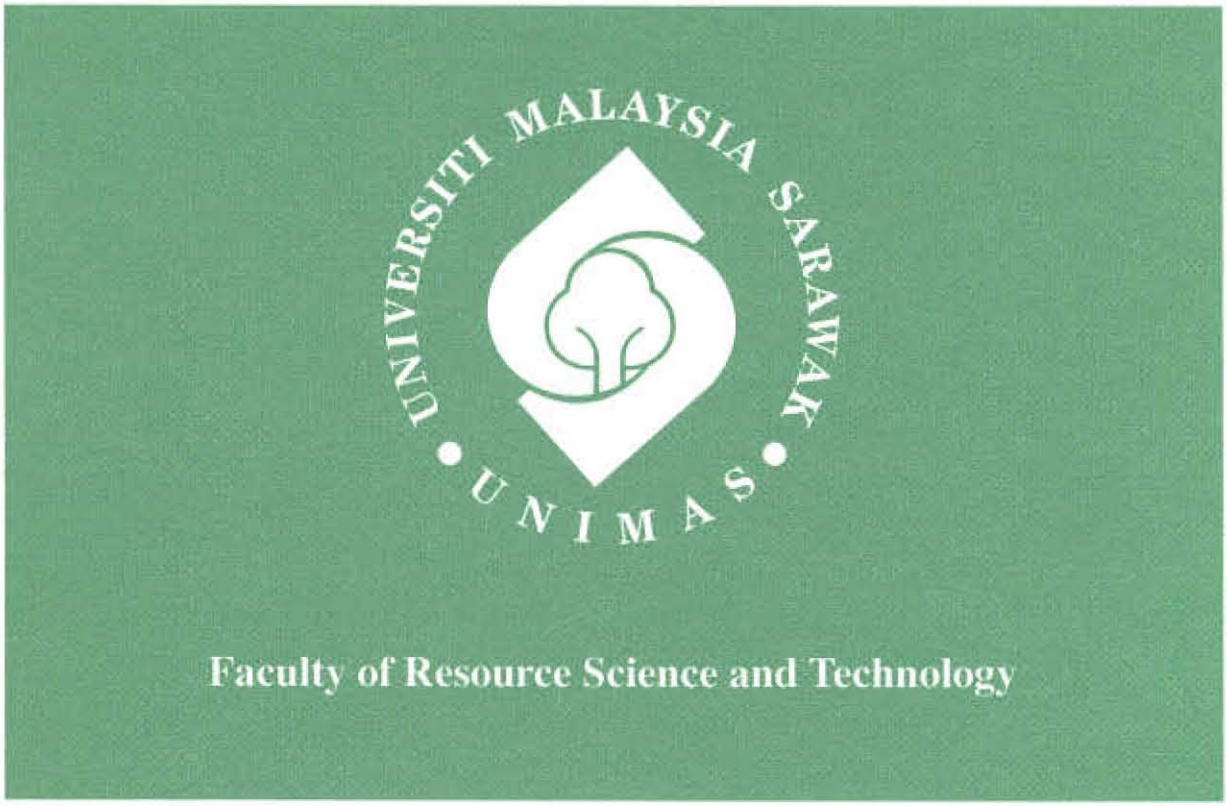

COMPARATIVE MORPHOLOGY OF BORNEAN SPECIES OF *ANSONIA* (AMPHIBIA: ANURA: BUFONIDAE)

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Bachelor of Science with Honours
(Animal Resource Science and Management)
2012

Comparative Morphology of Bornean Species of *Ansonia* (Amphibia: Anura: Bufonidae)

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23430

A report submitted in fulfillment of the Final Year Project II

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2012

Acknowledgements

Foremost, I would like to thank God for giving me the strength and patient upon my Final Year Project (FYP) completion progress. Next, I would like to give my appreciation to my supervisor, Prof. Dr. Indraneil Das for giving me useful advice and guidance including materials and specimens for my project. I'm grateful for participation of Mr. Pui Yong Min of the Institute of Biodiversity and Environmental Conservation (IBEC) for giving some useful advice and preparing the specimens for me. Thanks are due to Miss Lydia and Miss Roberta Chaya for teaching me how to use statistical methods, including Discriminate Function Analysis (DFA) and Cluster Analysis (CA). For laboratory work, I would like to document my appreciation for IBEC laboratory and External Laboratory (EL) for letting me doing my laboratory work and provide me with sufficient equipment. Not to forget, I would like to thanks my course mate for helping me during my field work- Khatijah bt. Ismail, Melynda Cheok, Zaty Rais, Shafiq and two master students which are Elvy and Fadzil. I would also like to thank Siti Nor Baizurah, Ikah, Paisal and Amaziasizamoria who guided me during the preparation of this Final Year Project. Last but not least for my family for giving me moral support.

Declaration

Hereby, I declare that this thesis is based on my original work except for quotations, citations and references which have been appropriately acknowledged. I also declare that a paper on this topic has not been submitted previously or concurrently for any other degree in UNIMAS or in any other institutions.

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List of Abbrevation

AG: axilla to groin

ED.: horizontal diameter of eye

et al.: and others

FA: forearm

HL: head length

HW: head width

ID: Indraneil Das

IN: internarial width

IO: interobital width

P: Pui

SJT: snout to jaw tip

Snout L: front margin of eye to tip of snout

SVL: snout-vent length

TBL: tibia length

TH: horizontal diameter of tympanum

UA: upper arm

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Comparative Morphology of Bornean Species of *Ansonia*

(Amphibia: Anura: Bufonidae)

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ABSTRACT

This project focused on the species of the genus *Ansonia* (family Bufonidae), which are also known as 'slender toads'. The genus distributed over Southeast Asia, and Borneo houses 12 species. These are *A. albomaculata*, *A. hanitschii*, *A. leptopus*, *A. longidigita*, *A. minuta*, *A. spinulifer*, *A. fuliginea*, *A. guibei*, *A. platysoma*, *A. torrentis*, *A. echinata* and including the recently rediscoverd (2011) *A. latidisca*. The objectives of this study are to discriminate between species by using morphology and morphometric parameters, and enhance identification keys for the genus. The results of Discriminate Function Analysis (DFA) showed that the strongest characters to discriminate the species are the ratio length of the head and warts type. By using only numerical data, *A. guibei* and *A. minuta* can be distinguished. By using binary data, *A. spinulifer* showed distinct group. For both analyses of Cluster analysis using ratios and raw data, failed to completely separate all the species into groups except for *A. spinulifer*. This may due to small sample size of some species and overlapping characteristics between species. Taxonomic keys are enhanced to identify all the species in the genus.

Keyword: *Ansonia*, discriminate function, morphology, morphometrics, taxonomic keys

ABSTRAK

Projek ini fokus kepada spesis genus Ansonia *(famili Bufonidae), yang juga dikenali sebagai kodok langsing. Genus ini mempunyai taburan di Asia Tenggara dan Borneo memegang 12 spesis. Spesis-spesis tersebut adalah* A. albomaculata, A. hanitschii, A. leptopus, A. longidigita, A. minuta, A. spinulifer, A. fuliginea, A. guibei, A. platysoma, A. torrentis A. echinata, *dan termasuklah* A. latidisca *yang baru ditemui sehingga tahun 2011. Objektif projek ini adalah untuk mendiskriminasikan di kalangan spesis dengan menggunakan morfologi dan parameter morfometrik, serta menambahbaikkan kunci pengenalan bagi genus ini. Hasil keputusan Diskriminasi Analisis Fungsi (DFA) menunjukkan bahawa karakter yang boleh mendiskriminasikan antara spesis adalah nisbah lebar dan panjang kepala, dan jenis ketuat.Untuk kedua jenis Analisis Kluster menggunakan nisbah dan data mentah, gagal membahagikan sepenuhnya spesis-spesis dalam kelompok. Ini mungkin disebabkan jumlah saiz sampel yang sedikit untuk sesetengah spesis dan karakter yang bertindih. Kunci taksonomi telah ditingkatkan bagi mengenalpasti kesemua spesis dalam genus.*

Kata kunci: Ansonia, *fungsi diskriminasi, morfologi, morfometrik, kunci taksonomi.*

1.0 Introduction

Borneo, at the heart of Sundaland, is rich in biodiversity, being covered with dense tropical rainforest with other habitats, such as mangroves, peat swamp, lowland dipterocarp forest, etc. (Davis *et al.,* 2011; Inger, 2005). Politically, Borneo consists of Sabah, Sarawak, Brunei and Kalimantan (Indonesia) and is the world third largest island. Home of thousand species of animals that include species as diverse as the Bornean clouded leopard, the Proboscis monkey, Sumatran rhino, Bornean pygmy elephant, and the Orangutan. Borneo also houses the largest flower in the world, the *Rafflesia*.

Borneo had yielded thousand species of herpetofauna, and new species are being discovered annually, with ongoing research. A total of 150 species of frogs had been identified in Borneo (Inger & Stuebing, 2005), and the island has attracted herpetologist since the mid-nineteenth century.

Six families for frogs are Bombinatoridae, Bufonidae (true toads), Ranidae (true frogs), Megophryidae, Microhylidae and Rhacophoridae. The distinct features of a frog are no tail, stocky body with long hind limb but short limb in front, large bulking eyes and very wide mouth (Inger & Stuebing, 2005). Frog and toad differ by skin texture, absent of warts in frog and wider body for toads. Toad have shorter hind limb compare to frog because they walk rather than hop. Frogs have longer hind limb and fully web toes for swimming. Frog also live near or in water while most of the toad live on land and only return to water for breeding.

One of the families of anuran amphibians is the Bufonidae (true toads). Bufonids have no teeth and their bodies are rough and consist of warts especially on the dorsum. They are the only tailless amphibian, and many have large parotid glands. These glands and their warts

secrete a white viscous fluid that deters predation and can cause death or paralysis to other small vertebrates. Similar to a majority of anuran amphibians, most bufonid species breed in streams.

According to Haas & Das (2011), the Family Bufonidae currently comprises 31 species on Borneo. Most obvious is the radiation of the genus *Ansonia* (stream toads) in rainforest habitats of Sabah and Sarawak. The following lists are the most recent 12 species of *Ansonia* found in Borneo including Sabah and Sarawak:

Table 1.0: List of *Ansonia* spesies in Borneo (Inger & Stuebing, 2005, Matsui *et al.*,2009 and Frost, 2011)

No.	**Scientific names**	**Common names**
1.	*Ansonia albomaculata* Inger	White-Lipped Slender Toad
2.	*Ansonia echinata* (Inger & Stuebing)	Sarang Stream Toad
3.	*Ansonia fuliginea* (Mocquard)	Montane Slender Toad
4.	*Ansonia guibei* Inger	Guibé's Slender Toad
5.	*Ansonia hanitschi* Inger	Kinabalu Slender Toad
6.	*Ansonia latidisca* Inger	Rainbow Toad
7.	*Ansonia leptopus* (Günther)	Brown Slender Toad
8.	*Ansonia longidigita* Inger	Long-Fingered Slender Toad
9.	*Ansonia minuta* Inger	Dwarf Slender Toad
10.	*Ansonia platysoma* Inger	Luidan Stream Toad
11.	*Ansonia spinulifer* (Mocquard)	Spiny Slender Toad
12.	*Ansonia torrentis* Dring	Gunung Mulu Stream Toad

The genus *Ansonia* (stream toads) ranges from the Philippine Islands, Peninsular Malaysia, Borneo, Sumatra, Thailand and Myanmar (Inger & Stuebing, 2005). The greatest diversity occurs in Borneo with 12 species known.

Some species can live in forest which spawn in streams and have torrent-adapted tadpoles. *Ansonia* commonly can be found in primary and old secondary forests, including selectively logging forest, hills and mountain (Inger & Stuebing, 2005). One species (*Ansonia leptopus*) is found in lowland forests (Mattison, 2007). These toads live on forest floor and will occasionally return to the stream to breed. They perch on the rocks, small shrubs, or herbaceous plant near steep slope of the river where fast water flows. The male will call from rocks or other vantage points next to rapids and their call is a high pitched-metallic trill or chirp.

With their sombre colouration, they can easily disguise and hard to see them when in the forest (Mattison, 2007). Their body colours are usually black and brown with indistinct pattern. They have rough skin and mostly covered with small, rounded wart.

These toads are called slender toad because of their elongated bodies and have relatively long legs. They are small (smallest range from 20 mm for male and 23 mm for female) and have dry and rough skin but lack of large parotoid gland behind the eyes and above the eardrum. Their tympanum is small but visible.

To identify and compare the species *Ansonia*, the work of Inger & Stuebing (1966, 2005 & 2009) is used. This book covers the species most likely to be seen and identifiable without the use of a microscope. Morphology of a species can be distinguished by special characteristics present in each species. Species of *Ansonia* can be difficult to differentiate

without locality data, for example to compare between *Ansonia* from Borneo and Peninsular Malaysia.

The comparative morphology of species include measurement of snout vent length (SVL), tibia length (TBL), head width (HW), head length (HL), snout length (Snout L), horizontal diameter of eye (ED), interobital width (IO), internarial width (IN), and other characteristics include feature of fingers, colours of abdomen, and so forth (Inger *et. al*, 2001). Photos taken are for image identification and simplify future study and correct measurement needed to be considered as important tools for identification. Even larval morphology is important to identify the species (Haas *et. al*, 2009).

1.1 Objectives

1. To discriminate the species of the genus *Ansonia* in Borneo using morphology and morphometrics.
2. To prepare an identification key to the species of genus *Ansonia* in Borneo.

2.0 Literature Review

2.1 Genus *Ansonia* (Family Bufonidae)

The genus *Ansonia* is a member of class Amphibian, order Anura, family Bufonidae. A total 12 species of *Ansonia* have been reported from Borneo. These are: white-lipped slender toad (*Ansonia albomaculata*), Kinabalu slender toad (*Ansonia hanitschii*), brown slender toad (*Ansonia leptopus*), long-fingered slender toad (*Ansonia longidigita*), dwarf slender toad (*Ansonia minuta*), spiny slender toad (*Ansonia spinulifer*), North Borneo stream toad (*Ansonia fuliginea*), Mesilau stream toad (*Ansonia guibei*), Luidan stream toad (*Ansonia platysoma*), Gunung Mulu stream toad (*Ansonia torrentis*), Rainbow Toad (*Ansonia latidisca*) and Sarang Stream Toad (*Ansonia echinata*) (Matsui *et al.*, 2009).

Matsui (2006) outlined the general history of anuran discovery in Sabah from the late 19th century until 2006. Some of the *Ansonia* species was described as *Bufo* species in 1880s and 1890s by Boulenger (1887). He reported from Gunung Kinabalu, a bufonid, *Bufo leptopus* (Günther, 1872), now known as *Ansonia leptopus* that was originally described from Matang, Sarawak. This record seems to be misidentification of *Ansonia hanitschi* Inger, 1960 or *Ansonia longidigita* Inger, 1960. In 1890s, Mocquard described four bufonids, *Bufo fuligineus* Mocquard (1890), now known as *Ansonia fuliginea* from north Borneo, *Bufo spinulifer* Mocquard (1890) from Kinabalu which now known as *Ansonia spinulifer*. Other two were described in the genera *Pedostibes* and *Pelophryne*.

The species *Ansonia echinata* was collected at Bukit Kana, Bintulu Division, Sarawak on 28 March 2007. The specimen found was all male and all was collected at the same locality and along the same stream where they were found perched on dead leaves at the edge of a

small stream (3 m wide) in primary rain forest. The specific name *echinatus*, Latin word for thorny, referring to the spinose tubercles on top of the snout and of the sides (Inger & Stuebing, 2009). *Ansonia albomaculata* was compared to *Ansonia echinata* which differs in lacking enlarged, spinose tubercles ventrolaterally, spinose tubercles on top of the snout, and mandibular spines under the jaw. Below the eye is a light streak that characterizes *A. albomaculata* (Inger, 1960) is absent in *A. echinata* (Inger & Stuebing, 2009). Similarly, Inger (1966) identified *Bufo leptopus* recorded by Mocquard (1890) as *Ansonia longidigita* Inger, 1960, originally described from Gunung Kinabalu.

An ongoing survey of the reptiles and amphibian in Tanintharyi Nature Reserve (TNR), Myanmar by members of the Nature and Wildlife Conservation Division (NWCD) of the Myanmar forest department, National Museum of Natural History, Smithsonian Institution (USNM), and California Academy of Science (CAS) had found new species of *Ansonia*, which also first *Ansonia* recorded in Myanmar. The species is phylogenetically closely related to *A kraensis* in Thailand but it can be distinguish from other *Ansonia* by comparing the morphology. The species was compared by combination of morphological character with available specimens in Museum, published description by Frost (2011), phylogenetically closely related according to Matsui *et al*. (2010) or geographically proximate species of *Ansonia* from Thailand and Malay Peninsula (Wilkinson *et al*., 2012).

Figure 1.0 shows the geographic range of *Ansonia* in Philippine Islands, Peninsular Malaysia, Borneo, Sumatra, Thailand and Myanmar (Inger & Stuebing, 2005).

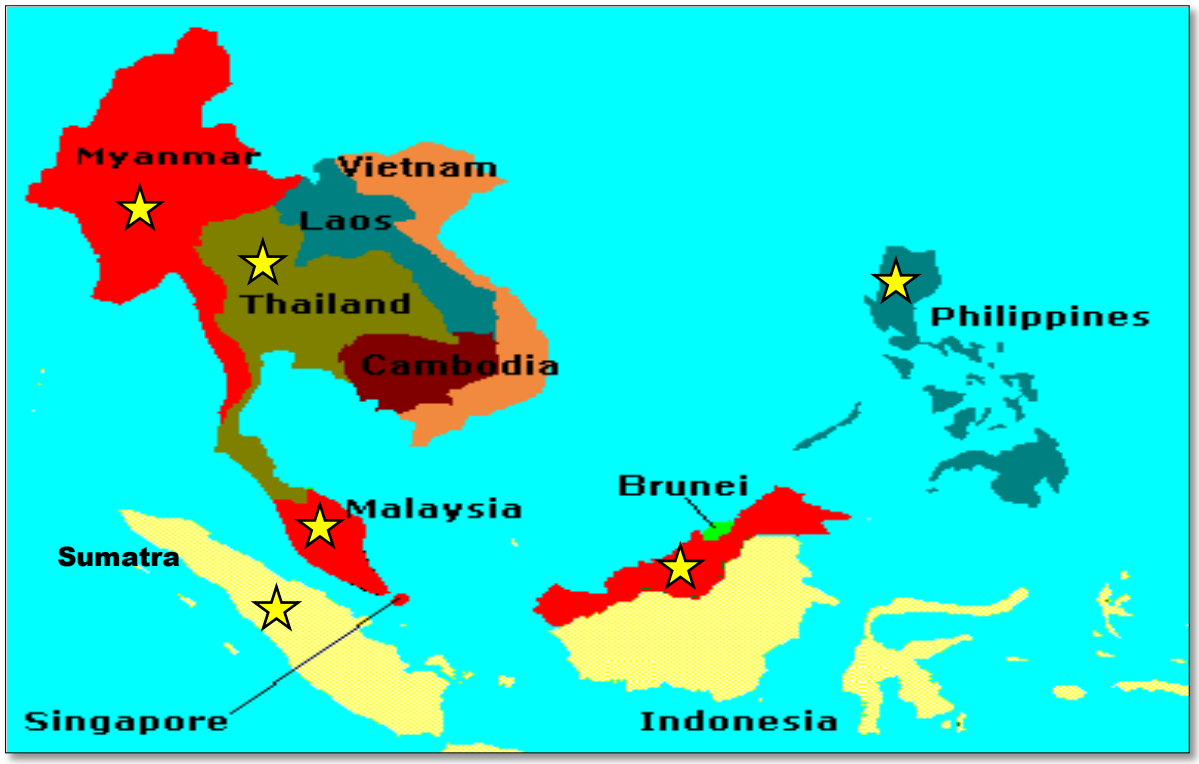

Figure 1.0: The geographic range of *Ansonia*
(Retrieved from: http://data.gbif.org/species/13183740)

2.2 Description of species

Descriptions of all 12 species were referred from Dring (1983), Inger (1966), Inger & Stuebing (2005), and Inger & Stuebing (2009).

The characters used in dichotomous identification keys for *Ansonia* species identification include: present of tympanum; first finger reaching disk of second or not when fingers are adpressed; sharp tarsal ridge; white spot below eyes; whitish band from eye to arm; tips of outer finger rounded/disks shape/expended; projecting snout; webbed toes; warts and so on. These keys are taken from the above authors for 12 species of *Ansonia*.

2.2.1 White-lipped Slender Toad, *Ansonia albomaculata* Inger

Ansonia albomaculata is a small species and stocky with snout projecting beyond mouth. The SVL range of male is from 20-28mm and female with 30-35mm.The tips of the fingers are rounded and first finger is shorter than the second finger. The toes are almost fully web with their tips of toes are rounded as well. The dorsal colour is dark brown to reddish brown with scattered red warts. They also have distinct white spot below the eyes and a light band runs from the corner of the eye to the armpit. The edge of the eyelid is red and the site of the head with dark red spots. The ventral colour is yellow-green with large black blotches anteriorly. The upper arm and anterior thigh covered with orange spots and narrow orange-red cross bar at the hind limb.

This species can be found in primary rain forest. By day, they will hide under leaves and logs and near slow forest stream for breeding during night (Inger, 1966).

2.2.2 Sarang Stream Toad, *Ansonia echinata* (Inger & Stuebing)

This species was a new found species in 2007 in Bintulu division of Sarawak. This is a small species with adult male range 20.0-21.3 mm, but no female had been collected. They have black spines under mandible. Their toes are at least three-fourths webbed and the tips are not expended for both toes and fingers. Large rounded tubercles at dorsal and those in dorsolateral region tipped with small black spines. The ventral is not smooth surface covered with rounded tubercles. Tubercles on the snout tipped with small black spines too.

Due to all main characteristics of tubercles and tipped with small black spines at the back and side of body, the name is given from *echinatus*, Latin word for thorny (Inger & Stuebing, 2009)

2.2.3 Montane Slender Toad, *Ansonia fuliginea* (Mocquard)

A. fuliginea is a moderate-sized species with male range from 32-36 mm and female with 38-44 mm. The head length and width is nearly equal and the snout is not projecting. The toes are incompletely webbed with rounded tips both fingers and toes. Dorsal covered with dense rounded warts and those on sides sometimes bearing numerous melanic spinules. Adult males have cluster of large black spines on the first (inner) finger.

This species is known only from elevations of 1800 to 3000 above sea level. They live in montane mossy forest and in the stunted forest above that zone (Inger, 1966).

2.2.4 Guibé's Slender Toad, *Ansonia guibei* Inger

This is also a moderate-sized species with male range from 31-32 mm and female with 34 mm. The toes of male are fully webbed, but unknown in female because no female had been recorded. The tips are swollen and round for both fingers and toes. The snout is not projecting and dorsal covered with many round or oval warts or tubercles. The colour of dorsal is blackish brown with light spots outlining an indistinct dark pattern. This species is unlike others in the genus, where they lack of vocal sacs in adult male. However, this species has an oblique flap of skin on each side of vent.

This species has been found on the forest floor and on banks of clear, rocky streams in montane forest. In Sabah, they are called Mesilau stream toad where the species are found on the East Ridge of Mount Kinabalu in Kinabalu National Park. "Guibé" is named in honor of Dr. Jean Guibé in his particular help with material of this genus for identification (Inger, 1966).

2.2.5 Kinabalu Slender Toad, *Ansonia hanitschi* Inger

This is a small slender species of *Ansonia* with flat-bodied and projecting snout. The male range from 20-28 mm and female is 28-35 mm. The tips of fingers are spatulated where they look like a spatula or slightly widened than the toes. The toes are about three-fourths webbed with rounded tips. Adult male have one or two rows of yellowish spinose tubercles under the mandibles. Dorsal covered with small rounded and few oval warts. The colour of dorsal is greenish grey to reddish brown, often with black diagonal bars or spots on the back and black crossbar on the limb.

This species has been found in the mountains of western Sabah, northern Sarawak, and the northeastern corner of Kalimantan (Inger, 1966).

2.2.6 Rainbow Toad, *Ansonia latidisca* Inger

This species was last seen in 1924 and just until recent, after 87 years "missing", the species was recently found again in 2011. *Ansonia latidisca* is the most colourful species in the genus. This is a large-size *Ansonia* with the SVL of male is about 35 mm and female is about 55 mm. Tips of fingers are dilated into truncate disks, but not for the toes. The toes have swollen tips and much narrower, with less webbed. The dorsal colour is very distinct with green-yellowish and brown elongated glandular ridge which covered with red-tips warts and other small tubercles.

This toad also known as Sambar Stream toad and they are found in montane forest with elevation about 1200 meters and above (Inger, 1966).

2.2.7 Brown Slender Toad, *Ansonia leptopus* (Günther)

This species is another large species of *Ansonia* where SVL of male is 30-40 mm and female with 45-65 mm. Tips of both fingers and toes are rounded with three-fourth webbed. Males have two or four rows of black spines under the mandibles. Dorsal colour is brown and slightly darker at the side. The entire back is covered with rough, small and rounded warts. Ventral is grey with darker mottling.

The locality of this species is 140 meters above sea level. It can be found on hilly primary forest or old secondary below 600 meters above sea level (Inger, 1966).

2.2.8 Long-fingered Slender Toad, *Ansonia longidigita* Inger

Ansonia longidigita is a species with long hind limbs. This is the largest species in the genus with male SVL range 35-50 mm and female with 45-70 mm. They have rounded tips on both fingers and toes with half webbed. Male have slightly more extensive webbing than female. Males also have three to six rows of spined under the mandible. Dorsal colour is clay brown to black, covered with small spined warts. The limbs have black crossbars on a clay brown background. The ventral is cream-coloured and on the belly is heavily brown or black spot.

This species is endemic to Borneo include Sabah, Brunei, Sarawak and northeastern of Kalimantan. They lived in hilly of primary and old secondary forest or selectively logged forests from 150-2200 meters above sea level (Inger, 1966).

2.2.9 Dwarf Slender Toad, *Ansonia minuta* Inger

This is a small slender toad with male SVL range from 20-23 mm and female with 23-28 mm. The tips of outer fingers are widened or spatulate and the toes are almost fully-webbed. Adult male have two or three rows of spines under the mandible. Dorsal colour is dark brown covered with small and round warts. Ventral colour is pale with black spots and yellowish dots. The side of the head has dark and light bars, with a whitish area below the eye.

This species lives in hilly, lowland rain forest below 700 meters above sea level. It has been found only in Sarawak and Kalimantan (Inger, 1966).

2.2.10 Luidan Stream Toad, *Ansonia platysoma* Inger

This is a small species of *Ansonia* with male range from 21.5-22.8 mm and female about 24 mm. Both tips of fingers and toes are spatulated with three-forth webbed in male and female less webbing. Dorsal colour is brown to olive and covered with smoother warts. The distinct feature for this is species is they have flat head and projecting snout.

They are also known as flat-bodies slender toad. The locality of this species is 1000 meters above sea level or mid-elevation hills up to montane forest (Inger, 1966).

2.2.11 Spiny Slender Toad, *Ansonia spinulifer* (Mocquard)

This is a medium-size species with male SVL range from 30-40 mm and female with 40-45 mm. Tips of outer fingers and toes are rounded with almost free of webbing. Dorsal colour is blackish brown to black with light streak along the side of the body covered with conspicuous, large, spiny warts which can easily felt. They also have a yellowish or whitish

oval or diamond-shape patch between shoulders. Ventral colour is dark grey or black with rough cream-coloured spots with heavy black marbling.

They are lowland stream toad and has been seen perched on shrubs and herbs only along swift flowing rocky stream in primary forest below 700 meters above sea level (Inger & Stuebing, 2005).

2.2.12 Gunung Mulu Stream Toad, *Ansonia torrentis* Dring

This is a moderate-sized species with male SVL range 30-33 mm and female is unknown. The tips of fingers are rounded, same goes to the toes with more swollen tips with three-fourth webbed. The snout is projecting. Dorsal colour is blackish brown and covered with numerous and rounded tubercles. The ventral colour is cream suffused with black and covered with large, grey-brown blotches separated by pale areas.

The type of locality is in lower montane forest at 1700-1800 meters above sea level. They also had known as Torrent slender toad (Dring, 1983).

2.3 Morphometrics

The etymology of the word ‘morphometrics’ have a straightforward meaning (Greek: *morph* = form, *metrikos* = measure) (Lestrel 2000). Morphometrics is a field concerned with studying variation and change in the form of size and shape of the organisms. Morphometric measure the physical parts of the body of organism, and the measurement are used for collecting data. In this study, the measurements and characteristics will be used to determine the comparative morphology among *Ansonia* species.

There are a few studies employ morphometric techniques for identification of Bornean frogs. Identification keys, morphology and images are traditionally important for identify species. Lack of identification keys or morphology characteristics as guide in the field sampling can causes difficulty in identifying species although they are similar in morphology.

Morphological variation within the order Anura is often thought to be less than that in other vertebrate orders. Two explanations have been suggested for this repetitive occurrence of similar morphotypes among distantly related species: convergence and morphological constraint (Emerson, 1988).

2.4 Discriminate Function Analysis (DFA)

DFA is used to determine which variables discriminate between two or more naturally occurring groups. DFA classify of unknown specimens into known groups to increase the discrimination between groups based on set of measurements. Discriminant maximizes the between-group variation and minimizes the within group variation. The greater the discriminant, the more accurately the unknown specimens can be place into a proper group. However, discrimination will degrade if overlap of closely related group increase or misclassification of individual cases belonging to each group increases (Oxnard, 2000).

2.5 Cluster Analysis (CA)

Cluster analysis has a wide variety of procedures to create a classification with observed "closer similarities" of each group (Aldenderfer & Blashfield, 1984; Dunteman, 1989). CA is technique of data analysis which divides data into groups of individual multivariate observations also known as classification (Krzanowski & Marriott, 1955). There are two steps in CA which the first is the measurement of some form of similarity or association between the entities to determine how many groups exist in the sample, and the second step is to profile the variables to determine the composition, which is done by applying discrminant analysis (Joseph *et. al.*, 1987).

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