical rainforests in the Indomalayan Archipelago (MacKinnon *et al*., 1996), thus providing moist environment for frogs to live all year round. Frogs have been found from mangrove to montane forest in Borneo, indicating their occurrence in almost at all type of ecosystem in the region (Inger & Stuebing, 2005). Current record of frog species in Borneo by Haas & Das (2012) is 166 species, which consist of eight (8) families (Bombinatoridae, Buforidae, Ceratobatrachidae, Dicroglossidae, Megophryidae, Microhylidae, Ranidae and Rhacophoridae). In addition, more species of frogs are newly discovered from Borneo (e.g. *Ingerana rajae* Iskandar, Bickford & Ariffin 2011, *Meristogenys stenocephalus* Shimada, Matsui, Yambun & Sudin, 2011, *Kalophrynus calciphilus* Dehling, 2011, *Microhyla nepenthicola* Das & Haas, 2010, *Ansonia*

echinata Inger & Stuebing, 2009) suggesting more discoveries are anticipated from the region.

2.2 Decoupling and metamorphosis in frogs

The development of amphibians is very unique as they undergo metamorphosis during their life cycle. In frogs however, the metamorphosis is very different compared to other amphibians because the larvae (tadpoles) has completely different morphology, breathing mode and lifestyle compared to the adult form (McDiarmid & Altig, 1999). Thus, leads to confusion in determining to which species the larvae belong to and how to morphologically differentiate between larval form of anuran species (Parmelee *et al*., 2002). The deviation of frogs' larvae from their adult forms giving them advantages in ecological aspects which eventually makes them very successful in their environment (Altig & Johnson, 1989).

Tadpoles are ephemeral, feeding and non-reproductive larvae of frogs (McDiarmid & Altig, 1999). Morphologically, they are very diverse and can be found in wide variety of microhabitat (Altig & Johnson, 1989). Duellman & Trueb (1994) describe tadpoles to have a very similar resemblance to a fish. It possesses dorsal and ventral fins, lidless eyes, terminal mouth and breath through gills. The microhabitats range is wide; tadpoles can be found in flowing river

streams, stagnant pools, inside water-containing tree holes and within pitcher plants (Inger & Stuebing, 2005; Leong & Chou, 1999). Tadpoles also have different feeding mode. Tadpoles can be herbivorous, detritivorous or carnivorous (Altig *et al*., 2007) and with some exhibits endotrophic lifestyle (McDiarmid & Altig, 1999).

2.3 Status of frogs larval knowledge

Although many frog species are being described each year, the studies focus mostly on the adults while information on its tadpoles is fairly limited (e.g. Grismer *et al*., 2004; Iskandar *et al*., 2011; Kraus, 2011). This lack of information is due to extreme decoupling in amphibians larva, in which little attention are given to the tadpoles in describing amphibians species. Additionally, a relatively small sample with narrow taxonomy, more morphological indicators and difficulty in handling preserved specimens contribute to less study conducted on tadpole compared to its adult form. (Inger, 1985). The knowledge on tadpoles is important for species identification, because they have different morphological forms from its parents (Duellman & Trueb, 1994). This species decoupling provide information in terms of taxonomy, ecology and more importantly, evolution of frogs (Rocek, 2003).

Parmelee *et al.* (2002) suggested there are three (3) major factors which make tadpoles difficult to identify. First, many species are conservative in their anatomy and some members of frog (e.g., Ranidae and Bufonidae) families have very similar larval form. Therefore, it is difficult to traditionally distinguish the larva of these frog species without molecular approach. Second, morphology among tadpoles of the same species can vary due to geographical factors and different developmental stages. Third, tadpoles exhibit different anatomies depending on the abiotic and biotic factor of their environment.

In Borneo, there are various species of frogs which lacks description on their tadpoles (Inger & Stuebing, 2005). Because of the wide range of microhabitats and lifestyles in tadpoles especially in Borneo, documentation that is specific on their tadpoles is limited. The only specific work available on the topic is that of Inger (1984), although this documentation is still in needs of extra information as it only covered 45-60 % of Bornean frog species. Haas & Das (2011) reported that there are currently 51 species of Bornean frogs in which their tadpoles are unknown. Some larval development within the genus *Microhyla* in Borneo had been documented, which are *Microhyla borneensis* (Leong & Chou, 1999) and *M. berdmorei* (Leong, 2004).

Frog tadpoles have proven to be important as in the case of the discovery of the species *Microhyla nepenthicola*. The tadpoles become the indicator for the

discovery when their genetic barcode reveals that they did not matched to any known adult frog species (Das & Haas, 2010). This eventually leads to the description of new species.

2.4 *Microhyla nepenthicola*

Common name for this tiny species is Matang Narrow-mouthed Frog. *Microhyla nepenthicola* Das & Haas 2010 has been described as "the smallest frog of southeast Asia" (Haas & Das, 2012). It is known to occur in the kerangas forest of Gunung Serapi, Sarawak, Malaysian Borneo. The miniaturization of the species is reported by Das & Haas (2010), in which their range of snout-vent length (SVL) is 10.6-12.8 mm in males, and 17.9-18.8 mm in females. Compared to other Microhylids, it possesses dorsum with low tubercles that are distinct on its flanks.

The mid-vertebral ridge is weak and broken, starting from forehead and continuing along body. On the forehead, the dermal fold is absent. The tympanic membrane and tympanic annulus are absent in this species. In males, Finger I is reduced to a nub proximal to Finger II. Its toe tips are weakly dilated and possess longitudinal grooves on its phalanges, which looks similar to two scale structures. The toes are webbed on IV basal, with narrow dermal fringes. It also

exhibit both inner and outer metatarsal tubercles. The dorsum are brown with hour-glass shaped mark appear on the scapular region.

M.nepenthicola is obligatory to carnivorous pitcher plant *Nephentes ampullaria*, in which it provides microhabitat for the eggs deposition, tadpoles development and the frog remains in pitcher clump area for breeding. The tadpoles are usually found suspended motionless in the pitcher liquid and quickly hides in undigested matter that contain in the pitcher when disturbed (Das & Haas, 2010). Apart from the tadpoles, many other organisms are found living inside the pitcher plant, suggesting that digestive properties of the pitcher liquid are utilized by the organisms as a protective barrier (Steiner, 2002). With the size of 7cm high and 5 cm wide, *N. ampullaria* is among the smallest pitcher plant in the genus (Clarke, 1997).

The tadpoles of *M.nepenthicola* have a total length range of 9-11.3 mm, with body length being 30% of the total length. At early stages, they have no external narial opening. The spiracle lies ventrally, approximately at mid-belly region. Melanocytes are scattered on the flanks, dorsum, dorsal head, and sides of tail, which can only be seen under magnification. The tadpoles of *M.nepenthicola* are hard to observe in the wild because they are near translucent under natural light. The eyes of the tadpole are laterally-positioned and dark coloured, making them contrast to their body coloration. The larvae lack

keratinized beaks and terminal mouth. *M. nepenthicola* is an all-year breeder (Das & Haas, 2010).

The larvae of this species are endotrophic, which is contributed by the stability of its microhabitat, where water in the pitcher plant *N.ampullaria* is available throughout the year. It develops inside the pitcher plant until adulthood. This becomes the key in separating them from its sister species *Microhyla borneensis* and *M. malang* where both of them develops outside pitcher plants (Das & Haas, 2010; Matsui, 2011). Currently, the available informations on *Microhyla nepenthicola* are morphological descriptions of adult and larva, larval microhabitat, distribution, ecology and description of call (Das & Haas, 2010).

2.5 Staging table of frogs larvae

Staging is a process where certain morphological features that are important in comparison of each different sequence in a life cycle of a tadpole are recorded. (McDiarmid & Altig, 1999). By using staging system, easy observation and comparison of the tadpoles that have different sizes and developmental period can be done, in addition, comparison between species that has completed similar development process can also be observed.