DIVERSITY OF PHASMIDS IN ULU KIMANIS AND MUAYA

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DECLARATION

I hereby admit that this dissertation is my own work except for certain quotations and references that has been duly cited.

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CERTIFICATION

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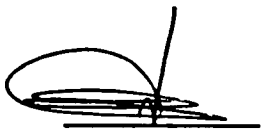

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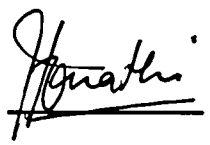

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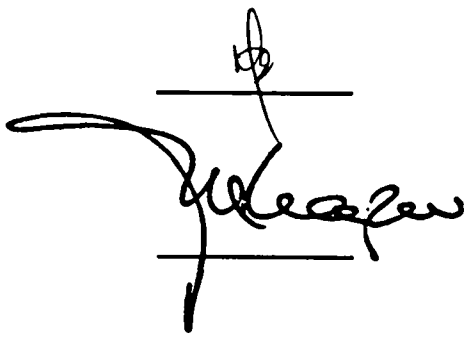

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ABSTRACT

The purpose of this study is to determine the diversity and species composition of phasmids at the two study sites. The objectives of this study were to compare the diversity of stick insect between Ulu Kimanis and Muaya, to determine the abundance of stick insect in Ulu Kimanis and Muaya and also to compare the species of phasmids between Ulu Kimanis and Muaya. The first study site of this research which is Utan Paradise is located at Ulu Kimanis, Papar. Its coordinate is N 05° 29.329' E 116° 01.085' and the altitude is approximately 706 m. The second study site is Muaya. The coordinate for this site is N 4° 53.552' E 115° 46.073' and has a elevation of around 720m. The methods used to capture these insects are direct searching during the night sampling period and sweep netting during the morning sampling session. A total of 229 specimens representing 38 species were collected. The biggest family collected was Bacillidae. The result showed that there was a difference in phasmids diversity at both areas where the Shannon-Wiener index value was 0.851 and 0.715 for Muaya and Ulu Kimanis respectively. Muaya also has a higher evenness of phasmids than Ulu Kimanis as shown by Rank/abundance plot and evenness index. According to Sorensen Similarity index result, both the areas have low similarity of species of phasmids. However, Mann-Whitney test showed that there was no significant dissimilarity of phasmids species between Ulu Kimanis and Muaya.

ABSTRAK

Tujuan kajian ini adalah untuk menentukan diversiti dan komposisi spesies serangga ranting di dua kawasan kajian. Objektif kajian ini adalah untuk membandingkan diversiti serangga ranting di antara Ulu Kimanis dan Muaya, menentukan kelimpahan serangga ranting di Ulu Kimanis dan Muaya dan juga membandingkan spesies serangga ranting antara Ulu Kimanis dan Muaya. Kawasan kajian pertama iaitu Utan Paradise adalah terletak di Ulu Kimanis, Papar. Koordinat kawasan ini adalah N 05° 29.329 E 116° 01.085 dan altitudnya adalah lebih kurang 706 m. Kawasan kajian yang kedua adalah Muaya yang terletak di Sipitang. Koordinat Muaya adalah N 4° 53.552' E 115° 46.073' and ia mempunyai altitud sekitar 720 m. Kaedah yang digunakan untuk mencari phasmid termasuklah teknik pencarian secara terus pada waktu malam dan kaedah jaring sapuan pada waktu pagi. Sebanyak 229 spesimen yang terdiri daripada 38 spesies telah dikumpul. Famili phasmid yang terbesar yang telah dikumpul adalah Bacillidae. Keputusan yang diperolehi menunjukkan bahawa terdapat perbezaan diversity phasmid di kedua-dua kawasan tersebut dimana indeks Shannon-wiener adalah 0.851 bagi Muaya dan 0.715 bagi Ulu Kimanis. Menurut keputusan indeks kesamaan Sorensen, dua kawasan tersebut mempunyai kesamaan spesies phasmid yang rendah. Namun begitu, keputusan daripada ujian Mann-Whitney menunjukkan bahawa tiada perbezaan yang ketara di antara spesies phasmids di dua kawasan tersebut.

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SYMBOL, ABBREVIATION AND UNIT LIST

°C	Degree celcius
Cm	Centimeter
C_s	Sorensen similarity index
H'	Shannon's diversity index
J'	The evenness index
m	Meter
p_i	The proportional abundance of each species at the site
R_M	Rank sums for Muaya sample
R_U	Rank sums for Ulu kimanis sample
U_M	U statistics for Muaya
U_U	U statistics for Ulu Kimanis

CHAPTER 1

INTRODUCTION

1.1 Background study

According to Bragg (2001), about 3,000 species of stick insects in the world have been described and 10% of them are found in Borneo. Several author have done the identification Bornean phasmids notably Günther (post 1908), Dohrn (1910), Gigilo-Tos (1910), Carl (1913), Hausleithner (1991), Zompro (1998), Seow-Choen (1998), and Brag and Chan (1993). Among the seven existing families of Phasmids, Timetidae and Pseudophasmatidae are not found in Borneo.

Phasmids of most countries are poorly studies (Brock, 1999). Most studies that have been done focused on their behaviour such as courtship and their physiology. There is very little research done and journal published about phasmids diversity.

1.2 General information of stick insects

Stick insects and leaf insects belong to Order Phasmida. Stick insects have long and slender bodies resembling a twig while leaf insects body are flattened and laterally dilated resembling a plant leaf. The head of phasmids may be rectangular or oval. Their antennae can range from very short (as short as their head) to very long

(longer than their body) and usually are very slender. Ocelli can only be found on some winged species and they usually present in three. Phasmids have chewing mouthpart where mandible used to pierce and consume leaves. Insects in this Order may be winged or wingless. They exhibit sexual dimorphism in which the female and male insects have a significant difference in their secondary characteristic such as body shape and colour (Johnson & Triplehorn, 2004).

Phasmida consume a large variety of dicotyledonous shrubs or trees. Some of the female of some species is known to eat fern. They chew leaf in a semi circular motion thus leaving the large rounded bite marks on the leaf (Seow-Choen, 1997). Stick insects can emit foul smelling substance from their thorax when they were threatened as a mean of defence and to ward off possible predators. The adults do not take care of their young and eggs. The eggs that they laid are left scattered on the ground. According to Brock (1999), egg of phasmids differ in size and shape and can range from perfectly round structure to long cigar structure. Most of the Phasmids are active at night which makes them nocturnal insects.

1.3 Justification of study

One of the reasons this study was chosen is to determine the diversity and species composition between Ulu Kimanis and Muaya forest reserve since there is no effort to assess phasmids diversity in both of the places previously. Besides that, since the phasmids collection in Borneensis is considerably lesser than other insect collection, this study can help to increase the amount of phasmids collection for future reference.

1.4 Scope of research

This research was limited to all species of phasmids found in Ulu Kimanis and Muaya forest reserve. The elevation in which this research was conducted is around 650-950m. The sampling was limited to the trail at both sites.

1.5 Research Questions

Some of the research questions that were answered include which site has higher diversity of stick insect, how abundance is phasmids in Ulu Kimanis and Muaya and also how similar are the species of phasmids between Ulu Kimanis and Muaya?

1.6 Objectives

The following were the objectives of this research

a. To compare the diversity of stick insect between Ulu Kimanis and Muaya
b. Determine the abundance of stick insects in Ulu Kimanis and Muaya
c. Compare the species of Phasmids between Ulu Kimanis and Muaya

1.7 Hypothesis

The null hypothesis for this study is that there will be significant difference in the species of phasmids between Ulu Kimanis and Muaya.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomic classification

Different author have different opinion on taxonomic classification of phasmids. Table 2.1 shows the taxonomic classification provided by Brock *et al.* (1999)

Table 2.1 Taxonomic classification of phasmids (Brock *et al.*, 1999)

Kingdom	Metazoa
Phylum	Arthropoda
Class	Insecta
Infraclass	Neoptera
Order	Phasmida

2.2 Classification of phasmids

There are seven families of phasmid in the world and about 3,000 described species. 10% of these species exist in Borneo (Bragg, 2001).

2.2.1 Timematidae

This family of Phasmids is also known as 'Timema Walkingsticks' (Johnson & Triplehorn, 2004). Compared to other family of walkingstick, the members of this family are much shorter and stouter. They have six short legs, three-segmented tarsi, and unequal pretarsal claws. Their colour varies from greenish to pink. Ten species have been identified for this family

and at least one species is known to be parthenogenic (Johnson & Triplehorn, 2004). This family does not exist in Borneo (Bragg, 2001).

2.2.2 Pseudophasmatidae

They are also known as 'Striped Walkingsticks' (Johnson & Triplehorn, 2004). The tergum of the first abdominal segment is completely fused while their middle and hind tibiae are broadly and deeply emarginated apically. Their natural colouration is brownish yellow for male and brown for female. Besides that, there is a dark median and two lateral dorsal stripes hence they were named striped walkingsticks. They are capable of squirting a thick, milky fluid through a defensive gland (Johnson&Triplehorn, 2004). This species is only found in Central and South America (Bragg, 2001) but does not exist in Borneo.

2.2.3 Heteronemiidae

They are also called the 'Common Walkingsticks' (Johnson & Triplehorn, 2004). They are the most stick like compared to the other families. Their antennae are slender filiform and indistinctly segmented. The ventral ridge of femore is smooth and the elevated ridge of middle and hind femora will not be evenly serrated if their antenna are short (Seow-Choen, 1997). When they are abundant enough, they are capable of seriously defoliating forest trees (Johnson & Triplehorn, 2004).

2.2.4 Phasmatidae

Phasmids located in this family are also known as 'Winged walkingsticks' (Johnson & Triplehorn, 2004) due to the presence of wings. There are two stout spines on the vertex which is found on the head (Johnson & Triplehorn, 2004). According to Seow-Choen (1997) members of this family usually have antennae that are shorter than the fore femora and distinctly segmented. However, some species have antennae that are longer than the fore femora but shorter than the body.

2.2.5 Bacillidae

This family includes three subfamilies which is Bacillinae, Pygirhynchinae and Heteropteryginae. The middle and hind tibiae of the members of this family have a distinct triangular depression at the apex of the ventral surface. The metanotum is longer than the median segment (Bragg, 2001). Wingless genera are more dominant in this family although winged genera occur as well (Seow-choen, 1997).

2.2.6 Phyllidae

This family has only three genera and not divided into subfamilies. Among these three genera, only the *Phyllium* are recorded from Borneo (Bragg, 2001). Members of this family are commonly known as leaf insects due to the similarity of their outer appearance to leaves. Their distinct characteristic includes body that is extremely dorso-ventrally flattened. The female insect has large tegmina that cover most of its abdomen while males have much shorter tegmina that barely cover the metathorax (Seow-Choen, 1997).

2.2.7 Aschiphasmatidae

All species in this family are quite small where they can grow up to only 8cm long. Winged and wingless species present in this family. One of the distinguishing characteristic of Aschiphasmatidae is the radial, sector and medial vein of their wings are completely fused (Bragg, 2001). This is a new family and is previously treated as part of Pseudophasmatidae.

2.3 Defence

Phasmids utilize various defensive strategies to escape from predator or to deter possible predator. Their defence mechanism can be divided into two categories which are passive or active. The passive defences are cryptic coloration, mimicry, body spines and nocturnal habit while active defences include spines, production of strong smelling secretion, and flash colour colouration which is a method of defence

where they flash their colourful wings in an attempt to ward off their predator (Bragg, 2001).

2.3.1 Passive defence

Their main passive defence is mimicry of leaves, twigs or bark since their body shape resemble these substances thus turning it into an effective camouflage. Other than mimicry, their nocturnal habit allowed them to escape from a lot of possible predator. They will feign death by remaining motionless for a period of time even though they were disturbed. According to Bragg (2001), this form of defence is known as Thanatosis. Another form of defence which is similar to Thanatosis is Catalepsy in which phasmida enter a state of immobilization and became insensible to stimulation. Phasmids are capable of autotomy, the shedding of their legs when caught. It is most common in Borneo's largest species, *Baculolonga kirbyi*.

2.3.2 Sound production

According to Bragg (2001), sound production through stridulation was first discovered by Wood-Mason in 1877. Three genera among the six genera found to produce sound occur in Borneo. *Haaniella* and *Heteropteryx* used their short wing to make rustling and clicking sound and is usually produced when they were threatened. Another genera that produce sound in Borneo is *Phyllium* that have been long known to produce sound by rubbing the thickened third segments of their antennae.

2.3.3 Smell production

The secretion of strong smelling or irritant odours is a form of defence widely used by phasmids. Only phasmids in the subfamily Aschiphasmatidae were observed to produce strong irritating secretions in the field. Other phasmids secretion smell was too weak to be sensed in the field. The reason why Aschiphasmatidae produce stronger deterrent smell than other subfamily might be due to their lack of spines that is large enough for defence (Bragg, 2001).

Trans,trans- and trans,cis-iridodial and nepetalactone are found to be the major constituent of the defensive spray of coconut stick insect, *Graffea crouani* (Roger *et al.*, 1979). *Megacrania tsudai*, a species of stick insect from Taiwan consumes the leaves of screw pine that contained actinidine and probably concentrated it for more than 3500 times in their gland tissue for defensive purpose (Ho & Chow, 1993).

Phyllium westwoodii belonging to Family Phyllidae is the first and the only species identified to spray opaque liquid that contained pyrazines. This liquid was a mixture of glucose, 3-isobutyl-2,5-dimethylpyrazine, 2,5-dimethyl-3-(2-methylbutyl)pyrazine, and 2,5 dimethyl-3-(3-methylbutyl)pyrazine (Dossey *et al.*, 2009). The odor of this spray is similar to chocolate. Alkyldimethyl-pyrazines are used by order Hymenoptera that include ant, wasp and bee as pheromone. Human uses pyrazine as flavour additive and odorant in food.

2.4 Predators

Many animals such as monkeys, rats, birds, frogs, lizards, spiders, praying mantis and ants have been recorded to preyed on phasmids besides being attacked by mites, parasitoid flies, and biting midges (Bragg, 2001).

Spider such as *Cupiennius salei* can even prey on phasmids that are 350 percent of the spider body length (Nentwig, 1990). According to Nentwig (1990), successful predation of *Cupiennius salei* on phasmids is dependent on the body weight factor rather than body length. Besides that, phasmids that secrete defensive secretion have lower acceptance compared to those without this defence mechanism.

2.5 Habitat

In Borneo, phasmids can be found in agricultural areas, lowland dipterocarp forest, mangrove forest, montane dipterocarp forest, mountain scrub, peatswamp forest,

and secondary forest (Bragg, 2001). According to Bragg (2001), the farther the location from urban area, the higher the abundance of phasmids will be.

2.6 Foodplant

Phasmids eat a wide variety of dicotyledonous shrubs or trees. Female of the species *Haniella grayii* are the only phasmids of Borneo that have been seen eating fern. Some plants that are not native to Borneo such as cocoa and acacia are also eaten by phasmids in their natural habitat (Bragg, 2001). The lists of native foodplant consumed by phasmids in Borneo are listed in Table 2.2.

2.7 Mate selection

Females may use pheromones to attract males for mating. Males will compete for females. This competition is in the form of fighting. In some species such as those from Timematidae family, the males will perform courtship and wait for acceptation of the female. Courtship is performed after the male and female species have been paired. Population of Timema walking-sticks are adapted to different host plants and this enable reproductive isolation between and within species of Timema walking-sticks. Even within the same species Timema walking-sticks (Timematidae), population inhabiting different host plant exhibit slightly different courtship behaviour. Courtship behaviour includes antennae waving from side to side and leg waving which involve kicking two or four posterior leg of the female to the side. Different species of Timematidae show significant difference in the frequency of antennae waving and leg waving. About 85 percent of courtship behaviour by the male will result in copulation attempt. If females reject males attempt, they will move their abdomen and do not allow males to make genital contact (Arbuthnott & Crespi, 2008). Figure 2.1 shows the dorsal view of Timematidae mating behaviour where the male is on top and female is at the bottom.

Table 2.2 List of foodplants consumed by Bornean Phasmids (Bragg, 2001)

Phasmids		Foodplant	
Subfamily	Species	Family	Species
Aschiphasmatinae	*Orthomeria superb*	*Urticaceae*	*Oreocnide rebescens*
	Dajaca monilicornis	Myrtaceae	Tristania sp.
	Dinophasma guttigerum	*Melastomatacaea*	*Melastoma malabathricum Linnaeus*
	Dinophasma sagitana	Melastomataceae	*Melastoma malabathricum*
	Dinophasma kinabaluensis	Melastomataceae	*Melastoma malabathricum*
Heteropteryginae	*Dares validispinus*	Dioscoreaceae	*Discorea* sp.
	Damates borneensis	Araceae	?
		Arecaceae (palmae)	*Daemonorops* sp.
	Epidares nolimetangere	*Melastomataceae*	*Clidemia hirta*
		Araceae	?
	Haaniella grayii	*Nepenthaceae*	*Nepentes ampullaria*
		Zingiberaceae	?
		Gleicheniaceae (fern)	*Dicranopteris linearis*
Lonchodinae	*Carausius sanguioligatus*	Rosaceae	*Rubus muluccana*
	Lonchodes amaurops	Myrsinaceae	*Embelia* sp.
	Lonchodes jejunus	Legumunoceae	*Acacia auriculariformis*
		Euphorbiaceae	*Mallotus* sp.
		Sterculiaceae	*Norrisia maior*
	Prisomera harmani	Rubiaceae	*Theobroma cacao*

	Phenachephorus auriculatus	Loganiaceae	*Norrisia maior*
	Prisomera nigra	Rubiaceae?	?
Necrociinae	*Asceles margaritatus* (Wingless variety)	Euphorbiaceae	*Macaranga* sp.
	Asceles margaritatus (Winged variety)	Euphorbiaceae	*Macaranga* spp.
			Mallotus sp.
Phylliidae	*Phyllium giganteums*	?	*Sacara thaipingensis*

*? = Uncertain species or have only been identified to family

2.8 Reproduction

All tropical species of phasmid reproduce sexually under normal conditions. However, some of them which reproduce sexually might reproduce parthenogenitically when no males were available for mating. Most of temperate climate phasmids usually reproduce by parthenogenesis (Bragg, 2001).

According to Lorenz *et al.* (1999), phasmid development and reproduction is dependent on juvenile hormone and ecdysteroids. Juvenile hormone is the main hormone regulating vitellogenin (egg yolk precursor protein) systhesis and uptake. Juvenile hormone and ecdysteroids (moulting and sex hormone) are necessary for normal egg development in almost all insect species. However for some reason or another, the Indian stick insect *Carausius morosus* does not need juvenile hormone for normal vitellogenesis which is normal yolk deposition (Lorenz *et al.*, 1999).

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