ECOLOGY AND SYSTEMATICS OF THE GENUS *LIPHISTIUS* (ARANAE: LIPHISTIIDAE) FROM PENINSULAR MALAYSIA

NURUL SYUHADAH BINTI MOHAMED DZARAWI

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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (EXCEPT MATHEMATICS & SCIENCE PHILOSOPHY)

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Name of Candidate : **Nurul Syuhadah Binti Mohamed Dzarawi**

I.C :

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ECOLOGY AND SYSTEMATICS OF THE GENUS *LIPHISTIUS* (ARANAE: LIPHISTIIDAE) FROM PENINSULAR MALAYSIA

Field of Study : **Molecular Ecology**

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ABSTRACT

The primitive trap door spider genus *Liphistius* is currently known from few region in South East Asia, including Malaysia. In the year 2012, Malaysian Government has listed all the fourteen species and one subspecies originated from this region as protected under the Malaysian Wildlife Conservation (Amendment of Schedule) Order 2012 to protect this group from commercial collectors. Unfortunately the trap door spiders are poorly studied because of the rarity of adult's specimens. The work on this group of conservation importance is also constrained by the difficulties in species identification. In this study the distribution of the genus *Liphistius* from Peninsular Malaysia was surveyed for the first time. A total of 33 population sites were investigated with new population records for several places in Terengganu, Perak, Pahang and Johor. Nine species and ten morpho species were examined in this study. All the species examined were described with information on type data, material examined and geographical distribution. The identified known species were *L. malayanus, L. desultor, L. murphyorum, L. endau, L. langkawi, L. kanthan, L. batuensis, L. laruticus* and *L. tempurung.* Images of adults, genitalia structures and distribution maps are provided. Molecular analysis was employed to help with the identification process and to clarify the species status. DNA barcoding method and neighbour joining analysis were employed to delimit the species. Bayesian inference and maximum parsimony analysis were then used to test the monophyletic and relationship of the genus *Liphistius* from Peninsular Malaysia. All the molecular methods conducted supported the existence of the five described species within *Liphistius* spp. in Peninsular Malaysia.

ABSTRAK

Labah-labah *trap door* genus *Liphistius* yang primitif hanya diketahui wujud di beberapa kawasan di Asia Tenggara, termasuk di Malaysia. Pada tahun 2012, Kerajaan Malaysia telah menyenaraikan kesemua 14 spesis dan 1 sub-spesis yang ditemui di rantau ini sebagai spesis dilindungi di bawah Perintah Pemuliharaan Hidupan Liar (Pindaan Jadual) 2012 untuk melindungi kumpulan ini daripada pengumpul komersial. Malangnya, labah-labah *trap door* tidak dikaji dengan baik disebabkan kesukaran untuk mendapatkan spesimen dewasa. Kajian ke atas kumpulan yang penting dari sudut pemuliharaan ini juga turut dihalang dengan kesukaran dalam mengidentifikasi spesis. Di dalam kajian ini, taburan labah-labah *Liphistius* di Semenanjung Malaysia di kaji buat pertama kali. Sebanyak 33 lokasi telah disiasat dengan penemuan lokasi yang baru di Terenggau, Perak Pahang dan Johor. Sembilan spesis dan sepuluh morpho-spesis telah diperiksa di dalam kajian ini. Kesemua spesis yang diperiksa telah dihuraikan dengan maklumat berkenaan jenis, bahan dikaji dan taburan geografi. Spesis yang berjaya diidentifikasi ialah *L. malayanus, L. desultor, L. murphyorum, L. endau, L. langkawi, L. kanthan, L. batuensis, L. laruticus* dan *L. tempurung.* Imej-imej spesimen dewasa, struktur genitalia dan peta taburan dibekalkan. Analisis molekular digunakan untuk membantu dalam mengenalpasti dan menjelaskan status spesis. Metod *barcoding* DNA dan analisis *neighbour joining* digunakan untuk membatasi spesis. Inferens Bayes dan *parsimony* maksimum turut digunakan untuk menguji monofiletik dan hubungan antara genus *Liphistius* di Semenanjung Malaysia. Kesemua kaedah molekular yang digunakan menyokong kewujudan lima spesis yang dihuraikan dalam *Liphistius* spp. di Semenanjung Malaysia.

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LIST OF SYMBOLS AND ABBREVIATIONS

DNA	:	Deoxyribonucleic acid
COI	:	Cytochrome c oxidase I
mtDNA	:	Mitochondrial DNA
PCR	:	Polymerase chain reaction
acc. no.	:	Account number
spp.	:	Species (plural)
sp.	:	Species (singular)
mm	:	millimetres
m	:	metre
NGO	:	Non-government organization
TA	:	Tibial apophysis
RC	:	Receptacular cluster
SA	:	Sub-tegular apophysis
PS	:	Posterior stalk
j	:	juvenile
MZUM	:	Museum of Zoology, University of Malaya
BMNH	:	British Museum of Natural History
ZMC	:	Zoological Museum of Copenhagen
AMNH	:	American Museum of Natural History
MHNG	:	Natural History Museum of Geneva
MCMC	:	Markov Chain Monte Carlo
TBR	:	Tree-bisection reconnection
NJ	:	Neighbour joining
MP	:	Maximum parsimony
BK	:	Book lung
BL	:	Body length
CW	:	Carapace width
CL	:	Carapace length
AW	:	Abdomen width
AL	:	Abdomen length
SW	:	Sternum width
SL	:	Sternum length

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Research Background

In the year of 2010, Wildlife Conservation Act 716 (refer to Appendix 3) has been enacted by the Malaysian parliament in order to protect some of the local Malaysian animal species from poachers and commercial collectors. Among the listed species in the legislation was one of the primitive segmented spiders, *Liphistius malayanus.* The species are listed in the First Schedule (Part One) which has categorized it as "Protected Wildlife". In April 2012, the conservation act was amended and with the exception of *L. tioman,* all *Liphistius* in the peninsula were listed as 'Protected Wildlife". *L. tioman* by law is a "Totally Protected Wildlife".

The spider genus *Liphistius* is currently only known in Laos, Myanmar, Thailand, Peninsular Malaysia and Sumatra Island. To date, forty nine *Liphistius* species and one subspecies are currently known (Schwendinger, 2013). Fourteen species and one subspecies are known in Peninsular Malaysia in which one species was also recorded from southern Thailand. They are *Liphistius batuensis, L. desultor*, *L. endau*, *L. johore*, *L. kanthan, L. langkawi, L. laruticus*, *L. malayanus, L. malayanus cameroni*, *L. murphyorum, L. panching, L. rufipes* (also found in Southern Thailand), *L. tempurung*, *L. tioman* and *L. yangae*.

Non-governmental organizations such as the Malaysian Nature Society and some nature-loving individuals has made claims that this species are threatened and heading towards extinction. However, there is no concrete empirical evidence to support these claims. According to Haupt (2003), a great number of species have been described without ever studying the range of variety of local populations. He also claimed that

Liphistius tendency to isolation has resulted in large number of population to differ morphologically. These reasons perhaps have contributed to the 'endemism' status of the *Liphistius* spiders.

Current literatures focused on the description of species (Schwendinger, 2009, 2013a; Schwendinger & Ono, 2011), and mainly on *Liphistius* spiders in neighboring country especially Thailand. Collecting new specimens from their actual habitat may impart further understanding on *Liphistius* biology and ecology therefore would provide adequate data for the conservation and management of the spider. Furthermore, a phylogenetic study on this group can quickly assist understanding on the species endemism.

In this study, *Liphistius* spiders were gathered from selected localities in Peninsular Malaysia, or at locations at which the spider has been previously described together with several new sites. The collections provided a good database of *Liphistius* distribution in Peninsular Malaysia and therefore provide insights for further studies in the future. Molecular analysis had been used to investigate the current status of *Liphistius* spp. that exists in Peninsular Malaysia. Molecular analysis on the specimen would allow better clarification on the endemicity of the species and further enhance the understanding of the species status which was previously described solely based on morphology. In *Liphistius,* its high level of endemicity yet understudied nature makes the use of molecular characters to determine its phylogenetic relationship particularly appropriate. Furthermore that molecular characters can be used to clarify species status and the relationship between spiders when morphological characters are difficult to differentiate.

1.2 Research Objective

This study is the first that combined both genetic and morphological methodology in determining and confirming the species identity of *Liphistius*. The objectives of the study are:

i. To provide the morphological descriptions and a dichotomic key for the known *Liphistius* species from Peninsular Malaysia.

ii. To investigate and record the distribution of the known *Liphistius* species from Peninsular Malaysia.

iii. To investigate the species status of the *Liphistius* spiders in Peninsular Malaysia using partial cytochrome c oxidase subunit I (*COI*).

1.3 Limitations of Study

Although this study has reached its aim, there were some unavoidable limitations. Firstly, all species type specimens that originated from Peninsular Malaysia were not deposited in Malaysia. Thus, the species identification is very difficult because reference could not be made to the original source. Limited budget allocated for overseas travel to Masters candidates limited the chance to examine the type specimen. In addition, the type specimens were also stored separately in a few museums. It would be better in the future study that this problem could be overcome to obtain a better result.

Secondly, the field works conducted was not only limited by the weather conditions, but also challenges in transportation and accommodation which require an organized strategy. In addition, field works also require permits from the authorities such as the Forestry Department, Wildlife Department and National Park. Hence, it was very difficult to complete a collection from all over Peninsular Malaysia within 24 months of study.

Thirdly, this study also involved working in the laboratory. The limited time has caused only one gene, *COI* which was obtained from only one primer pair to be used. Many species collected from the field also could not have their sequences amplified and the results were not able to be repeated due to the time constraint.

CHAPTER 2: LITERATURE REVIEW

2.1 Spider research in Malaysia

In 2009, Norma-Rashid and Li listed 425 species of spiders in Peninsular Malaysia, including 20 new records from the mangrove areas. In 2014, the checklist was updated to include an additional 219 new records (Dzulhelmi et al., 2014a). The updated checklist includes 70 new records of spider species that were primarily collected in the field. In total, there were 644 spider species that were currently known from Peninsular Malaysia. The numbers of species described however was only 2% of the total number of the described spider species in the world (45681 species according to World Spider Catalog, 2015).

Malaysia is known to be a mega-biodiversity country, therefore the numbers of listed species are believed to be far from complete as there are large possibilities of more unknown taxa, of which, many could be endemic. Deeleman-Reinhold (2001) noted that the great majority of Southeast Asian spiders are probably forest dwellers and that spiders in tropical Asian forests are poorly known. The same under-representation issue for spider species was also observed in other Southeast Asian countries, with uneven level of progress in spider's research between the countries (Jaeger, 2012; Song & Zhang, 2002).

Historically, the 'colonial masters' play very important roles in the advancement of research on spiders in the Peninsula. In the middle of 19th century, a large number of spider specimens from Penang was collected by van Teylingen, a Dutchman who brought the specimens back to the Zoological Museum of Copenhagen. The specimens were then described to be *Liphistius desultor* in the year 1849 (Haupt, 2003). Following this, many

other discoveries were made in Malaya and Borneo. The 2009 compiled list had also indicated that the known species were mainly collected from specific localities such as Fraser's Hill, Cameron Highlands, and in the National Parks (Norma-Rashid & Li, 2009).

In the recent years, especially post-independence, progress in spider's research in Malaysia has been slow. Occasionally, new records of spider species were made in places frequented by tourists (see Norma-Rashid & Li, 2009).This suggested that the rich biodiversity in the country still fascinates local and foreign enthusiasts. The most recent effort was the compilation of spider's checklist in Sabah by local researchers (Dzulhelmi et al., 2014b). This could be the starting point in moving the research forward.

What is crucial in spider research is the difficulty in species identifications, which depend mainly on morphological descriptions. In addition to that, the lack of locally trained experts and sufficient grant allocations had also hindered research progress. Much progress was due to the enthusiasm of experts from abroad with good financial backing. Collaborative efforts between local and experts from abroad could be the necessary way to further enhance research works and a way to move forward.

2.2 The spider fauna of Malaysia

It is estimated that only one half to one third of total existing spider species have been described (Platnick & Raven, 2013). The spider fauna of Malaysia has never been examined in its entirety despite of its significant importance on the biodiversity conservation. From the current spider checklist compiled as shown in Table 2.1, it can be observed that there are 31 families represented by less than 10 species with twelve families represented by only a single species.

However, this data should not be considered as a representation of the spiders' species status due to the possibility of some misunderstanding of type locality, misidentification

and uncertainty of the spiders' existence in Peninsular Malaysia (Norma-Rashid & Li 2009). To make things worse, there are several states in Malaysia which have never been sampled for spiders. Therefore, detailed information on the distribution of the spiders in this region is also lacking. Thus, it can be said that the data does not reflect the actual species number, and that we are definitely lacking in collection efforts.

What is more interesting is the existence of local endemics such as the trap door spider genus *Liphistius*. Fourteen species and one sub-species have been recorded from Peninsular Malaysia, and with an exception of one species, *Liphistius rufipes,* others were only found here. Thus the *Liphistius* spider is protected under the Malaysian Wildlife Conservation (Amendment of Schedule) Order 2012. The legislation was enacted mainly due to illegal exotic pet trade which has become an increasing cause for concern in Malaysia. A few years ago, a *Liphistius* is believed to be able to fetch a price of RM90 per individual (see Appendix1) and illegal trade is apparently a direct threat to the *Liphistius* populations in Malaysia.

Table 2.1: Spider families recorded from Malaysia indicating the number of species (including sub species) in each family, asterisk (*) indicating family with update species number from (Norma-Rashid & Li, 2009, Dzulhelmi *et al*., 2014)

Family	Species	Family	Species
Anapidae	2	Nesticidae	1
Araneidae*	94	Ochyroceratidae	3
Barychelidae	5	Oonopidae	14
Cithaeronidae	1	Oxyopidae*	11
Clubionidae*	16	Palpimanidae	2
Corinnidae*	18	Philodromidae	1
Cryptothelidae	3	Pholcidae*	22
Ctenidae	4	Pisauriidae	10
Ctenizidae	1	Prodidomidae	1
Deinopidae	1	Psechridae	7
Desidae	3	Salticidae*	140
Dictynidae	2	Scytodidae	6
Gnaphosidae*	4	Selenopidae	1
Hahniidae	1	Sparassidae*	27
Hersilidae	1	Stenochilidae	2
Hexathelidae	1	Symphytognathidae	1
Linyphiidae*	19	Telemidae	2
Liocranidae	19	Tetrablemmidae	12
Liphistiidae	15	Tetragnathidae*	32
Lycosidae	18	Theraphosidae	16
Mimetidae	1	Theridiidae*	37
Miturgidae	2	Theridiosomatidae	4
Mysmenidae	2	Thomisidae*	29
Nemisiidae	3	Uloboridae*	9
Nephilidae	5	Zodariidae*	13
		Total: 50	644

2.3 Trap door spider genus *Liphistius* and the biological aspects

The *Liphistius* is a genus of primitive spider in the family Liphistiidae which are found only in Myanmar, Laos, Thailand, and Malaysia and in Sumatera, Indonesia (World Spider Catalog, 2016). They were classified as primitive spiders as they retain segmented abdomen and appendage like spinneret (Xu et al., 2015b). The *Liphistius* had also retained the primitive number of eight separate spinnerets (Platnick & Sedgwick, 1984). Even though *Liphistius* possessed such a significant position among the spiders taxonomy, they are currently under-represented in the literature. In the past 15 years, only a few studies have been conducted on this particular group (Foelix et al., 2010; Michalik, 2007; Schwendinger, 2009, 2013; Schwendinger & Ono, 2011; Schwendinger & Pape, 2000).

Their common name, trap door spider, points to their nest structure, which best characterized them and making them a unique spider (Figure 2.1). Members of this genus are most commonly found constructing their nest either in road banks, on cave walls or on the tree trunks in the forest. Several strands of silk radiated (known as radials) from the door and according to Murphy & Murphy (2000), there are seven radials originating from the burrow's entrance. The radials are used as fishing lines to detect their prey. The trap door is usually camouflaged with soil and moss, and for species that are found in caves, the trap door is made from surrounding material.

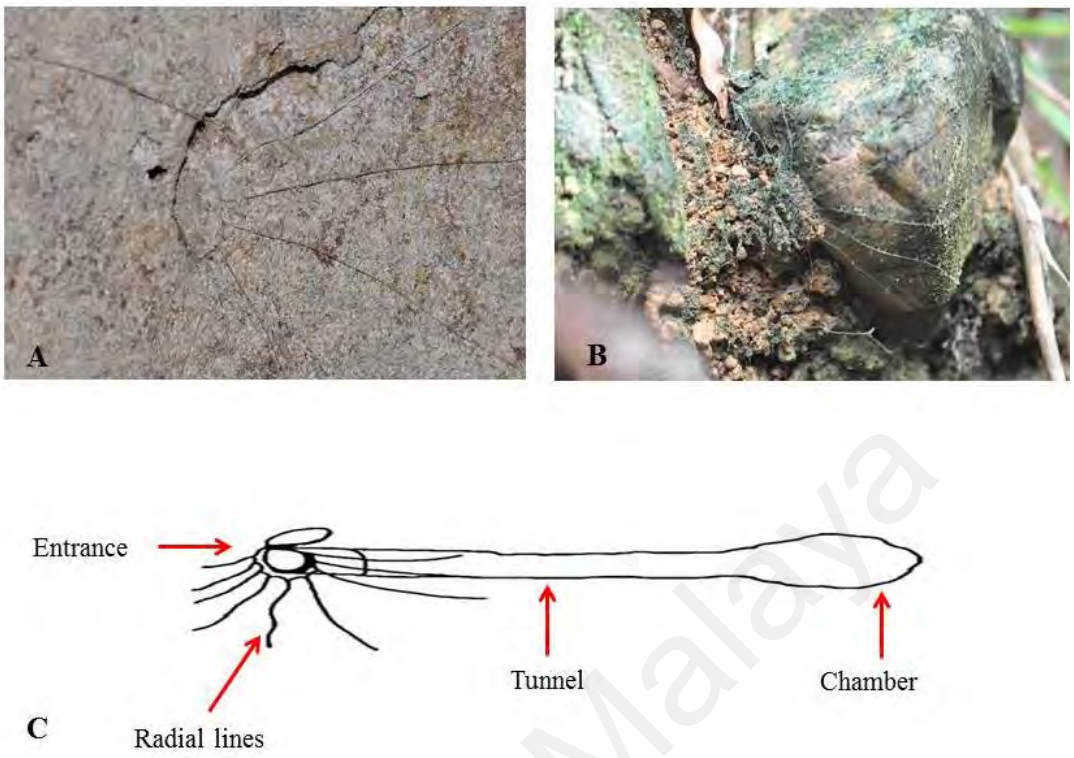

Figure 2.1 *Liphistius* spp. nest examples, [A] Nest of *L. kanthan,* [B] Nest of *L. endau* and [C] Illustration of the nest construction of *L. desultor* collected in this study.

To date, a total of 32 *Liphistius* species has been described from Thailand, two species from Myanmar, one species from Sumatera, two species from Laos and 14 species and one sub species from Peninsular Malaysia. A study conducted on the family Liphistiidae suggested that liphistiids spider are confined to their burrow and rarely move around, and phylogenetic and biogeographic analysis confirm that they are dispersal-limited and highly genetically structured (Xu et al., 2015c). Therefore, it is not surprising that this genus has high potential for endemism. However, very little is known about the ecology, diversity, and phylogenetic relationships of these spiders and this is especially true for the species that are known in Peninsular Malaysia. Many species were only known from a single locality. This may be due to poor collection and lack of research (Platnick & Sedgwick, 1984). For example, *L. kanthan* and *L. tempurung* are currently only reported from Kanthan Cave and Tempurung Cave respectively. However, both

species are described based only on single sex specimens (Platnick et al., 1997), therefore, the validity of the species status could still be debatable.

The main factor that causes the lack of research in *Liphistius* is probably the difficulty with species identification. According to Xu et al. (2015b), all existing classification schemes of liphistiid spiders were dominated by a few selected characters and opinions rather than phylogenetic analysis. In nature, it is relatively easier to find adult females than adults males, with six out of 50 *Liphistius* species are only being known from females only (World Spider Catalog, 2015). However, liphistiids female have simple genitals with extraordinarily intraspecific variation (Xu et al., 2015a), and thus making species delimitation based on female morphology extremely challenging (Haupt, 2003; Tanikawa, 2013; Tanikawa & Miyashita, 2014)

2.4 *Liphistius* spider in Peninsular Malaysia

There are currently 14 species and one sub-species described from Peninsular Malaysia. They are *L. batuensis* Abraham, 1923; *L. desultor* Schiödte, 1849; *L. endau* Sedgwick & Platnick, 1987; *L. johore* Platnick & Sedgwick, 1984; *L. kanthan* Platnick, 1997; *L. langkawi* Platnick & Sedgwick, 1984; *L. laruticus* Schwendinger, 1997; *L. malayanus* Abraham, 1923; *L. malayanus cameroni* Haupt, 1983; *L. murphyorum* Platnick & Sedgwick, 1984; *L. panching* Platnick & Sedgwick, 1984; *L. rufipes* Schwendinger, 1995; *L. tempurung* Platnick, 1997; *L. tioman* Platnick & Sedgwick, 1984; and *L. yangae* Platnick & Sedgwick, 1984. Although many of the described species of *Liphistius* are thought to exhibit relatively restricted distributions, there are some species that can be found to be widely distributed in Peninsular Malaysia. The *L. malayanus,* is widely distributed in the central peninsula and *L. desultor,* can be found in several states in the north peninsula.

Within the Peninsular Malaysia, the entire *Liphistius* fauna had clearly been overlooked and was understudied. Out of six *Liphistius* species that were described from females only, five originated from the peninsula (*L. kanthan, L. tempurung, L. johore, L. endau* and *L. yangae*). In the previous revision study on *Liphistius* made by Platnick and Sedgwick (1984) only two *Liphistius* species were known from Thailand compared to nine species described from Peninsular Malaysia. However, starting from 1988, more studies have been conducted in Thailand yielding to the current total of 32 species (Figure 2.2). These data indicate that the number of study affected the number of species described.

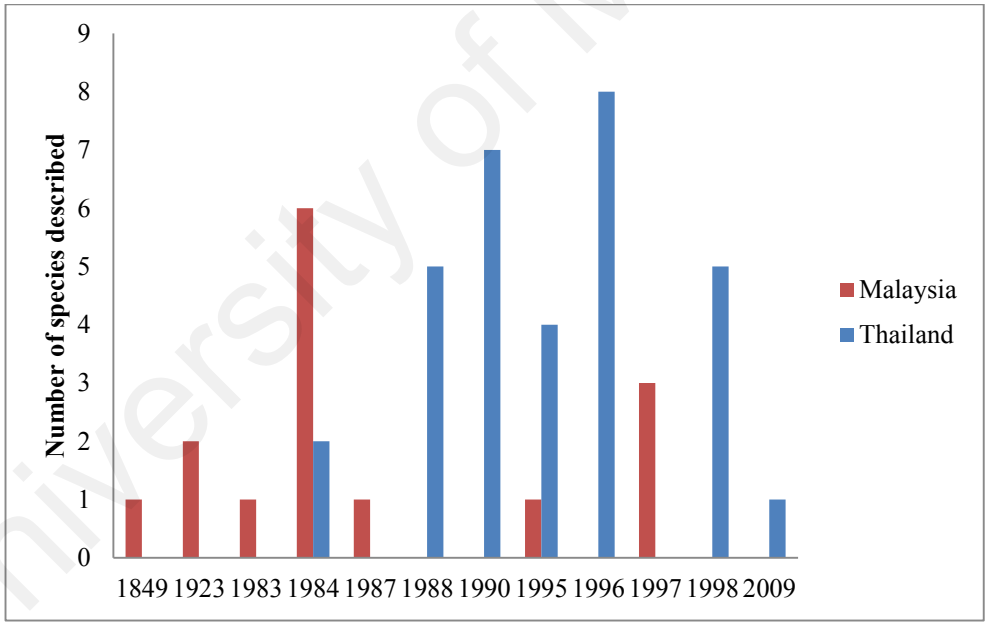

Figure 2.2 Comparisons in number of *Liphistius* spp. described from Thailand and P. Malaysia in the last 160 years

Owing to their primitive features and extremely high endemicity, this group of spiders has attracted a number of interested parties. The spiders were not only hunted by exotic pet collectors, but they were also being 'guarded' by conservationist in Malaysia. In 'guarding' the spiders, conservationists in some cases, provided inaccurate information

to the public. For example, in a news item from The Star in 2005 (see Appendix 2), *Liphistius* spiders were erroneously branded as tarantula, and they claim that *L. malayanus* can only be found in Fraser's Hill. Based on the current literatures, we can say that this claim has been in fact, exaggerated. Even when all *Liphistius* spider in Peninsular Malaysia are listed and protected under the law; there are insufficient basic studies for all the species. None of the species has been investigated thoroughly in terms of their habitat, distribution and their species status.

2.5 Taxonomy and systematics of *Liphistius*

The trap door spider genus *Liphistius* belongs to the suborder Mesothelae which comprises of a single family, Liphistiidae and can only be found in Southeast Asia. The suborder Mesothelae gets its name from the median position of the spinnerets on the venter of the abdomen. It is believed that the Mesothelae are the most evolutionary primitive spider (Ubick et al., 2015). The family Liphistiidae is divided into two subfamilies, Liphistiinae (*Liphistius*) and Heptathelinae (*Ganthela, Heptathela, Qiongthela, Ryuthela, Songthela, Vinathela*) (Schwendinger & Ono, 2011; Xu et al., 2015b).

In contrast to the members of the subfamily Heptathelinae, Liphistiinae burrows are closed with a trapdoor; with signal lines radiating from the entrance. According to Platnick and Sedgwick (1984), *Liphistius* spiders can be identified using the following characters: 1) the male palp retains a tibial apophysis (Figure 2.3 [A]); 2) the female genitals have a poreplate and an unpaired receptacular cluster (Figure 2.3 [B-C]) and 3) the presence of clavate trichobothria on the tarsi and metatarsi of all legs and on the palpal tarsi.

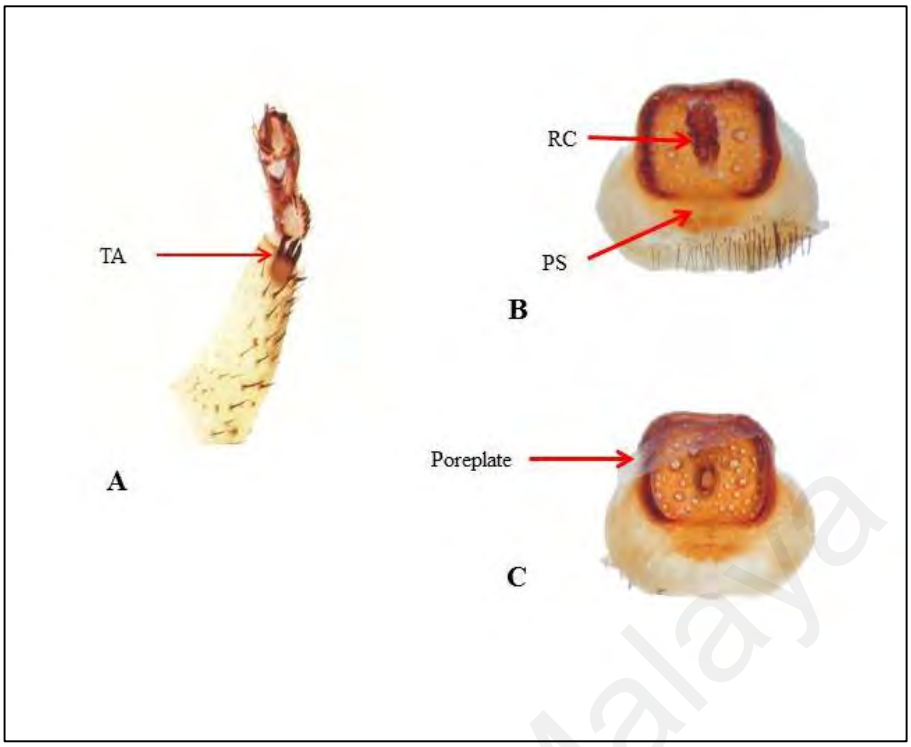

Figure 2.3[A] *Liphistius* spp. male palp, lateral view, [B] *Liphistius* female internal genitalia, ventral view, [C] *Liphistius* female internal genitalia, dorsal view. TA; tibial apophysis, RC; receptacular cluster, PS; posterior stalk.

Platnick and Sedgwick (1984) proposed two species group by using the morphology of the internal female genitalia to distinguish between the two. The first group consists of *L. birmanicus, L. lordae, L. bristowei, L. trang, L. yangae, L. langkawi, L. murphyorum, L. desultor* and *L. sumatranus,* has the ventral receptacular cluster to be narrow and confined to the central portion of the poreplate. In the second group, containing *L. malayanus, L. batuensis, L. panching, L. tioman* and *L. johore,* the ventral receptacular cluster is wide, occupying a substantial portion of the width of the poreplate. However, owing to the possibility that the condition is just a modified form of the other, the monophyly of the species groups is not yet established. Female genitalia however, are fairly variable in most species examined, and are therefore of only limited value for identification (Schwendinger, 1990; Schwendinger, 1995b). Haupt (2003) also mentioned that the continuous molting of adult females make the use of the receptacular cluster for identification to be problematic.

Schwendinger (1990) suggested that there are three distinct groups within the *Liphistius* in Thailand and Myanmar; 1) the *bristowei*-group; 2) the *birmanicus*-group and 3) the *trang*-group. Three *Liphistius* species from Peninsular Malaysia, *L. langkawi, L. murphyorum* and *L. desultor* are claimed to be closely related to the *trang*-group. Schwendinger (1995b) proposed that the use of for example, the scale-like plate on the ventral embolus edge of the male palp is more useful to distinguish species within the *trang*-group. Two species from Peninsular Malaysia, *L. laruticus* and *L. kanthan* are said to belong to the *trang*-group of species from Thailand and northern Malaysia, which feature a lateral thickening on the ventral surface of the poreplate (Platnick et al., 1997). Whereas *L. tempurung* appears to be closer to central and southern Malaysian species (Platnick et al., 1997).

From the species group classification, we can assume that the relationships among the species in *Liphistius* are ambiguous, but recent studies conducted by Xu et al. (2015c) shows that *Liphistius* is a monophyletic taxa. However, the number of *Liphistius* species used in the study is limited, thus may not represent the genus *Liphistius* as a whole.

2.6 Distribution boundaries of *Liphistius* spp.

Many members of the spider infraorder Aranaemorphae are able to disperse great distances by aerial ballooning (Greenstone et al., 1987), but in the most primitive spiders, Mesothelae, ballooning is absent because of their lifestyle and the lack of convection and wind speed gradient in their natural habitat (Bell et al., 2005). Due to their poor dispersal abilities, many species of the family Liphistiidae are strongly endemic and range restricted (Xu et al., 2015c). Other than liphistiids spiders, mygalomorphs also rarely balloon, which make their powers of dispersal to be limited to walking. It is understood that the mygalomorph juveniles do not walk far, resulting clumping populations (Ubick et al., 2005). It is also known that, species with small distribution is most likely to be

threatened by habitat loss (Harvey, 2002). Nowadays, much of these spider habitats including for the liphistiids have been changed through land clearing.

Another important limiting factor for the distribution of *Liphistius* spp. is moisture (Haupt, 2003). According to Schwendinger (1993) it was shown that many *Liphistius* species were remarkably ill-adapted to desiccations, especially species from higher elevations as compared to those from lower altitudes (Schwendinger, 1993). Therefore they are vulnerable to disturbance and it is very important that preventive measures be implemented so to avoid extinction.

2.7 Importance of molecular systematics to *Liphistius* study

Since the 18th century, Linnaeus taxonomy has been widely accepted to be used for biological taxonomy in describing species and categorizing organism into taxa based on hierarchal classification system. Nevertheless, Linnaeus taxonomy is only based on the structural similarities of the different organisms. Over time, the understanding of the relationship between organisms has changed, with the widespread acceptance of evolution theory (Darwin, 1859). Since then, scientists have proposed that taxonomic classification to reflect evolutionary relationship. This systematics sub-discipline is known as phylogenetic (the study of evolutionary relationships among organism). Nowadays, phylogenetic analysis are based on cladistics argumentation (Agnarsson et al., 2013). Previously, morphological characters have been widely used to derive phylogenies. With the advance of DNA sequencing, the use of molecular techniques have been commonly accepted.

Molecular systematics is the use of molecular genetics to study the evolution of relationship among organisms. The technique provides researchers with the capability to evaluate organisms non-invasively (an important element when dealing with endangered species) and offer independent and relatively objective assessment of phylogeny needed

to infer the phylogeny (Boon et al., 2001). Mitochondrial DNA (mtDNA) analysis is a powerful tool for evolutionary studies and can provide insights into the population's structure, gene flow, hybridization, biogeography and phylogenetic relationship (Moritz et al., 1987). Molecular systematics is an essentially cladistic approach; it assumes that classification must correspond to phylogenetic descent, and that all valid taxa must be monophyletic.

To date, there are only a few molecular phylogeny researches on the family Liphistiidae (Tanikawa, 2013; Xu et al., 2015c). Much worse, the phylogenetic relationship and monophyly of species within the genus *Liphistius* has not been thoroughly tested, and no published molecular study has yet been undertaken to include all known *Liphistius* species. Phylogenetic studies using molecular data (Xu et al., 2015c) suggested that the genus *Liphistius* is monophyletic; however this assumption was made based on limited samples. Attempts to describe and classify species in the genus have been primarily made on the basis of morphological characters (Platnick et al., 1997; Platnick & Sedgwick, 1984; Schwendinger, 1987; Schwendinger, 2009, 2013; Schwendinger, 1995b, 1996, 1998; Schwendinger & Ono, 2011; Sedgwick & Platnick, 1987; Sedgwick & Schwendinger, 1990) with no published morphological cladistic analysis. Therefore, immediate research is greatly needed at this crucial moment.

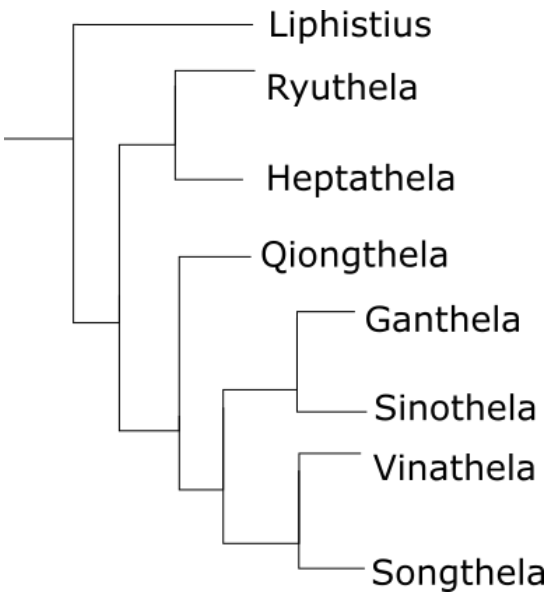

Figure 2.4 A simplified genus level phylogeny adapted from Xu et al. (2015c)

The ability to identify species is extremely important in the field of biology. It has been mentioned earlier that all existing classification schemes for Liphistiidae were not dominated by sound phylogenetic analysis (Xu et al., 2015b). Relying only on the morphological characters may cause problems, as it may result in misidentification of cryptic species. Moreover, morphological characters for spiders are only apparent at adult stage. The development of molecular analysis in the study of the group Liphistiidae can help to alleviate some of these problems.

2.7.1 DNA barcoding method

DNA barcoding (Hebert et al., 2003) was proposed as a taxonomic method that uses short DNA marker to aid species discovery and identification. This method differs from molecular phylogeny where its main objective is not to determine the evolutionary relationship but to identify an unknown sample (Hebert & Gregory, 2005; Schindel & Miller, 2005). The DNA barcoding method employ a 'barcoding gap' that delimit

candidate species based on non-overlapping values of intraspecific and interspecific genetic distances (Hendrixson et al., 2013)

The most commonly used barcode region for animals is a short segment of approximately 600 base pairs of mitochondrial gene *COI.* The *COI* gene is a good target because of its presence in all animals (Barrett & Hebert, 2005) and performed enough sequence divergence to regularly allow differentiation between closely related species (Hebert, 2003).

Although this method has received criticism among conventional systematists, the DNA barcode has shown to be useful in separating and identifying species from across the range of spider species (Blagoev et al., 2013; Harvey et al., 2012; Hendrixson et al., 2013; Xu et al., 2015a). It is understood that efforts to identify *Liphistius* through morphology are problematic due to the rarity of adult's specimens and also due to unavailability of the taxonomic key. Therefore this method would hopefully be able to solve the problems in the *Liphistius* spider research.

CHAPTER 3: SYSTEMATICS STUDY OF THE GENUS *LIPHISTIUS* SCHIÖDTE, 1849 IN PENINSULAR MALAYSIA WITH NOTE ON THEIR GEOGRAPHICAL DISTRIBUTION

3.1 Abstract

Trap door spiders *Liphistius* spp. are the only representatives of the mesothelids spider in South East Asia which are protected in Peninsular Malaysia. In this study, the *Liphistius* spider's current distributions were studied by visiting the recorded locations and also potential habitat throughout Peninsular Malaysia. Sampling was conducted during the daytime with samples manually collected from their burrow. From the total numbers of 56 adults collected, nine species have been identified based on the genital structure of male and female adults. The species were *L. malayanus, L. desultor, L. murphyorum, L. endau, L. langkawi, L. kanthan, L. tempurung, L. batuensis* and *L. laruticus.* There were ten morpho-species which were divided base on their population sites and could not be identified to species level. All species identified were described, with information on type data, material examined, distribution range and the morphological descriptions. A key to adults *Liphistius* was proposed based on the known species collected. Images of *Liphistius* adults, genital structures and distribution maps were also provided. In this study, the knowledge on the geographical distribution of *Liphistius* spp. in Peninsular Malaysia has expanded. Several new populations have been found in Terengganu, where this spider has never been reported before (with possibilities of the spider being new species). This study shows that the genus is widely distributed in Peninsular Malaysia with many more potential localities.

3.2 Introduction

The Liphistiidae are limitedly distributed family which is only found in few regions in Southeast Asia. They are known for their pivotal position in the phylogeny of spiders (Platnick & Gertsch, 1976), however, very little is known about the ecology, diversity, and phylogenetic relationships of these spiders. The genus *Liphistius* was established by Schiödte in 1849 based on female specimen collected from Pulau Pinang, Malaysia. The type specimen of this genus was described as *Liphistius desultor*, in the belief that it was so primitive that it even lacked of spinnerets (Haupt, 2003). Trap door spider of the genus *Liphistius* are characterised by clavate trichobothria, a retrolateral apophysis on the male palpal tibia, and internal female genitalia consisting of a ventral receptacular cluster and dorsal poreplate (Platnick & Sedgwick, 1984).

It can be said, from the literature review presented in the previous chapter, that morphological examinations conducted for species *Liphistius* is lacking clarity. A recent study conducted by Xu et al. (2015b) on Liphistiidae group agrees on this, when it stated that the classification of the Liphistiidae group is subjected to a few selected characters and judgments rather than solely on phylogenetic analysis. According to Wiens (2001), many aspects of morphological character analysis are controversial, including the way in which characters are constructed. He also mentioned that practitioners of morphological phylogenetic tend not to be open about their methodology, specifically on how morphological characters are selected. This obstacle has led to a serious problem for the next generation of scientists, when they want to further study *Liphistius*.

In the next chapter, molecular data were used to test the monophyly of the *Liphistius* species collected in this study. Based on the analysis in the following chapter, and on morphological characters provided here, this chapter described *Liphistius* species that are found in Peninsular Malaysia.

Some of the *Liphistius* described were collected from caves, and many of them were taken from road or path cut in the forest, and also from less accessible hills and mountains throughout the region (Platnick & Sedgwick, 1984). What makes this group so interesting is the enormously small distribution ranges that many species reveal, mainly from the species that were collected from the caves. Many species were only known from a single locality. However, based on recorded data, there is at least two common species which are widely distributed in Peninsular Malaysia; *L. malayanus* and *L. desultor*. Both species can be found in highland and lowland forest (*L. desultor* was recorded from higher elevation of Bendera Hill in Pulau Pinang). *L. malayanus* was also found in Genting Highland and Fraser Hill.

Fourteen species and one sub-species are recognized to be originated from Peninsular Malaysia, in which all of the species has a very limited geographical distribution. The species described were; *L. batuensis* (Abraham, 1923b), *L. desultor* (Schiödte, 1849b), *L. endau* (Sedgwick & Platnick, 1987), *L. johore* (Platnick & Sedgwick, 1984), *L. kanthan* (Platnick et al., 1997), *L. langkawi* (Platnick & Sedgwick, 1984), *L. laruticus* (Platnick et al., 1997), *L. malayanus* (Abraham, 1923a), *L. malayanus cameroni* (Haupt, 1983), *L. murphyorum* (Platnick & Sedgwick 1984), *L. panching* (Platnick & Sedgwick,1984), *L. rufipes* (Schwendinger, 1995b), *L. tempurung* (Platnick, Schwendinger & Steiner, 1997), *L. tioman* (Platnick & Sedgwick, 1984) and *L. yangae* (Platnick & Sedgwick, 1984).

Distribution data are important to the setting of conservation priorities. Published data on the distribution of *Liphistius* are scarce and incomplete; this is mostly true for the species that occur in Peninsular Malaysia. Therefore, the objectives for this chapter are:

1. To provide the morphological descriptions and a dichotomic key for the known *Liphistius* species from Peninsular Malaysia.

2. To investigate and record the distribution of the known *Liphistius* species from Peninsular Malaysia.

3.3 Materials and Methods

Specimens were collected from their type locality that was identified from literature material (Platnick et al., 1997; Platnick & Sedgwick, 1984; Schwendinger, 1995a, 2009). The selected forest throughout Peninsular Malaysia was surveyed for *Liphistius* nest. A total of 33 localities were used for mapping purpose. A total of 26 locations have been identified from the literatures (Table 3.1) while other places were based on verbal information gathered from volunteers of Malaysian Nature Society, other lab members and also on the accessibility of the sites. Fieldworks were undertaken from 27th February 2010 to 15th April 2012 to collect *Liphistius* specimens and to investigate their distribution for molecular and morphological studies. The general habitat type and the locations coordinate and altitude were also recorded. The spider specimens were collected directly from their burrow during daytime using forceps and a small sweep net.

Specimens were examined using a Nikon stereomicroscope. Digital images were taken with a Nikon digital camera attached to the microscope eyepiece. Descriptions were generated with the aid of the available taxonomic references and modified where appropriate. Both females and males (if available) were described in this study. The voucher specimens are preserved in 70% ethanol and label were added to the vials. The voucher specimens will be deposited in Museum of Zoology, University of Malaya for future reference.

Following the method by Schwendinger (2013), female genitalia were removed using a sharp needle. Tissue was cleaned away using fine needles and the genitalia were then placed in 10% KOH solution for few minutes to clean away remaining tissue. Specimens were then transferred to distilled water and then to 70% ethanol for examination under a dissecting microscope.

Table 3.1 *Liphistius* species described locations and cited references.

No	Species	Location no	Locality	Reference
1	*L. langkawi*	1	a cave 5 km. east of Pekan Kuah, Langkawi	Platnick & Sedgwick, 1984
		2	Gua Landak	Schwendinger, 2009
		3	road side near Pantai Beringin	Schwendinger, 2009
		4	Air Terjun Temurun	Schwendinger, 2009
2	*L. murphyorum*	5	Bukit Bendera	Platnick & Sedgwick, 1984
3	*L. desultor*	6	Mount Jerai	Platnick & Sedgwick, 1984
		7	Penang Hills	Platnick & Sedgwick, 1984
		8	Teluk Bahang	Platnick & Sedgwick, 1984
		9	Maxwell Hill	Platnick & Sedgwick, 1984
4	*L. malayanus*	10	Gunung Angsi	Platnick & Sedgwick, 1984
		11	Cameron Highland	Platnick & Sedgwick, 1984
		12	Fraser Hill	Platnick & Sedgwick, 1984
		13	Kepong	Platnick & Sedgwick, 1984
		14	Klang Gate Reservoir	Platnick & Sedgwick, 1984

Table 3.1 Continued.

No	Species	Location no	Locality	Reference
5	*L. malayanus cameroni*	15	Mount Brinchang	Haupt, 1983
6	*L. batuensis*	16	Batu Caves	Platnick & Sedgwick, 1984
		17	Templar Park Cave	Platnick & Sedgwick, 1984
7	*L. panching*	18	Gua Panching	Platnick & Sedgwick, 1984
8	*L. tioman*	19	Gua Sinah and Gua Panah	Platnick & Sedgwick, 1984
9	*L. johore*	20	Sungai Rengit	Platnick & Sedgwick, 1984
10	*L. tempurung*	21	Gua Tempurung	Platnick *et al*, 1997
11	*L. kanthan*	22	Gua Kanthan	Platnick *et al*, 1997
12	*L. laruticus*	23	Bukit Larut	Platnick *et al*, 1997
13	*L. yangae*	24	Kaki Bukit	Platnick & Sedgwick, 1984
14	*L. endau*	25	Sungai Jasin	Sedgwick & Platnick, 1987
15	*L. rufipes*	26	Bukit Baring	Schwendinger, 1995

3.4 Results

3.4.1 Geographical distribution

A total of 33 population sites from nine states in Peninsular Malaysia were investigated in this study. This study covered all the habitat types known for *Liphistius* spider to exist including forest, cave and island habitats. Details of each collection site, such as coordinate, altitude and habitat are shown in Table 3.2. New population records were identified based on previous collection details.

Interestingly, new population records from Terengganu have been found in this study. They were at Pasir Raja Forest Reserve, Kenyir Lake, Lata Tembakah Amenity Forest and Lata Belatan Amenity Forest. Other new population records were also observed from Perak. *Liphistius* spiders were collected from Temenggor Forest Reserve, Ulu Kinta Amenity Forest and Lata Kekabu Amenity Forest. Locations of *Liphistius* from Selangor was not considered as new record as the species collected were *L. malayanus* and it is understood from the literature that the species distribution were from the central of Peninsular Malaysia.

Meanwhile, there were three locations identified from the references material that did not yield any specimens (Figure 3.2). The locations were Tioman Island and Panching Cave in Pahang and Pengerang in Johor. An empty nest was successfully spotted in Tioman Island, however, a three days search failed to discover any occupied nest. Two attempts were made to survey the *Liphistius* spp. existence in Panching Cave and Pengerang however failed. The species are believed to have moved into the deeper side of the cave due to human disturbance from visitors of a Hindu Shrine at the cave entrance.

Rapid development in Pengerang has caused the forest to be cut down. There was an only small patch of forest left, which has been reserved for military activities.

A total of 144 individuals were collected in this study. Of the total, 56 specimens were adults while others were juvenile and sub adult specimens (Figure 3.1). They were only seven male individuals collected in this study, which were collected from Cameron Highland, Genting Highland, Kenyir Lake and Lata Tembakah Amenity Forest (Table 3.2). Nine *Liphistius* species with ten other morpho species were able to be identified from this study.

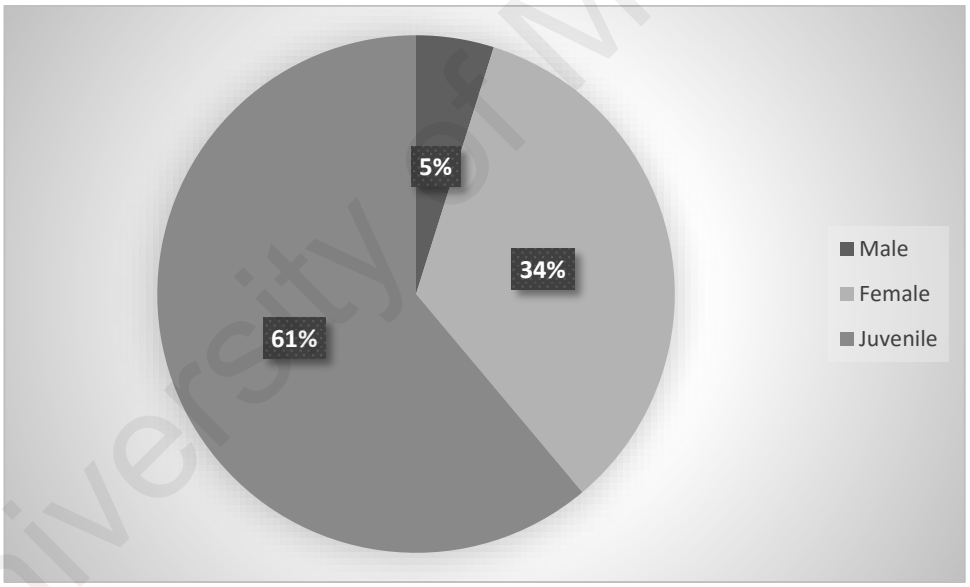

Figure 3.1 Pie chart showing the percentage comparison of male and female adults and juveniles collected.

Table 3.2 List of species collected in this study with relevant information: locality, sex, coordinates, altitude and the species habitat

Species	Locality	Sex	Coordinates	Altitude (m)	Habitat
L. batuensis	Anak Takun Cave	1♀	N03°17.847' E101°38.087'	81	Cave
L. desultor	Penang Botanic Garden	2♀	N05°26.445' E100°17.390'	31	Forest
	Telok Bahang Amenity Forest	1♀ 1j	N05°26.682' E100° 13.219'	53	Forest
	Mount Jerai	1♀ 7j	N05°48.715' E100° 26.342'	433	Forest
	Maxwell Hill	1♀ 8j	N04°51.701' E100°47.998'	105	Forest
L. endau	Endau Rompin National Park	3♀ 7j	N02°31.651' E103°23.876'	4	Forest
	Kota Tinggi Waterfall	2♀	N01°49.000' E103°49.000'	36	Forest
L. kanthan	Kanthan Cave	6♀	N04°45.685' E101°07.322'	87	Cave
L. laruticus	Maxwell Hill	3♀ 8j	N04°51.701' E100°47.998'	1195	Forest
L. langkawi	Mount Raya	1♀ 1j	N06°23.189' E099°48.495'	807	Forest
	Bukit Putih Cave	3♀ 2j	N06°20.292' E099°52.460'	45	Cave
	Porcupine Cave	3j	N06°18.244' E099° 51.519'	45	Cave
L. malayanus	Ulu Gombak Forest Reserve	3♀ 2j	N03°19.487' E101°45.215'	300	Forest
	Kemensah Waterfall	5j	N03°12.736' E101°46.086'	78	Forest
	Ulu Bendul Amenity Forest	2♀ 2j	N02°43.636' E10 °04.519'	122	Forest
	Fraser Hill	2♀ 3j	N03°43.041' E101°44.255'	1268	Forest
	Genting Highland	2♀ 1♂ 1j	N03°25.904' E101°47.087'	1716	Forest
	Mount Tahan	1♀	N04°38.907' E102°11.509'	128	Forest
	Taman Rimba Ampang	2♀ 1j	N03° 9.054' E101°47.761'	137	Forest
L. murphyorum	Telok Bahang Amenity Forest	2♀ 1♂	N05°26.682' E100° 13.219'	53	Forest
L. tempurung	Tempurung Cave	2♀	N04°24.986' E101°11.212'	54	Cave

Table 3.2 Continued

Species	Locality	Sex	Coordinates	Altitude (m)	Habitat
L. sp. 1	Pasir Raja Forest Reserve	2♀ 3j	N04°35.516' E102°56.726'	310	Forest
	Kenyir Lake	1♀ 2♂	N04°57.907' E102° 50.465'	226	Forest
	Felda Tekam	6j	N03° 58.63' E102°43.676'	260	Forest
L. sp. 2	Parit Waterfall	2♂ 3j	N04°28.463' E101°23.047'	1464	Forest
L. sp. 3	Lata Tembakah Amenity Forest	2♀ 1♂ 9j	N05°35.189' E102°26.968'	42	Forest
L. sp. 4	Temenggor Forest Reserve	3♀ 2j	N05°33.530' E101°36.701'	846	Forest
L. sp. 5	Ulu Kinta Amenity Forest	2♀ 4j	N04° 40.098' E101°11.668'	136	Forest
L. sp. 6	Perlis State Park	2♀ 2j	N06°42.074' E100°12.059'	141	Forest
L. sp. 7	Ganesh Cave	3j	N03°14.224' E101°41.042'	60	Cave
L. sp. 8	Mount Brinchang	2j	N04°31.497' E101°23.345'	1804	Forest
L. sp. 9	Lata Belatan Amenity Forest	1j	N05° 38.347' E102°35.278'	37	Forest
L. sp. 10	Lata Kekabu Amenity Forest	2j	N05° 2.677' E100°56.962'	86	Forest

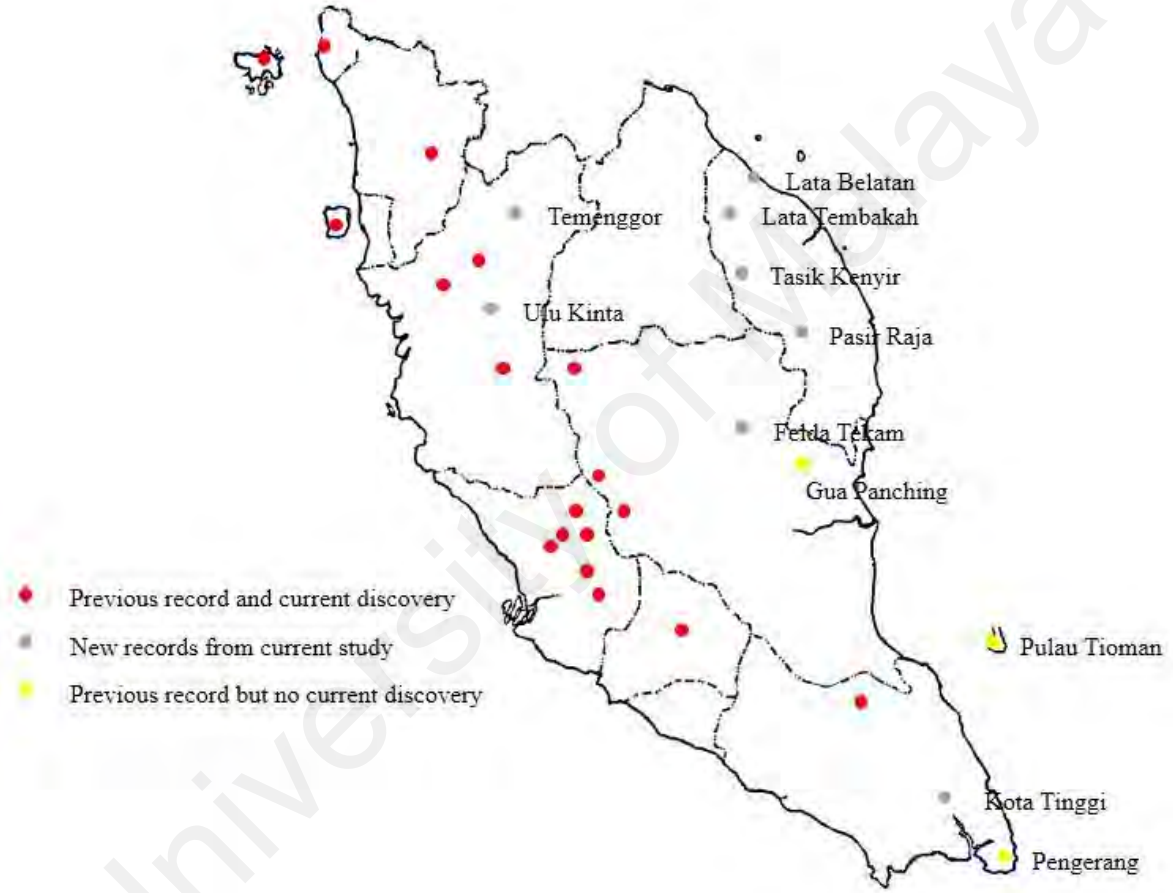

Figure 3.2 Distribution map for *Liphistius* spp. from previous record and current study.

3.4.2 Systematics

Genus *Liphistius* Schiödte, 1849

Figure 3.3

Type species: *Liphistius desultor* Schiödte, 1849

Diagnosis. *Liphistius* spiders construct signal lines radiating from the burrow entrance, the male palp possesses a tibial apophysis, and the female genitals have a poreplate and unpaired receptacular clusters. The spiders are also characterised by the presence of clavate trichobothria on the tarsi and metatarsi of all legs and the palpal tarsi

Description. Total length (excluding chelicerae) = 9-37 mm (Platnick & Sedgwick, 1984); male with retrolateral tibial apophysis; female genitalia with a poreplate and unpaired receptacular clusters.

Distribution. Indonesia (Sumatra), Laos, Malaysia, Myanmar, Thailand

Liphistius batuensis

Figure 3.4

Types. Males and females syntypes from Batu Caves, Selangor, in BMNH, not examined

Diagnosis. Female of this species can be recognized by the short, wide, medially narrowed poreplate (Platnick & Sedgwick, 1984).

Description. FEMALE (LS007). Carapace pale yellow; sternum dark pale yellow; chelicerae dark brown; legs pale yellow; abdomen pale yellow; 8 spinnerets. Measurements: BL 13.0, CL 5.50, CW 4.50; SL 3.0, SW 2.5; AL 6.5, AW 4.5; Leg I 14.0 (4.5+2.0+3.0+3.0+1.5), Leg II 15.0 (5.0+2.0+3.0+3.0+2.0), Leg III 16.0 (5.0+2.0+3.0+4.0+2.0), Leg IV 21.5 (6.0+2.0+5.0+6.0+2.5). Internal genitalia with broad posterior stalk indistinctly fused to short, wide, medially constricted poreplate, and wide median receptacular cluster protruding far anterior of poreplate (Figure 3.4 [C-D]).

Material examined. Anak Takun Cave, Selangor: 1 female [N03°17.847' E101°38.087'], 81m, manual collection, 20 June 2010 (Nurul Syuhadah & Rosli, H, LS007).

Notes. Only one sample was collected in this study and the internal genitalia were unclear when cleaned with KOH. The species was assumed to be *L. batuensis* due to the specimen locality.

Distribution: Known only from Batu Caves and Templar Park caves in Selangor (Figure 3.23).

Liphistius desultor

Figure 3.5

Types. Female holotype from Penang Hills, Penang Island, no date, collected by van Teylingen, in ZMC, not examined.

Diagnosis. It is a large species with bicolored legs. Females can be distinguished by the squared poreplate (Platnick & Sedgwick, 1984).

Description. FEMALE (LS034). Carapace orange; sternum brown with black margin; chelicerae dark brown; legs with trochanter and femora orange, more distal segments dark brown; abdomen light brown with dark brown tergites; 8 spinnerets. Measurements: BL 25.0, CL 10.0, CW 11.0; SL 6.5, SW 2.5; AL 14.0, AW 10.0; Leg I 33.0 (11.0+6.0+7.0+6.0+3.0), Leg II 34.0 (11.0+6.0+7.0+7.0+3.0), Leg III 35.0 (10.0+6.0+8.0+8.0+3.0), Leg IV 48.0 (14.0+7.0+10.0+13.0+4.0). Internal genitalia with broad posterior stalk, without anterior lobes but with thickened anterior margin on poreplate, and narrow median receptacular cluster (Figure 3.5 [C-D]).

Material examined. Penang Botanical Garden, Pulau Pinang: 2 females [N05°26.445' E100°17.390'], 31m, manual collection, 11 November 2010 (Nurul Syuhadah & Sharaani, LS021, LS034); Mount Jerai, Kedah: 1 female [N05°48.715' E100° 26.342'], 433-684m, manual collection, 9 February 2012 (Nurul Syuhadah & Sharaani, LS118), 7 juveniles (LS117, LS119, LS120, LS121, LS122, LS123, LS124); Maxwell Hill, Perak: 1 female [N04°51.701' E100°47.998'], 105m, manual collection, 10 February 2012 (Nurul Syuhadah & Sharaani, LS130).

Distribution. Known only from Penang Island and nearby mainland localities in Northern Malaysia (Figure 3.22).

Liphistius endau

Figure 3.6

Type. Female holotype from the banks of Sungai Jasin, Ulu Endau, Johor (10 Nov 1985), deposited in AMNH. (Not examined).

Diagnosis. Relatively longer length of the opening to the receptacular clusters as well as by the presence of three posterior stalks of receptacular on ventral surface of the poreplate (Sedgwick & Platnick, 1987).

Description. FEMALE (LS087). Carapace dark brown; sternum dark brown with black margin; chelicerae dark brown; legs dark brown; abdomen light brown with dark brown tergites; 8 spinnerets. Measurements: BL 29.0, CL 14.0, CW 13.0; SL 9.0, SW 2.5; AL 15.0, AW 10.0; Leg I 15.0 (5.0+2.0+3.0+3.0+2.0), Leg II 19.0 (6.0+3.0+4.0+4.0+2.0), Leg III 18.5 (6.0+2.0+4.0+4.0+2.5), Leg IV 25.0 (7.0+3.0+5.0+6.0+4.0). Internal genitalia with larger poreplate bearing long, divided opening to receptacular clusters, receptacula clustered on three stalks (one anteromedian, two post laterals; Figure 3.6 [C-D]).

Material examined. Endau Rompin (Peta) National Park, Johor: 3 females [N02°31.651' E103°23.876'], 46m, manual collection, 4 October 2011 (Nurul Syuhadah, LS087, LS088, LS094), 7 juveniles (LS089, LS090, LS091, LS092, LS093, LS095, LS096); Endau Rompin (Selai) National Park- manual collection (Rasul, LS148); Kota Tinggi Waterfall, Johor: 2 females [N01°49.000' E103°49.000'], 36m, manual collection, 21 February 2012 (Nurul Syuhadah & Sharaani, LS140, LS141).

Distribution: Known only from Johor (Figure 3.21)

Liphistius kanthan

Figure 3.7

Types. Female holotype from Kanthan Cave (Sept. 1996; H. Steiner), deposited in AMNH, not examined.

Diagnosis: The female having lateral thickenings on the ventral surface of the poreplate with the poreplate being wider anteriorly than posteriorly. The female is having numerous receptacular on the median invagination (Platnick et al., 1997).

Description. FEMALE (LS023). Carapace pale yellow; sternum light yellow; chelicerae yellow-dark brown; chelicerae light yellow; abdomen light brown, legs light yellow; 8 spinnerets. Measurements: BL 11.0, CL 4.2, CW 4.0; SL 2.0, SW 1.5; AL 6.0, AW 4.5; Leg I 10.0 (3.5+1.5+2.0+2.0+1.0), Leg II 12.0 (4.0+2.0+2.5+2.5+1.0), Leg III 12.5 (3.5+2.0+2.5+3.0+1.5), Leg IV 17.0 (5.0+2.0+3.0+4.5+2.5). Internal genitalia with posteriorly narrowed posterior stalk; poreplate with medially invaginated anterior margin, receptacular cluster narrow (Figure 3.7 [C-D]).

Material examined. Kantan Cave, Perak: 6 females [N04°45.685' E101°07.322'], 87m, manual collection, 27 November 2010 (Nurul Syuhadah, LS023, LS024, LS026, LS027, LS032, LS033).

Distribution: Known only from Kanthan Cave, Perak (Figure 3.23).

Liphistius laruticus

Figure 3.8

Type. Male holotype (collected Jan 20, 1994) and female allotype (collected Jan 20, 1994) from an elevation of 1380m on Maxwell Hill, Perak, deposited in MHNG (not examined). Paratypes from the same locality collected and reared by P. Schwendinger: from elevation 1380m, 1 male (collected Jan 20, 1994), and from elevation 1150m, 1 female (collected Feb 5, 1991), deposited in AMNH. (Not examined).

Diagnosis: Female, small size compared to *L. desultor,* with dark-colored and having annulated legs. The female poreplate is cross-oval in shape (Platnick et al., 1997).

Description. FEMALE (LS128). Carapace brown; sternum light yellow; chelicerae dark brown; chelicerae light yellow; abdomen light brown, legs light yellow; 8 spinnerets. Measurements: BL 11.5, CL 5.5, CW 4.0; SL 2.0, SW 1.5; AL 6.5, AW 5.0; Leg I 10.0 (3.5+1.5+2.0+2.0+1.0), Leg II 12.0 (4.0+2.0+2.5+2.5+1.0), Leg III 12.5 (3.5+2.0+2.5+3.0+1.5), Leg IV 17.0 (5.0+2.0+3.0+4.5+2.5). Internal genitalia with cross-oval poreplate; ventral pores and racemose receptacular cluster distinctly elevated (Figure 3.8 [C-D]).

Material examined. Maxwell Hill, Perak: 3 females [N04°51.701' E100°47.998'], 1195-1389m, manual collection, 10 February 2012 (Nurul Syuhadah & Sharaani, LS125, LS128, LS136), 8 juveniles, manual collection (LS126, LS127, LS129, LS134, LS135, LS137, LS138, LS139).

Distribution: Known only from high altitudes of Maxwell Hill, Perak (Figure 3.22).

Liphistius langkawi

Figure 3.9

Types. Male holotype and female paratype from a cave 5 km, east of Pekan Kuah, Langkawi Island (June 9, 1981), deposited in AMNH (not examined).

Diagnosis. Female can be distinguished by the presence of three anterior and two posterior lobes on the poreplate (Platnick & Sedgwick, 1984).

Description. FEMALE (LS039). Carapace pale yellow; sternum yellow; chelicerae pale yellow; abdomen light gray; legs light yellow; 8 spinnerets. Measurements: BL 11.5, CL 6.0, CW 5.0; SL 2.5, SW 2.0; AL 6.0, AW 4.5; Leg I 10.0 (3.5+1.5+2.0+2.0+1.0), Leg II 12.0 (4.0+2.0+2.5+2.5+1.0), Leg III 12.5 (3.5+2.0+2.5+3.0+1.5), Leg IV 17.0 (5.0+2.0+3.0+4.5+2.5). Internal genitalia with broad posterior stalk fused to laterally expanded sidepieces, three anterior and two posterior lobes on poreplate, and narrow median receptacular cluster (Figure 3.9 [C-D]).

Material examined. Mount Raya, Kedah: 1 female [N06°23.189' E099°48.495'], 807m, manual collection, 9 January 2011 (Nurul Syuhadah, LS039); 1 juvenile, manual collection (LS043); Putih Hill's Cave, Kedah: 3 female, manual collection (LS040, LS041, LS042); Porcupine Cave, Kedah: 3 juveniles [N06°18.244' E099° 51.519'], 45m, manual collection, 6 January 2011 (Nurul Syuhadah, LS044, LS045, LS046).

Distribution: Known only from Langkawi Island (Figure 3.22).

Liphistius malayanus

Figure 3.10; Figure 3.13

Types. Female holotype from Gunong Angsi, Negeri Sembilan, Dec 1922, elevation 2500 feet, collected by F. Norris, in BMNH, not examined.

Diagnosis. Males can be distinguished by the large embolus and broadly rounded tegular apophysis, females by the anterolateral expansions of the poreplate (Platnick & Sedgwick, 1984).

Description. MALE (LS009). Carapace dark brown; sternum dark brown with black margins; chelicerae dark brown; legs brown; abdomen dark brown; 8 spinnerets.

Measurements: BL 20.0, CL 10.0, CW 10.0; SL 5.0, SW 4.0; AL 1.0, AW 5.0; Leg I 26.0 (7.0+3.0+6.0+7.0+3.0), Leg II 28.5 (7.0+3.5+6.0+8.0+4.0), Leg III 32.5 (7.5+4.0+8.0+9.0+4.0), Leg IV 35.0 (9.0+4.0+7.0+10.0+5.0). Palp as in Figure 3.13 (C). FEMALE (LS010). Carapace light brown; sternum dark brown with black margins; chelicerae dark brown; legs brown; abdomen dark brown; 8 spinnerets. Measurements: BL 25.0, CL 15.0, CW 10.0; SL 8.0, SW 2.0; AL 10.0, AW 6.0; Leg I 24.0 (8.0+4.0+5.0+5.0+2.0), Leg II 24.5 (8.0+4.5+5.0+5.0+2.0), Leg III 25.5 (7.5+4.0+5.0+6.0+3.0), Leg IV 33.5 (10.0+4.5+6.0+9.0+4.0). Internal genitalia with wide posterior stalk, two anterolateral expansions on poreplate, and wide median receptacular cluster Figure 3.10 [C-D]).

Material examined. Ulu Gombak Forest Reserve, Selangor: 3 females [N03°19.487' E101°45.215'], 300m, manual collection, 27 February 2010 (Nurul Syuhadah, LS001), 17 October 2010 (LS018, LS019); Fraser Hill, Pahang: 2 females [N03°43.041' E101°44.255'], 1268m, manual collection, 3 June 2010 (Nurul Syuhadah, LS008, LS012), 3 juveniles (LS015, LS016, LS017); Genting Highland, Pahang: 1 male [N03°25.904' E101°47.087'], 1716m, manual collection, 14 July 2010 (Nurul Syuhadah, LS009), 2 females (LS010, LS011), 1 juvenile, manual collection, 22 November 2011(Nurul Syuhadah, LS097); Ampang Forest Reserve, Selangor: 2 females [N03° 9.054' E101°47.761'], 137m, manual collection, 13 July 2010 (Nurul Syuhadah, LS013, LS014), 1 juvenile (LS104); Ulu Bendul Amenity Forest, Negeri Sembilan: 1 females [N02°43.636' E102°04.519'], 122m, manual collection, 12 July 2010 (Nurul Syuhadah, LS020), 1 female collected on 19 January 2011 (Nurul Syuhadah), 2 juveniles collected on the same day (LS115, LS116); Mount Tahan: 1 female [N04°38.907' E102°11.509'], manual collection, 6 September 2012 (Rasul, LS145).

Distribution. This species is only known from Selangor and Pahang (Figure 3.21).

Liphistius murphyorum

Figure 3.11; Figure 3.14

Type. Male holotype and female paratype from Penang Island, Malaysia, July 20, 1982, collected by W. Sedgwick, deposited in AMNH- not examined

Diagnosis. Males having a ventral sub-tegular apophysis (Figure 3.14); female internal genitalia with the presence of small lobes on the anterior margin of the poreplate (Platnick & Sedgwick, 1984).

Description. FEMALE (LS037). Carapace light brown with dark brown marking; sternum light brown; chelicerae dark brown with some yellowish at the lower part; legs light brownish yellow with brown annulations on femora, tibiae and metatarsi; abdomen light brown; 8 spinnerets. Internal genitalia as in figure 4.x. Measurements: BL 10.0, CL 5.5, CW 5.0; SL 3.0, SW 1.0; AL 4.0, AW 3.5; Leg I 11.0 (3.5+2.5+2.0+2.0+1.0), Leg II 11.5 (3.5+2.0+2.5+2.5+1.0), Leg III 12.5 (3.5+2.0+2.5+3.0+1.5), Leg IV 16.0 (4.0+2.0+3.5+4.0+2.5). Internal gentalia with short, broad posterior stalk, four small lobes on poreplate, and narrow median receptacular cluster (Figure 3.11 [C-D]).

Material examined. Telok Bahang Amenity Forest, Pulau Pinang: 1 male [N05°26.682' E100° 13.219'], 53m, manual collection, 10 November 2010 (Zaidee, LS022), 2 females, manual collection collected on the same data (LS035, LS037).

Distribution: Known only from Pinang Island, Malaysia (Figure 3.22).

Liphistius tempurung

Figure 3.12

Types. Female holotype from Gua Tempurung, Perak (May 18, 1996; H. Steiner), deposited in AMNH (not examined).

Diagnosis: Female of this species having a rectangular poreplate with an extended receptacular cluster. They also have anterolateral corners on the poreplate and large posterior stalk (Platnick et al., 1997).

Description. FEMALE (LS030). Carapace brownish yellow; sternum light yellow; chelicerae light yellow with some brownish at the lower part; legs light brownish yellow with darker rings on femora, tibiae and metatarsi; abdomen light brown; 8 spinnerets. Internal genitalia as in figure 4.x. Measurements: BL 15.0, CL 5.5, CW 5.0; SL 3.5, SW 1.0; AL 6.0, AW 4.0; Leg I 12.0 (4.0+2.0+2.5+2.5+1.0), Leg II 12.0 (4.0+2.0+2.5+2.5+1.0), Leg III 12.5 (3.5+2.0+2.5+3.0+1.5), Leg IV 17.5 (5.0+2.0+3.5+4.5+2.5). Internal genitalia with wide posterior stalk; poreplate with incised anterolateral corners, receptacular cluster large, protruding anterior of anterior margin of poreplate (Figure 3.12 [C-D]).

Material examined. Tempurung Cave, Perak: 2 females [N04°24.986' E101°11.212'], 54m, manual collection, 26 November 2010 (Nurul Syuhadah, LS028, LS030)

Distribution: Known only from Tempurung Cave, Perak (Figure 3.21).

3.4.2.1 Taxonomic key

Based on the nine known species collected in this study, a simple taxonomic key was constructed using the female internal genitalia structure:

1a Receptacular cluster is narrow and confined to the central portion of the poreplate……2

1b Receptacular cluster is wide, occupying a substantial portion of the width of the poreplate……....6

2a Presence of anterior lobes on the poreplate…….....……..3

2b No anterior lobes on the poreplate…….…….…..4

3a With three anterior lobes……..*L. langkawi*

3b With four anterior lobes……..*L. murphyorum*

4a Having lateral thickening on the ventral of the poreplate……...*L. kanthan*

4b Without lateral thickening on the ventral of the poreplate……5

5a Square shape poreplate……..……..……..……*L. desultor*

5b Oval shape poreplate……..……..……*L. laruticus*

6a Triangular posterior stalk…….....……...*L. tempurung*

6b Posterior stalk not triangular……...…….…….……7

7a With two anterolateral expansion on the poreplate……..……..…*L. malayanus*

7b Without anterolateral expansion…….…...……8

8a Receptacular cluster expanded outside the anterior poreplate .....….*L. batuensis*

8b Receptacular cluster within the poreplate…….…….……9

9 Receptacular cluster with three stalks…….....……...*L. endau*

3.4.2.2 Unidentified species

Although the following spiders could not be identified due to identification constraints, they are listed with their localities and with short description of their external and/or internal morphology characters. Some of the species are further discussed in Chapter 4.

***Liphistius* sp. 1**

Figure 3.15 (A-D); Figure 3.16 (A-B); Figure 3.19(C); Figure 3.20

Material examined. Pasir Raja Forest Reserve, Terengganu: 2 females [N04°35.516' E102°56.726'], 310m, manual collection, 18 September 2011 (Rasul, LS076, LS077), 3 juveniles collected on 14 February 2011 (Shaarani, LS049, LS050) ; Kenyir Lake, Terengganu: 2 males and 1 females [N04°57.907' E102° 50.465'], 226m, manual collection, 15 April 2012 (Rasul & Shikin, LS142, LS143, LS144) ; Tekam, Pahang: 6 juveniles [N03° 58.63' E102°43.676'], 260m, manual collection, 22 May 2011 (Rasul & Sharaani, LS059, LS067, LS068, LS069, LS070, LS071).

Diagnosis. The species body colour is black and dark brown, which resembles *L. malayanus* and *L. endau* group. The internal genitalia of female samples from Pasir Raja Forest Reserve resembles *L. endau* with longer length of the opening to the receptacular clusters , but differ with a round shape opening on the upper part (Figure 3.16 [A-B]). The species are also differing from *L. endau* by the presence of two posterior stalks of receptacular on ventral surface of the poreplate. The male palp resembles *L. malayanus* with large embolus but differ in tegular process formation (Figure 3.19 [C]).

The species are identified based on DNA barcoding, and likely represents species on their own (see Chapter 4), which probably a new species and will be further discuss in future.

Liphistius sp. 2

Figure 3.19 (A-B)

Material examined. Paritfall, Cameron Highland, Pahang: 2 males [N04°28.463' E101°23.047'], 1464 m, pitfall trap, 6 February 2010 (Nurul Syuhadah & Marisi, LS002, LS003); 3 juveniles, manually collected on a road bank, 28 April 2011 (Nurul Syuhadah, LS057, LS058, LS060).

Body coloration: Males, black; juveniles, light brown with annulated leg. The male palp resembles *L. malayanus* with large embolus but differ in the tegular process formation.

Liphistius sp. 3

Figure 3.15 (E-F)**;** Figure 3.16 (C-D)

Material examined. Lata Tembakah Amenity Forest, Terengganu: 1 male [N05°35.189' E102°26.968'], 42 m, manual collection, 18 October 2011 (Atikah & Marisi, LS099), 2 females, manual collection, 19 January 2011 (Nurul Syuhadah & Shikin, LS108, LS109), 1 juvenile collected on 4 October 2009 (Rosli H, LS004), 2 juveniles manually collected on a tree bark, 17 February 2010 (Rosli H, LS005, LS006), 6 juveniles manually collected on 19 January 2011 (Nurul Syuhadah & Shikin, LS106, LS107, LS110, LS111, LS112, LS113).

Diagnosis. The male examined are smaller compared to other species in this study. The female coloration resembles species in *L. malayanus* and *L. endau* group with dark brown in carapace colour. The female abdomen colour is light brown with dark brown tergites plate. Female internal genitalia resemble *L. malayanus* with wide median receptacular cluster but differ in narrower posterior stalk.

Liphistius sp. 4

Figure 3.17

Material examined. Temenggor Forest Reserve, Perak: 3 females and 2 juveniles [N05°33.530' E101°36.701'], 846 m, manual collection, 9 May 2011 (Rasul & Sharaani)

Diagnosis. Large species with orange femora in females. The poreplate shape resembles *L. desultor* but differ in short and clumping receptacular cluster (Figure 3.17 [E-F]).

Liphistius sp. 5

Figure 3.18 (E-F)

Material examined. Ulu Kinta Amenity Forest, Perak: 2 females and 4 juveniles [N04° 40.098' E101°11.668'], 136 m, manual collection, 29 September 2011 (Nurul Syuhadah, LS080, LS081, LS082, LS083, LS084, LS085).

Diagnosis. Resembles *L. desultor* in having a square poreplate but differ without heavy sclerotized portion on the ventral part. The species are identified through DNA barcoding (see Chapter 4) and likely represents species of their own.

Liphistius sp. 6

Figure 3.17; Figure 3.18

Material examined. Perlis State Park, Perlis: 1 female: **[**N06°42.074' E100°12.059'], 141 m, manual collection, 3 March 2011 (Rasul & Rosli H, LS055), 1 female from same location collected on 3 June 2011 (Shikin & Rasul , LS073) and 2 juveniles (LS072, LS074).

Diagnosis. Resemble *L. desultor* with narrow median receptacular cluster but differ in having a round shape poreplate (LS055 sample,Figure 3.18[A-B]). However sample of LS073 (Figure 3.18[C-D]) collected from the same location resemble of *L. yangae* in poreplate shape and receptacular cluster formation. More specimens are needed for comparison before we can confirm the species, as they are possibly of variation in poreplate shape.

Liphistius sp. 7

Material examined. Ganesh Cave, Selangor: 3 juveniles **[**N03°14.224' E101°41.042'], 60 m, manual collection, 8 December 2010 (Nurul Syuhadah, LS038, LS048, LS052).

Diagnosis. A small species with dark colour carapace. The species which are collected near to *L. batuensis* locality, however, the dark colour doesn't likely represent a cave species. Therefore they were likely to represents species on their own and should be further studied in future.

Liphistius sp. 8

Material examined. Mount Brinchang, Pahang: 2 juveniles [N04°31.497' E101°23.345'], 1804 m, manual collection, 27 April 2011 (Nurul Syuhadah, LS056, LS061).

Diagnosis. The species resemble *L. malayanus* juveniles with having annulated legs. However, due to the limitation for identification the species are treated as unknown.

Liphistius sp. 9

Material examined. Lata Belatan Amenity Forest, Terengganu: 1 juveniles [N05° 38.347' E102°35.278'], 37 m, manual collection, 18 January 2011 (Nurul Syuhadah & Shikin, LS105).

Diagnosis. The species resemble *L. malayanus* juveniles with having annulated legs. However, due to the limitation for identification the species are treated as unknown.

Liphistius sp. 10

Material examined. Lata Kekabu Amenity Forest, Perak: 2 juveniles [N05° 2.677' E100°56.962'], 86m, manual collection, 27 September 2011 (Nurul Syuhadah & Sharaani, LS078, LS079).

Diagnosis. The species resemble other Northern species color with having orange carapace and annulated legs. However, due to the limitation for identification the species are treated as unknown.

3.5 Discussions

3.5.1 Taxonomic characters and interspecific relationship of *Liphistius* from Peninsular Malaysia

Two characters that are used to describe *Liphistius* species from Peninsular Malaysia are: 1) the structure of the male tibial apophysis and 2) the female internal genitalia (Platnick et al., 1997; Platnick & Sedgwick, 1984; Schwendinger, 1995a). Within Peninsular Malaysia, several relationship can be recognized. Two species, *L. murphyorum* and *L. desultor* were primarily recognized as closely related based on the ventral receptacular cluster formation (Platnick & Sedgwick, 1984). This two species were also proposed to be closely related to the trang-group of Thailand, which characterized for example with detached embolic part and the sclerotized one with 3 longitudinal ridges (Schwendinger, 1990).

However, a latter study suggested that *L murphyorum* is to be a different phylogenetic group from other *Liphistius* as they show a presence of sub-tegular apophysis in the male palp (Schwendinger, 2009). The study explained that the presence of a subtegular apophysis on the pedipalp organ of *Liphistius* male is quite rare. Only two species from Peninsular Malaysia share this specific character with the other one being *L. langkawi.* Schwendinger's current theory has been highly supported by the molecular analysis carried out in this current study as it shows that *L. murphyorum* form a separate clade from *L. desultor*, suggesting that the two species were distinct (Figure 4.1).

The second relationship that are recognized were between *L. malayanus* and *L. endau*. Schwendinger (1990) recognized that *L. malayanus* and *L. endau* possess different type of poreplate and embolic sclerites and suggested that it would be placed in a separate group from *L. desultor* and *L. murphyorum* when revised in the future. Platnick *et al.*

(1984) distinguish *L. malayanus* from the first group (*L. murphyorum* and *L. desultor*) by the ventral receptacular cluster that is wide which occupies a substantial portion of the width of the poreplate. *L. endau* was however described based on female specimen only and no male specimen has been described so far. Schwendinger (1995) questioned the identification that is based only on female specimens due the presence of considerable variation observed in the shape of female genitalia in *Liphistius* species from Thailand. As mentioned by Haupt (1983, 2003), the continuous moulting of adult females has caused the use of receptacular cluster for cladistic analysis to be problematic. However, the original assignment for *L. endau* species can be accepted based on its well-supported clade in the molecular analysis presented in the next chapter. Still, the interspecific relationship between this two species require a more detail study as the genetic analysis has shown that they belong to a species complex group (please refer to the next chapter analysis).

3.5.2 *Liphistius* distribution range

The study on the distribution of the spider genus *Liphistius* is extremely important for a successful conservation management of the group in Peninsular Malaysia. In Malaysia, the spider groups were understudied despite of its vital position in conservation issue. There were few species collected in this study that might be new species or new records which could not be identified due to lack of expertise. Research on *Liphistius* spider remained slow, not only in Malaysia but also other regions, loaded by the fact that identification of these primitive spider is difficult and requires the knowledge and experience of a well-trained arachnologists. Another important factor for this problem is the fact that the taxonomic key of *Liphistius* species has yet to be established.

Although a revision studied was done for all *Liphistius* species by Platnick and Sedgwick (1984), there is no organized effort to study on the *Liphistius* distribution from Malaysia specifically. As such, the current study serves to preliminarily review the existing liphistiids distribution checklist and provide updates when and where necessary. This study also confirmed the occurrence of *Liphistius* species in Terengganu and significantly extended the current distribution of *Liphistius* in Peninsular Malaysia. The discovery of *Liphistius* samples from inaccessible primary forest such as in Pasir Raja Reserve Forest and also in Temenggor Forest Reserve has open up for more potential localities. Detailed analysis of populations and habitat preferences were beyond the scope of this study, although they need to be explored in future investigation.

There is no doubt that the data collected in this study is not enough to represent the true distribution of the genus *Liphistius* in Peninsular Malaysia. Prior to this study, most *Liphistius* were collected around populations that are highly accessible and well known to tourist. This study has identified new localities in forest reserves and protected forests, for instance, Pasir Raja Forest Reserve, which is a primary forest reserve. This study can be said to be a preliminary report on the actual distribution of *Liphistius* spiders in Peninsular Malaysia. However, more extensive fieldworks are needed to study the distributional range of the species from this region.

This study has also provides taxonomic description, digital images and geo-referenced distributional maps for nine known species and ten unknown species from Peninsular Malaysia. However, due to small sample size, some of the specimen was described based on single specimen. In order to provide a better understanding on the classification of the genus, a cladistic analysis on this genus is much needed. Therefore the remaining known and unknown species need to be examined thoroughly in the future.

3.5.3 Plates

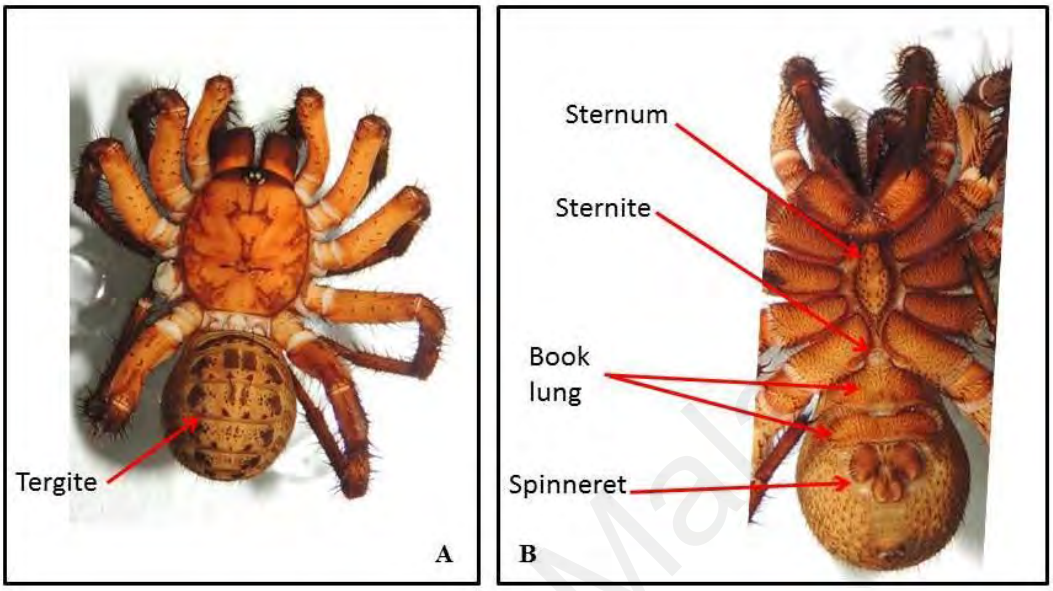

Figure 3.3: General morphology of *Liphistius,* A, Female *Liphistius desultor* Schiödte, 1849 (LS021), dorsal view, B, same, dorsal view

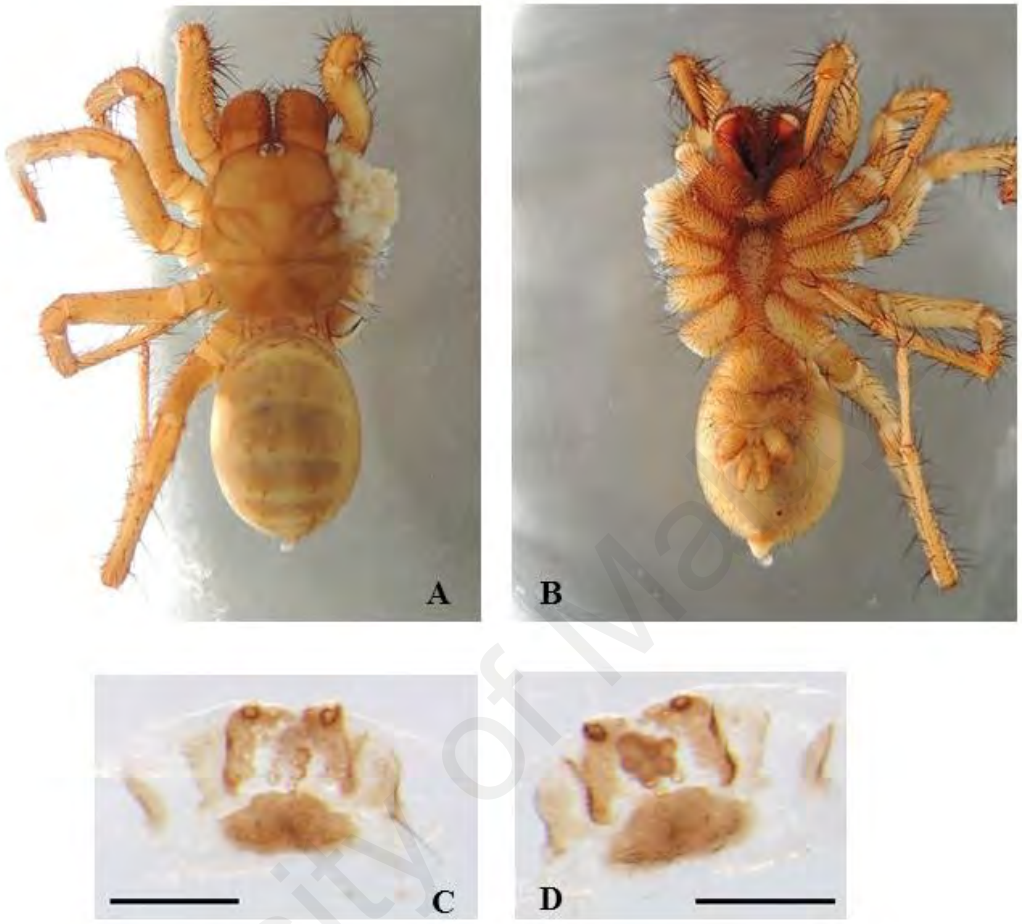

Figure 3.4: *Liphistius batuensis.* Examined female (LS007), A, dorsal view. B, ventral view. C, internal female genitalia, dorsal view. D, internal female genitalia, ventral view. Scale line = 0.5mm

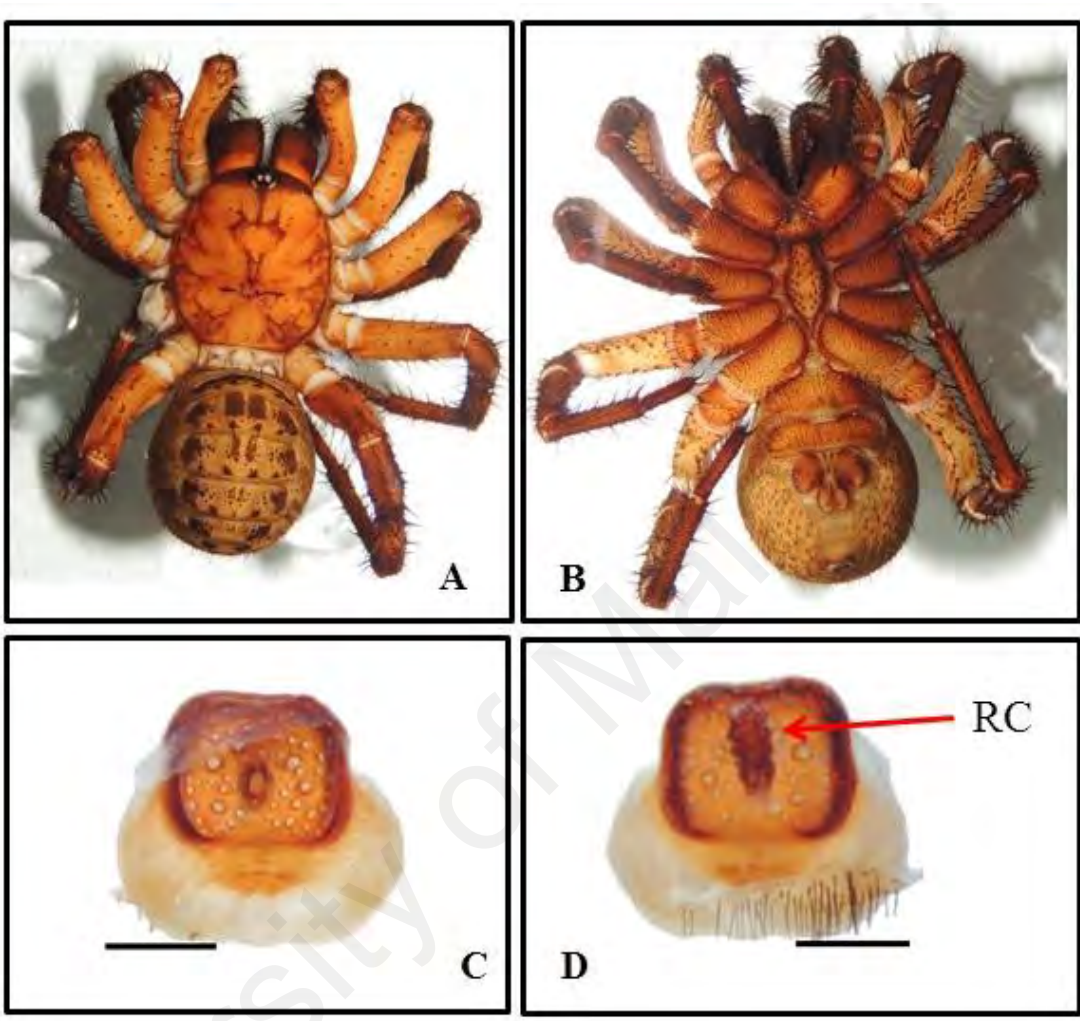

Figure 3.5: *Liphistius desultor*. Examined female (LS034), A, dorsal view. B, ventral view. C, internal female genitalia, dorsal view. D, internal female genitalia, ventral view. Scale line = 1.0mm

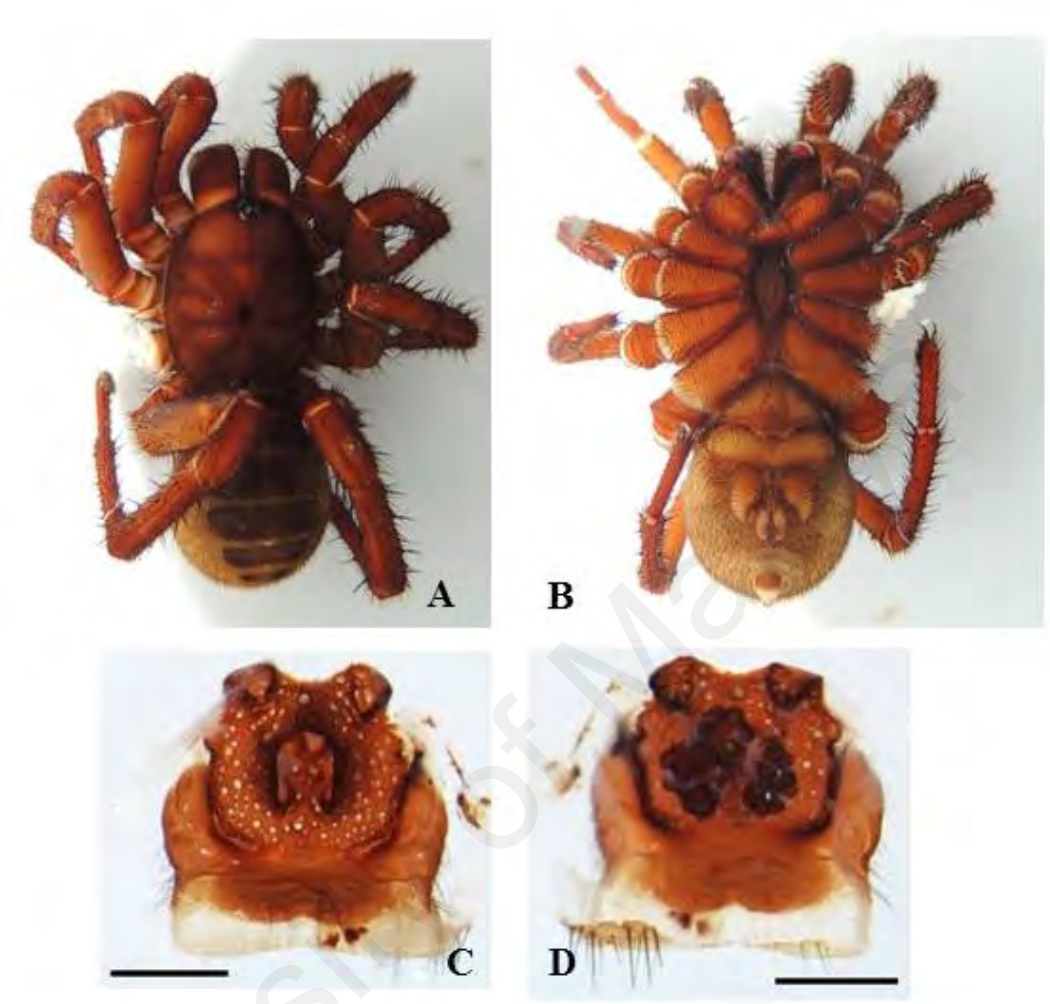

Figure 3.6: *Liphistius endau.* Examined female (LS087), A, dorsal view. B, ventral view. C, internal female genitalia, dorsal view. D, internal female genitalia, ventral view. Scale line = 1.0mm

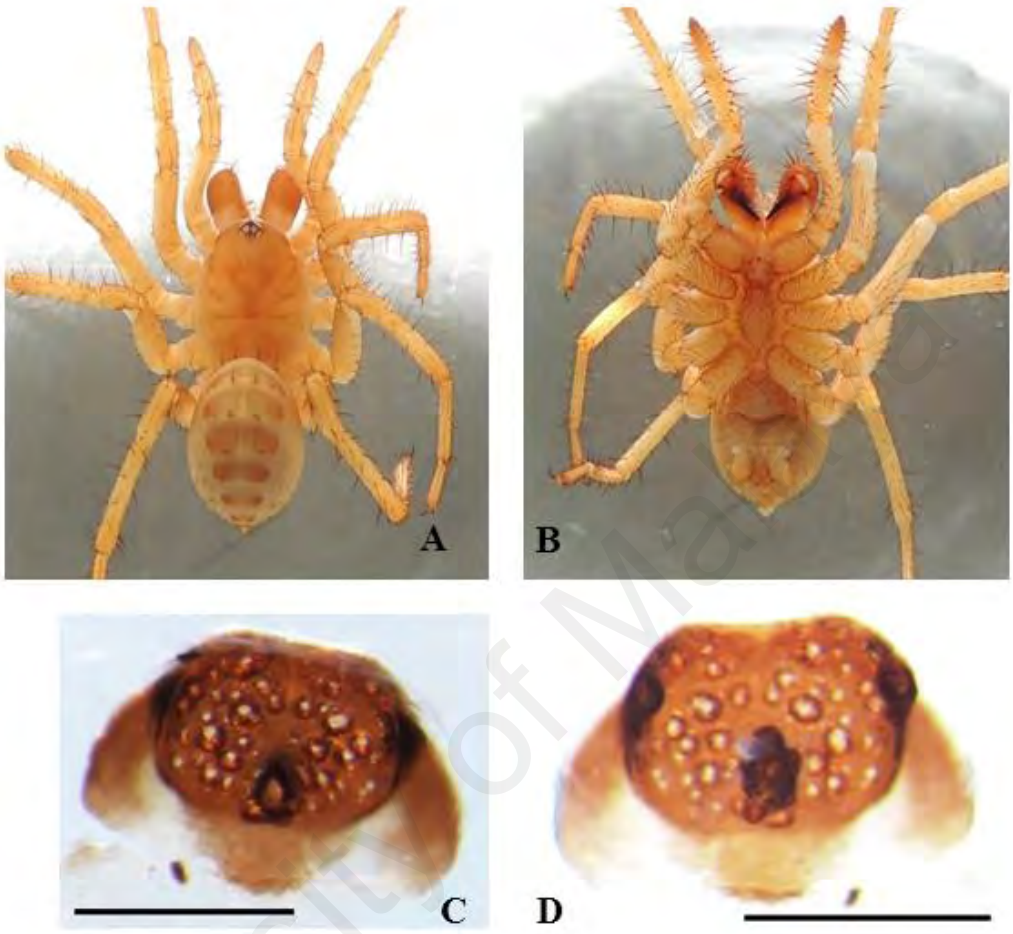

Figure 3.7: *Liphistius kanthan.* Examined female (LS023), A, dorsal view. B, ventral view. C, internal female genitalia, dorsal view. D, internal female genitalia, ventral view. Scale line = 0.5mm

Figure 3.8 *Liphistius laruticus*. Examined female (LS128), A, dorsal view. B, ventral view. C, internal female genitalia, dorsal view. D, internal female genitalia, ventral view. Scale line = 0.5mm

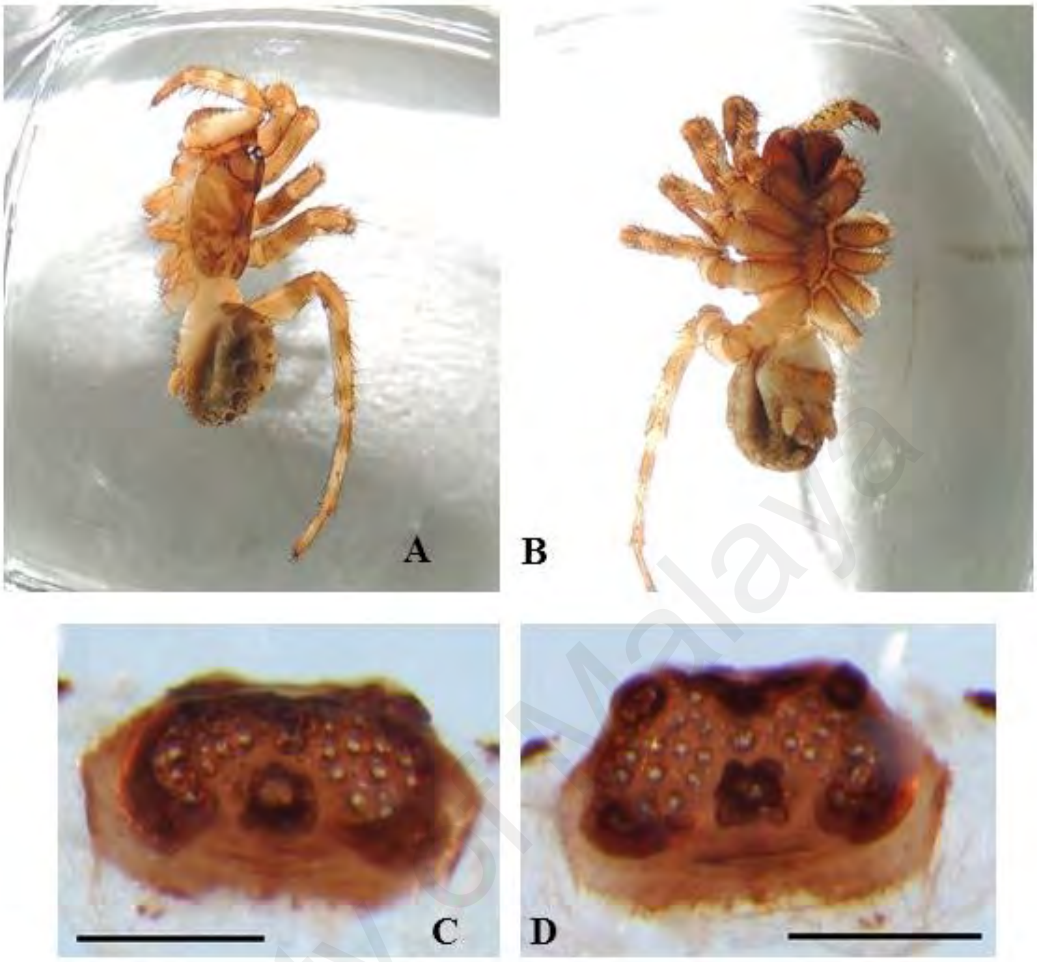

Figure 3.9 *Liphistius langkawi.* Examined female (LS039), A, dorsal view. B, ventral view. C, internal female genitalia, dorsal view. D, internal female genitalia, ventral view. Scale line = 0.5mm

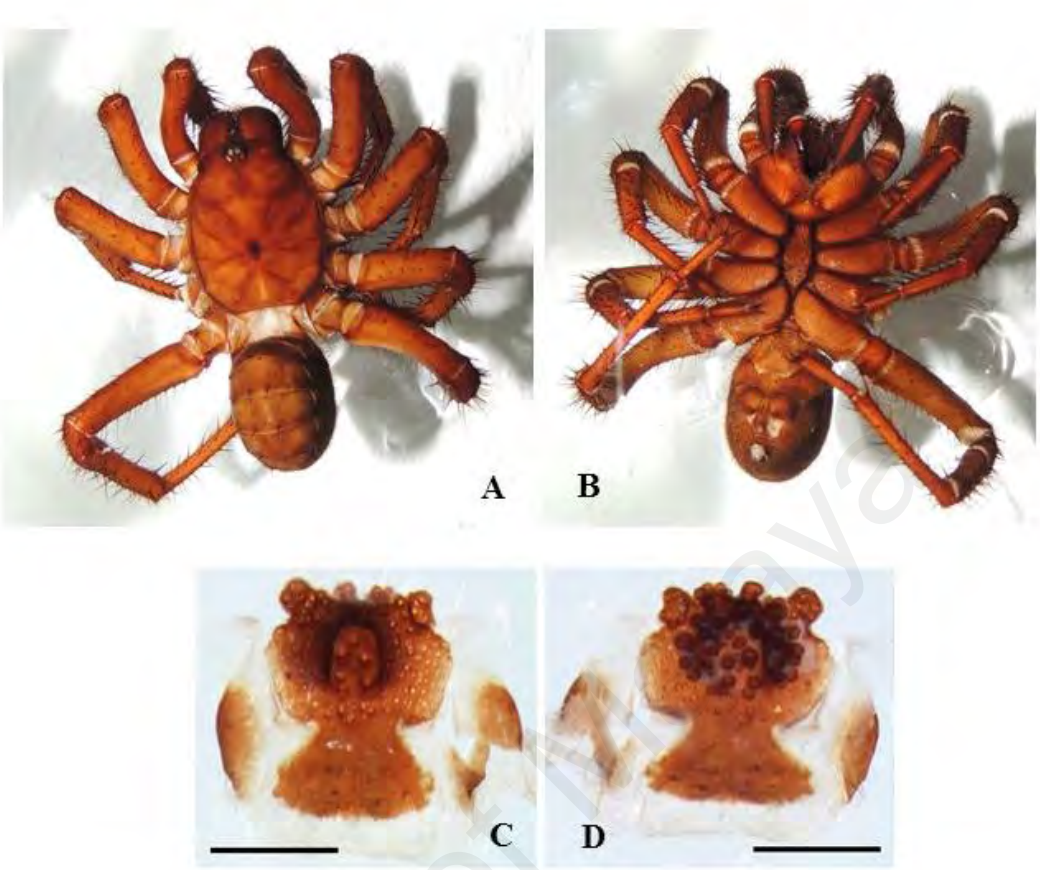

Figure 3.10 *Liphistius malayanus*. Examined female (LS010), A, dorsal view. B, ventral view. C, internal female genitalia, dorsal view. D, internal female genitalia, ventral view. Scale line = 1.0mm

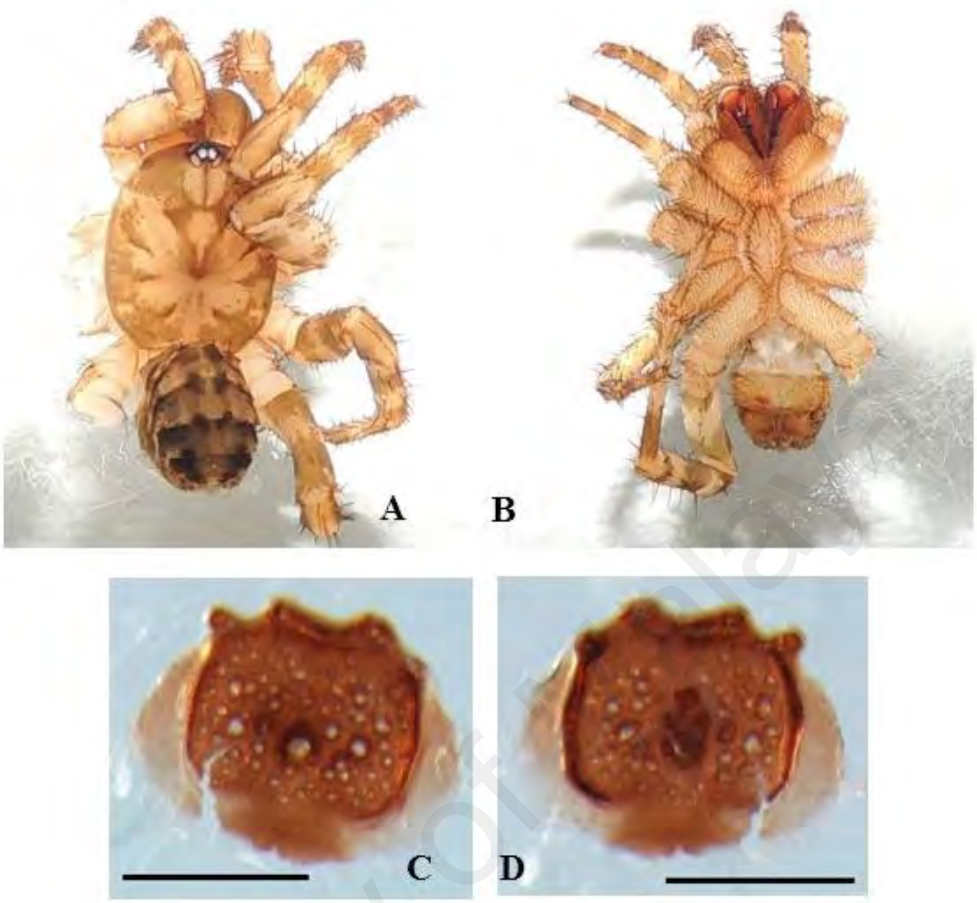

Figure 3.11 *Liphistius murphyorum*. Examined female (LS037), A, dorsal view. B, ventral view. C, internal female genitalia, dorsal view. D, internal female genitalia, ventral view. Scale line = 0.5mm

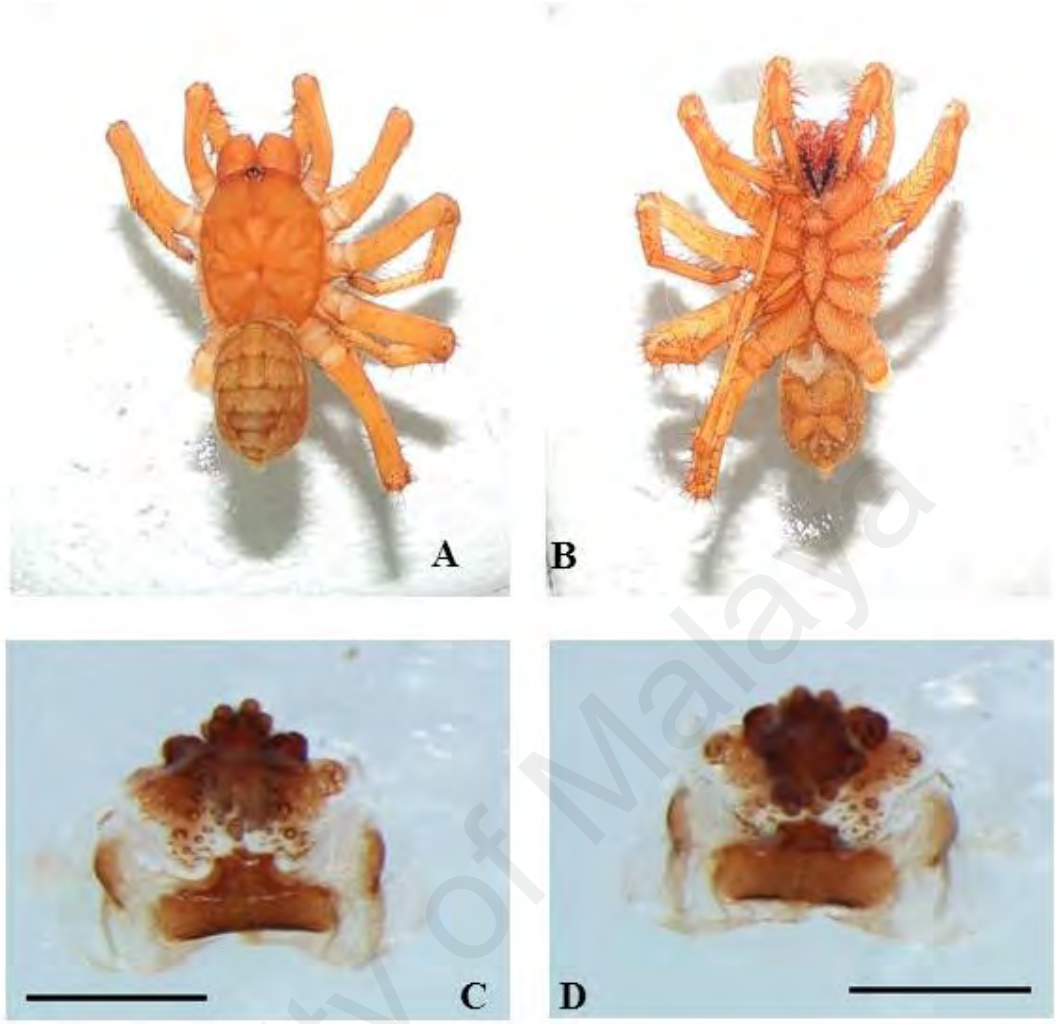

Figure 3.12 *Liphistius tempurung.* Examined female (LS030), A, dorsal view. B, ventral view. C, internal female genitalia, dorsal view. D, internal female genitalia, ventral view. Scale line = 0.5 mm

Figure 3.13 *Liphistius malayanus.* Examined male (LS009), A, dorsal view. B, ventral view. C, left palp lateral view.

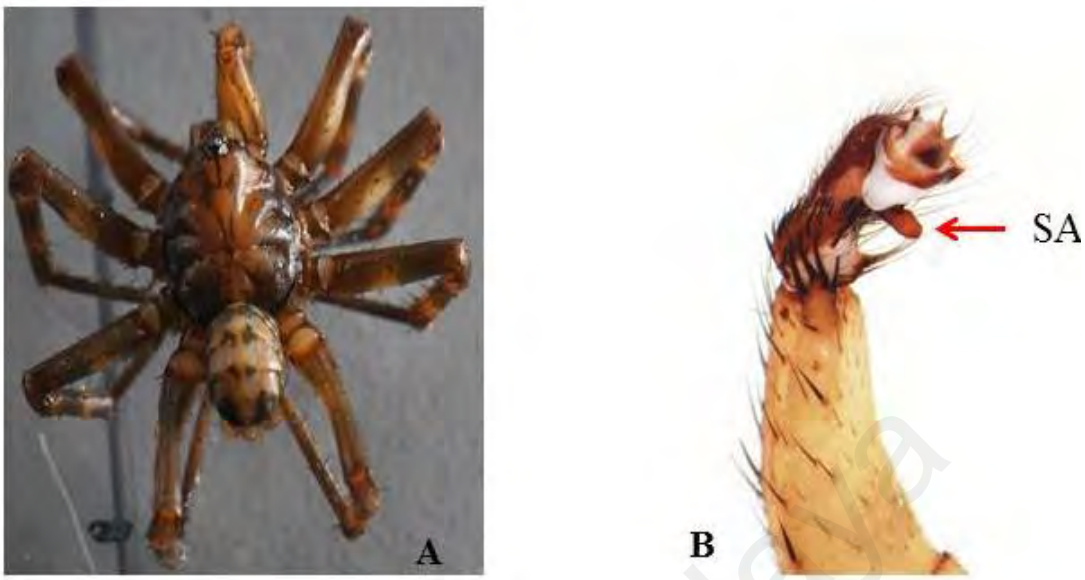

Figure 3.14 *Liphistius murphyorum*. Examined male (LS022), A, dorsal view. B, right palp lateral view. SA: sub-tegular apophysis

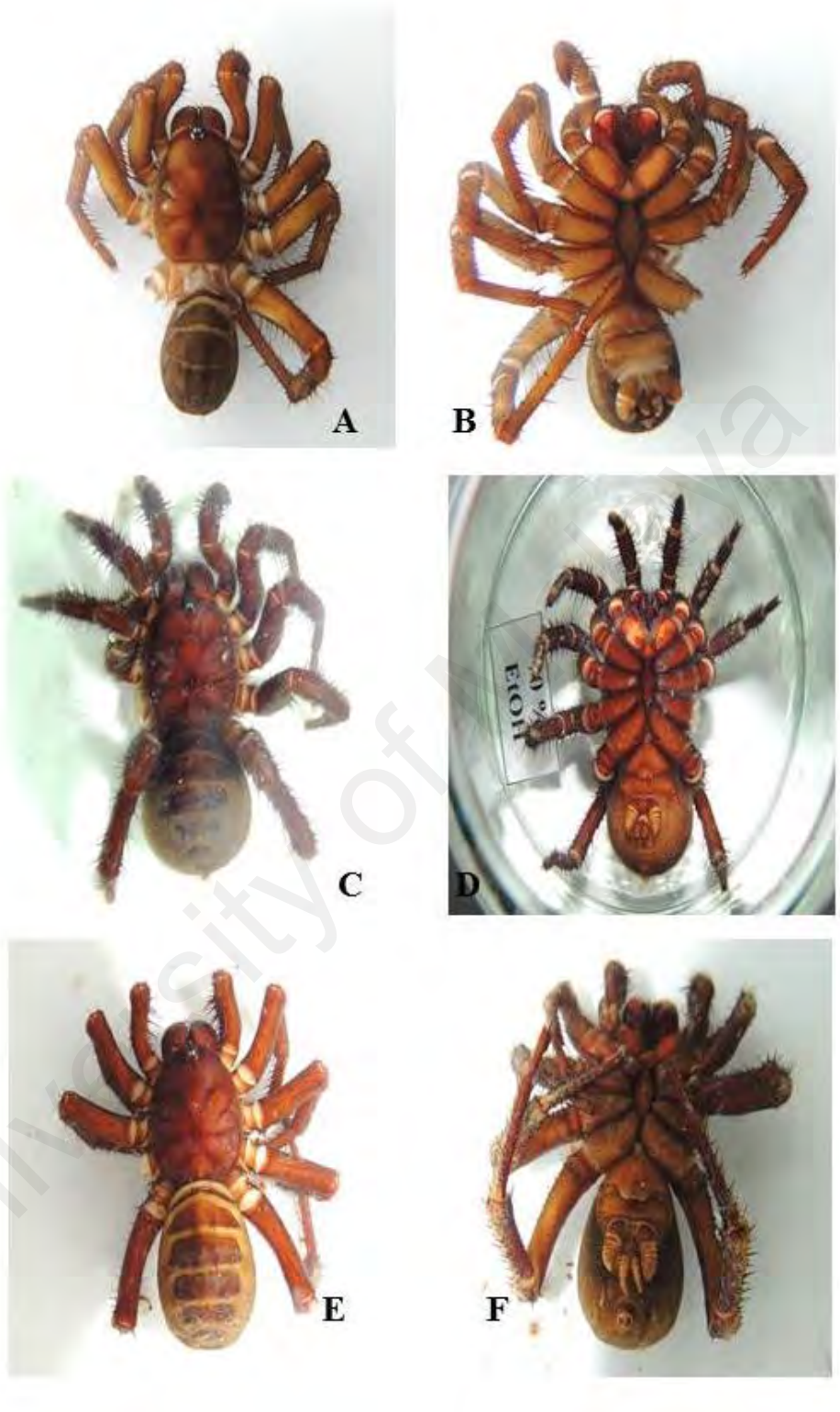

Figure 3.15 Unidentified female *Liphistius* spp. from Terengganu [A-D] *Liphistius* sp. 1, [E-F] *Liphistius* sp. 3; [A] LS142, collected in Kenyir Lake, dorsal view, [B] LS142 ventral view, [C] LS076, collected in Pasir Raja Forest Reserve, dorsal view, [D] LS076 ventral view, [E] LS109, collected in Lata Tembakah Amenity Forest, dorsal view, [F] LS109 ventral view

Figure 3.16 Internal genitalia of unidentified female *Liphistius* spp. from Terengganu [A-B] *Liphistius* sp. 1, [C-D] *Liphistius* sp. 3; [A] LS076, dorsal view, [B] LS076, ventral view, [C] LS109, dorsal view, [D] LS109, ventral view. Scale line = 1.0 mm

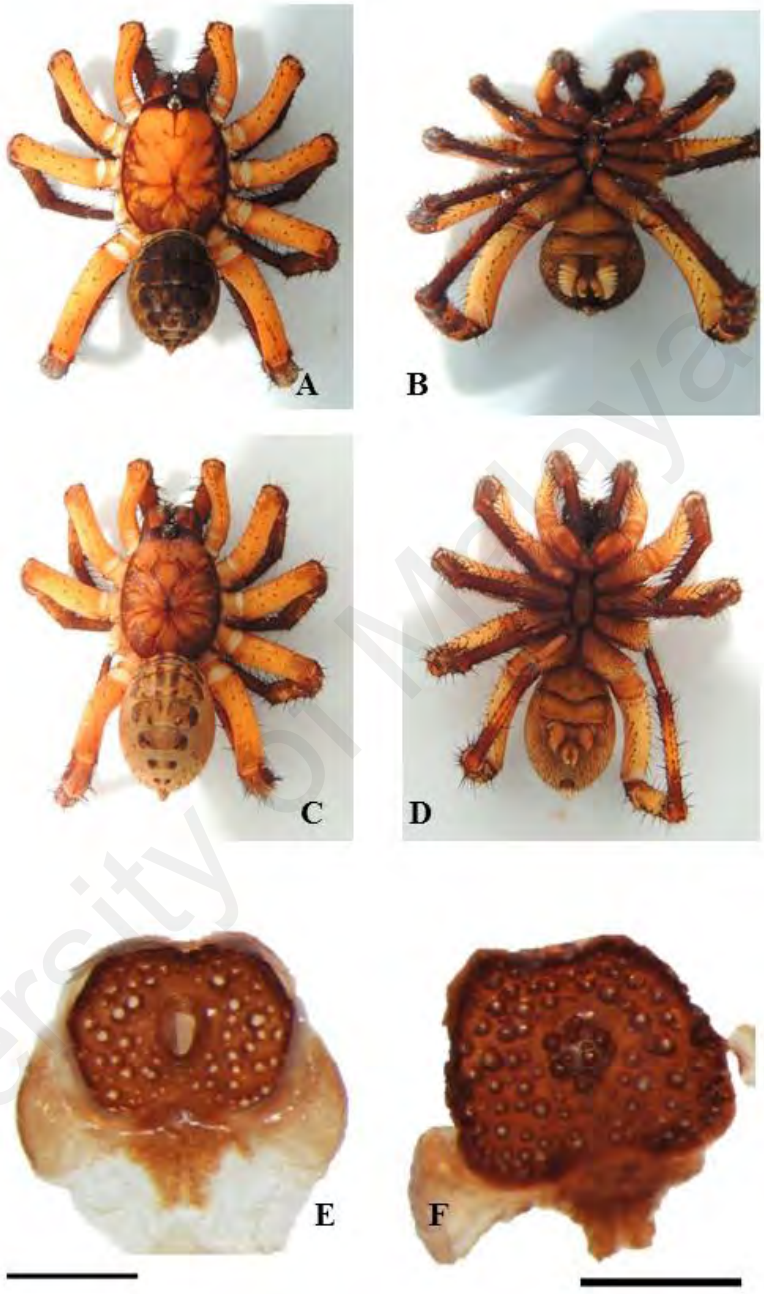

Figure 3.17 Unidentified female *Liphistius* spp. from Northern Malaysia [A-B] *Liphistius* sp. 6, [C-F] *Liphistius* sp. 4; [A] LS055, collected in Perlis State Park, dorsal view, [B] LS055 ventral view, [C] LS062, collected in Temenggor Forest Reserve, dorsal view, [D] LS062 ventral view, [E] *L.* sp. 4 dorsal view, [F], *L.* sp. 4 ventral view. Scale line = 1.0mm

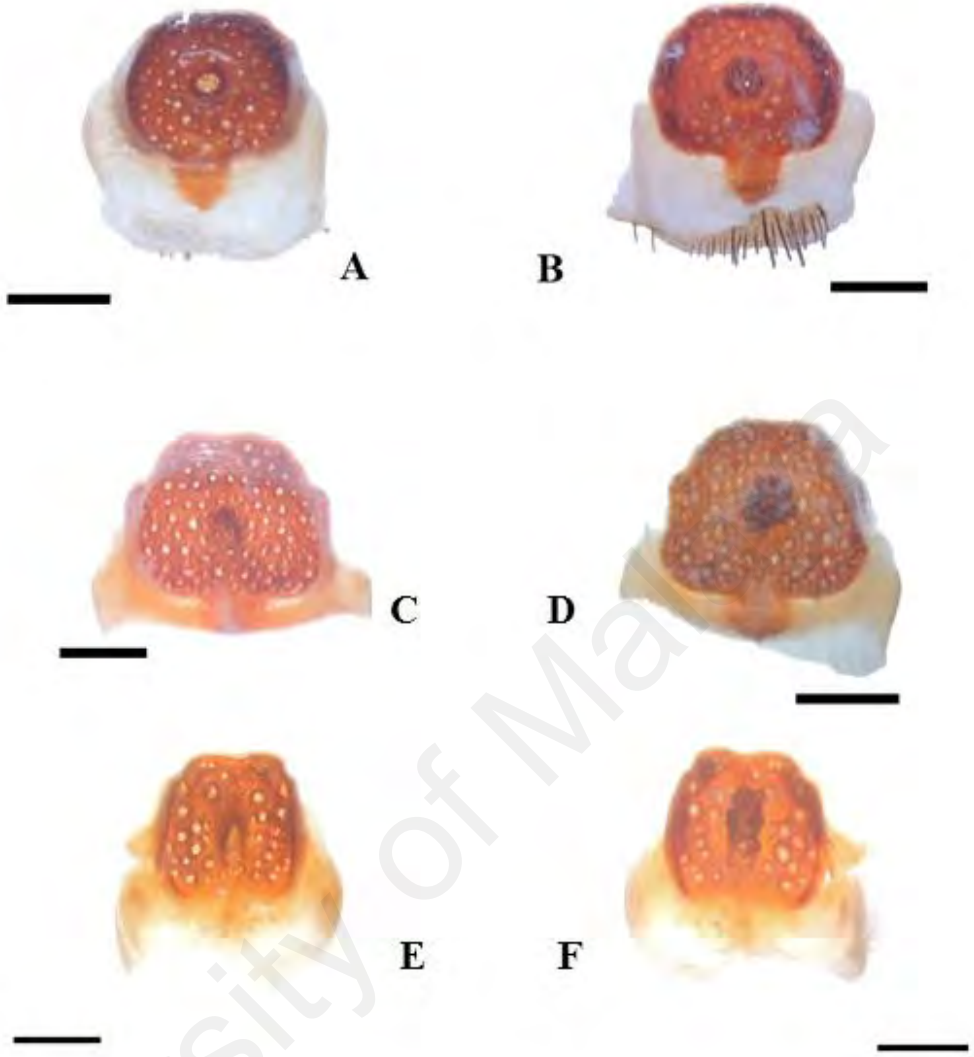

Figure 3.18 Internal genitalia of unidentified female *Liphistius* spp. from Northern Malaysia [A-D] *Liphistius* sp. 6, [E-F] *Liphistius* sp. 5; [A] LS055, dorsal view, [B] LS055 ventral view, [C] LS073, dorsal view, [D] LS073 ventral view, [E] LS081, dorsal view, [F] LS081, ventral view. Scale line = 1.0mm

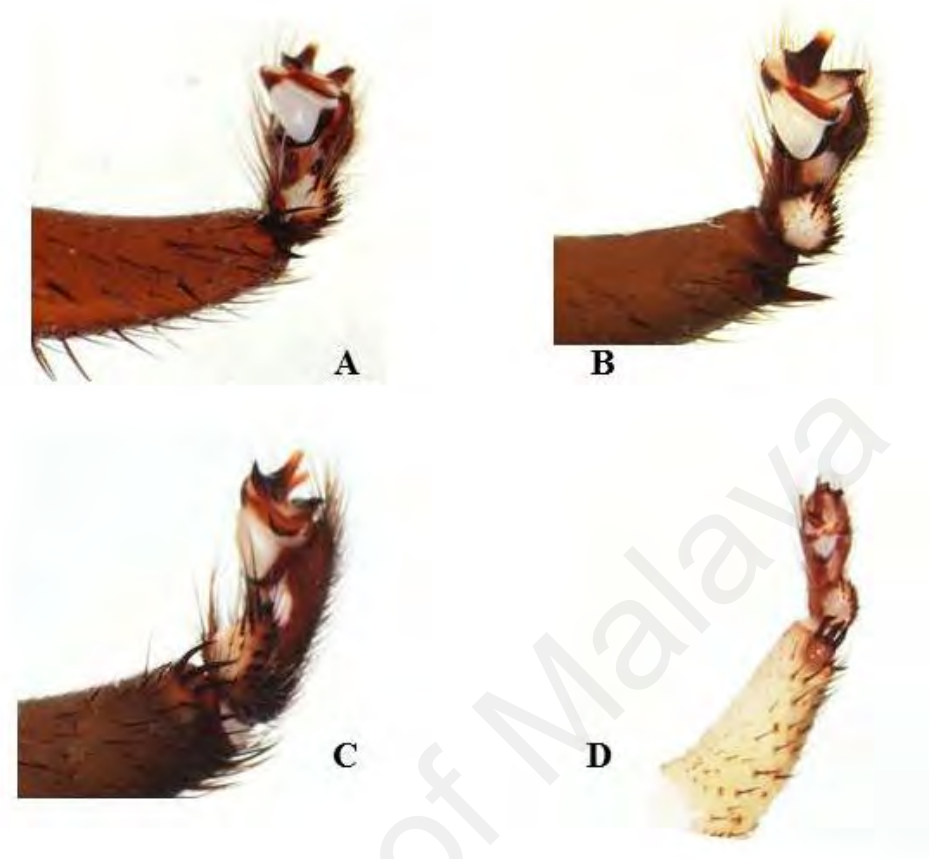

Figure 3.19 Unidentified male palp, lateral view [A] *Liphistius* sp. 2, LS002, [B] *Liphistius* sp. 2, LS003, [C] *Liphistius* sp. 1, LS144, [D] *Liphistius* sp. 3, LS099

3.5.4 Maps

Figure 3.20 Map of Peninsular Malaysia showing collected distributions of *Liphistius* sp. 1 (•), *Liphistius* sp. 3 (■) and *Liphistius* sp. 9 (◊) in Terengganu and Pahang

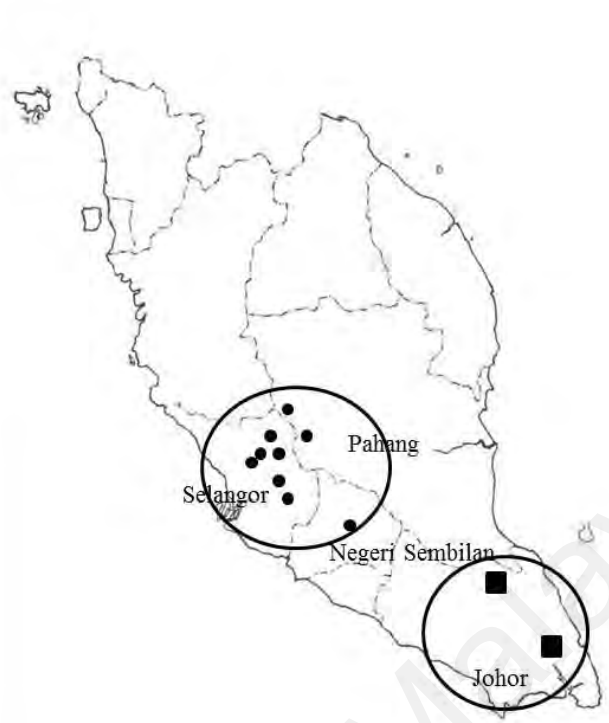

Figure 3.21 Map of Peninsular Malaysia showing collected distributions of *L. malayanus* (•) and *L. endau* (■).

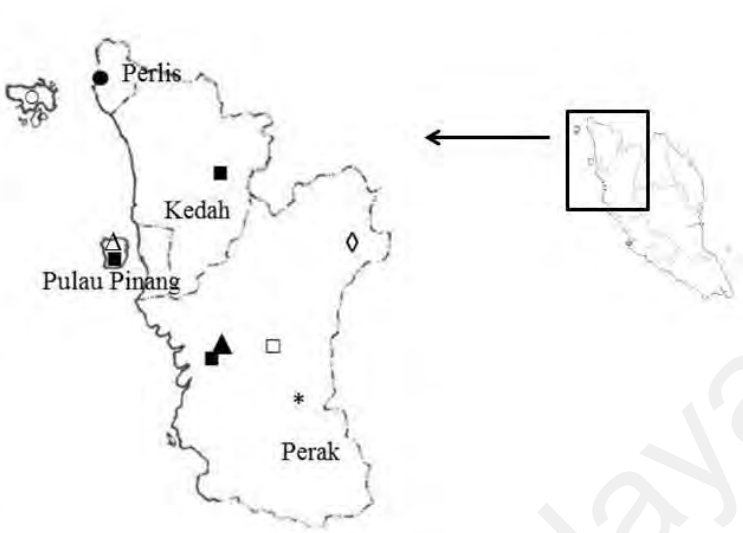

Figure 3.22 Map of Northern P. Malaysia showing collected distributions of *L. desultor* (■), *L. murphyorum* (Δ), *L. laruticus* (▲), *L. langkawi* (○), *Liphistius* sp. 4 (◊), *Liphistius* sp. 5 (*), *Liphistius* sp. 6 (•), and *Liphistius* sp. 10 (□).

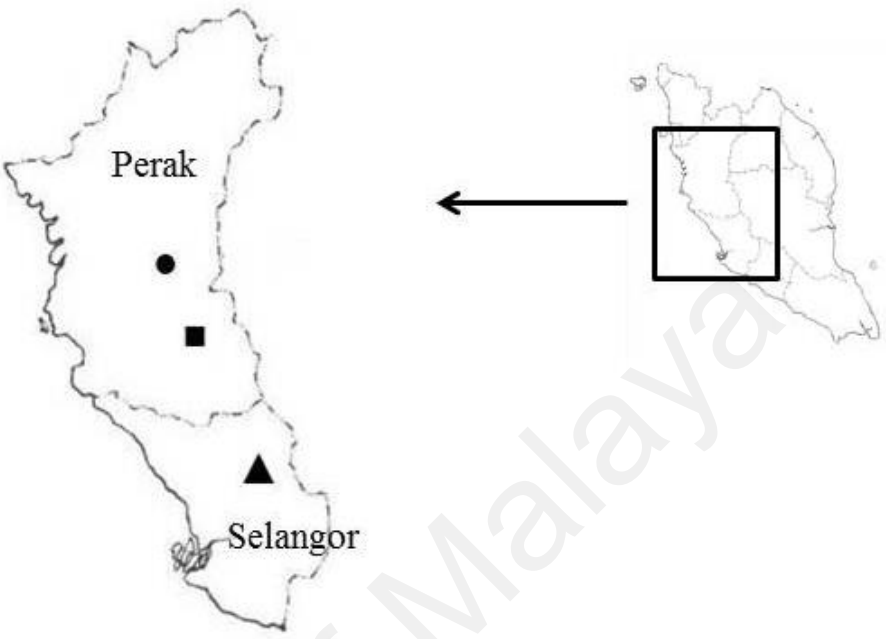

Figure 3.23 Distribution map of *Liphistius* spp. collected from caves: *L. kanthan* (●), *L. tempurung* (■) and *L. batuensis* (▲).

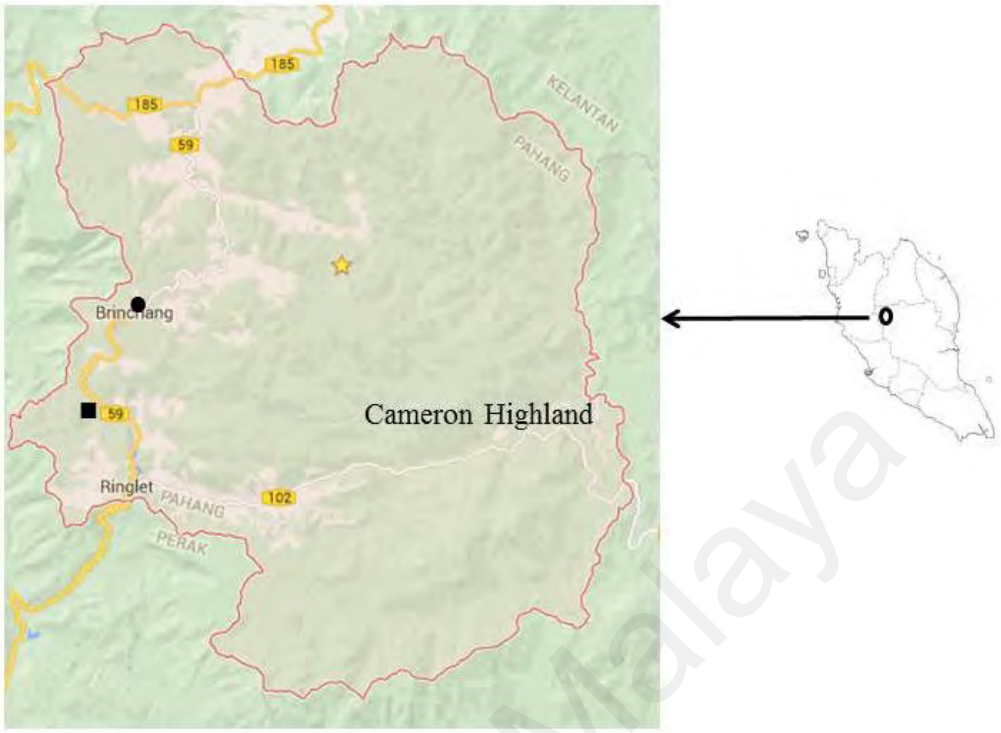

Figure 3.24 Distribution of *L.* sp. 2 (■) and *L.* sp. 8 (●) from Cameron Highland**.**

CHAPTER 4: MOLECULAR SYSTEMATICS OF THE GENUS *LIPHISTIUS* (ARANAE: LIPHISTIIDAE) FROM PENINSULAR MALAYSIA

4.1 Abstract

The taxonomic status of *Liphistius* spider remains uncertain because no cladistic analysis study has been conducted so far. The phylogeny of *Liphistius* was further investigated using molecular data to test for the species status. Data sets from cytochrome oxidase subunit I (COI) of the five identified species and two unknown genus *Liphistius* species in the Peninsular Malaysia were generated and were conducted for the first time. The species status of the five known and two unknown species were clarified using DNA barcoding. The sequence data were also phylogenetically analysed using maximum parsimony (MP) and Bayesian inferences (BI). Our results indicate that *Liphistius* spider of Peninsular Malaysia is a monophyletic group with the BI analysis exhibiting better performance in inferring the phylogeny of *Liphistius* than the MP. The DNA barcoding analyses also supported the existence of the seven putative species. Although some taxa are still missing from the analysis, the study can be the basis for future reconstruction of the phylogeny of *Liphistius*.

4.2 Introduction

Although there have been a number of studies at higher taxonomic levels which included representatives of *Liphistius* species (Bond et al., 2012; Tanikawa, 2013; Xu et al., 2015c), no comprehensive molecular systematics of the genus has been described. Therefore, the monophyly of the genus and phylogenetic relationships of the species have not been completely established, and the taxonomy status of the species within the genus remains uncertain. Effort to describe and classify species in the genus has been mainly on morphological characters only e.g (Platnick & Sedgwick, 1984; Schwendinger, 2009; Schwendinger, 2013), and there is no morphological cladistic analysis of relationship within the genus. Phylogenetic studies using molecular data suggested the genus is monophyletic (Xu et al., 2015c); however, this conclusion was based on limited samples of two of the currently known species.

The taxonomic status of the species within the genus has remained unsettled. Six species are described based on female samples only; *L. endau, L. johore, L. kanthan, L. tempurung* and *L. yangae* from Malaysia and *L. jarujini* from Thailand. Given the morphological variability within the genus have never been fully studied, molecular analysis are extremely needed to test for the presence of cryptic species and resolve phylogenetic hypothesis.

Various DNA sequences of mitochondrial gene regions have been applied to resolve spider phylogenies at species level e.g. (Hamilton et al., 2011; Planas et al., 2013). The aim of the present study is elucidate on the relationships within *Liphistius* by carrying out the first molecular phylogenetic analysis of the genus using *COI* DNA fragments.

This study used the partial cytochrome c oxidase subunit I *(COI)* gene to investigate the phylogenetic relationships of *Liphistius* species in Peninsular Malaysia and to test the monophyly of the genus *Liphistius* in Peninsular Malaysia.

4.3 Materials and Methods

4.3.1 Data collection

This chapter deals with the taxonomy of nine known species and five unknown species within the trap door spider genus *Liphistius* in Peninsular Malaysia. Samples were collected manually from the burrows. Fieldworks were undertaken from 27th February 2010 to 15th April 2012 to collect *Liphistius* specimens for analysis. Specimens were preserved in 80% ethanol immediately to reduce the risk of desiccation and were stored at 4°C.

Specimens identified for DNA sequencing were selected on the basis of their maturity, their sex and their location. The COI sequence of *Liphistius* sp. (LM01) obtained by Tanikawa (2013), GenBank acc. No. AB778257.1 was used as a reference for the specimens used in this study. Collection information is listed in Table 4.1 for each sample used in the molecular analysis. One to three legs of each specimen were stored individually in microcentrifuge tube preserved with absolute ethanol and kept in -20°C freezer prior to phylogenetic analysis. All specimens were assigned with an identification number and labels were added to the vials. Voucher specimens were deposited at the Museum of Zoology, University of Malaya.

4.3.2 Molecular and laboratory protocols

Whole genomic DNA was extracted from one or two legs per individual using the I-genomic CTB DNA Extraction Kit protocol for animal tissue. The partial fragments of COI gene were amplified using the universal primer pair LCO1490, HCO2180 (Folmer et al., 1994). Unfortunately, this primer pair was not capable to amplify across *Liphtius* spp. used in this study, resulting with only seven species which included two unknown species from 38 specimens. Alternative suitable primers were also not used due to time constrain, has been mentioned in Chapter 1 (1.3).

Polymerase chain reaction (PCR) amplification of target gene regions were achieved using the following PCR cocktail (30 µL final volume): 2.40 µL dNTPs, 3.00 µL of 10X *i*-Taq PCR buffer, 0.60 µL of *i*-Taq™ plus DNA Polymerase (iNtRON Biotech); 15.0 µL ultra-pure water; 0.75 µL of each 10 mM/µL primer; and 7.5 µL genomic DNA. PCR parameters included an initial 95 °C denaturation followed by 35 cycles of 45 sec at 95 °C, 45 sec at 56.3 °C, 45 sec at 72 °C with a final 5 min extension at 72 °C. In order to minimize miss-priming and maximize amplifications success prior to sequencing, both PCR mixture and annealing temperature were optimized for different samples. The DNA fragments were sequenced in both directions.

The presence of PCR products in PCR reactions was confirmed using 1% agarose gel. Before electrophoresis started, 5 µL of PCR product from each PCR tube was loaded into separate wells of the gel. The electrophoresis tank was connected to the power supply (BioRad) at 80 volt for about 35 minutes to allow the migration of DNA from negative pole to positive pole. Next, the gel was then transferred into ethidium bromide (EtBr) solution and soaked for 30 minutes. Finally the gel was viewed under the UV light to detect the presence of band using Alpha Imager™ 2200 translluminator.

If PCR products were detected, the selected band with the correct size was excised from the agarose gel with a clean scalpel. The excised gel was sliced into small pieces and was transferred into 2 ml microcentrifuge tube. The gel were then purified using LaboPass PCR clean-up Kit according to protocol provided by the manufacturer.

The success of gel purification was again determined by 1% agarose gel electrophoresis, with product visualised by staining with EtBr. The clean product was subjected to sequencing reactions to both directions performed by First Base Laboratories Sdn Bhd using supplied PCR primers.

4.3.3 Sequence analysis

Prior to data analysis, all sequence reads were checked against chromatograph data using Sequence Scanner v1.0 (Applied Biosystem™) to ensure the high quality of the sequence and to remove ambiguous bases. Each chromatogram for the forward and reverse sequence for each sample was checked by eye for errors and assembled using MEGA 5.2 (Tamura et al., 2011). The obtained DNA sequences were aligned using the Basic Local Alignment Search Tools (BLAST) (Altschul et al., 1990) to check for homology to other spiders COI sequences in order to ensure that the desired COI region had been obtained. A consensus sequence was created for each specimen.

Mitochondria DNA (mtDNA) sequences were verified for protein coding frame shifts, for the presence of stop codons or frame shift that may indicate the amplification of pseudogenes (Lopez et al., 1994; Song et al., 2008; Zhang & Hewitt, 1996) by using MEGA 5 (Tamura et al., 2011). All nucleotide sequences were then aligned using Clustal W algorithm included in the MEGA 5 software and were trimmed to a length of 552 bp.

The aligned sequences of COI were submitted to GenBank database in batches. After the sequence alignment, the software DnaSP v5.10 (Librado & Rozas, 2009) was used to summarize haplotype information.

4.3.4 DNA barcoding analysis

To define the species status, the COI dataset was analyzed using DNA barcoding analysis (Barrett & Hebert, 2005; Hebert et al., 2003), and neighbour-joining (NJ) analysis (Saitou & Nei, 1987). The unknown species were divided into two putative species based on combination of haplotype data, morphological identification and geographic information. A total of 40 specimens representing seven nominal species and one outgroup species were analyzed (Table 4.1).

In the DNA barcoding gap analysis, overlap between the mean intraspecific and interspecific Kimura two-parameter (K2P) were examined using MEGA5.2. Subsequently, NJ trees were constructed from nucleotide sequences under Kimura 2 parameter (Kimura, 1980), and reliability of branches was tested using the nonparametric bootstrap test (Felsenstein, 1985).

4.3.5 Phylogenetic analysis

Two methods were used to infer phylogenetic relationships: maximum parsimony (MP) (Farris, 1970, 2008), and Bayesian Inference (BI) (Huelsenbeck & Ronquist, 2001). MP analyses was computed using MEGA 5 software and BI with MrBayes (Huelsenbeck & Ronquist, 2001). In the case to confirm the divergence pattern, *Ryuthela ishigakiensis* (GenBank accession number AB778250.1) was included as outgroup taxa.

The maximum parsimony criterion was applied using 1000 random addition sequence heuristic replicates with tree-bisection-reconnection (TBR) branch-swapping. To estimate branch support on the recovered independent and combined topologies, non-parametric bootstrap values (Felsenstein, 1985) were calculated with MEGA5. For bootstrapping analyses 1000 pseudoreplicates were generated with 10 random additions of taxa.

A Bayesian tree was also estimated using MrBayes software. The corresponding evolutionary model including the rate matrix and base frequencies obtained from Modeltest3.7 (Posada & Crandall, 1998) were appended in nexus file. The analysis was run using four Markov Chain Monte Carlo (MCMC) chains and was run for 2,000,000 generations, samplings of every 100^{th} generations. The first 25% of the generated trees were discarded, as confirmed by visualisation of the log likelihood trace and the average standard deviation of the split frequencies being <0.01. Posterior probabilities were calculated and reported on a 50% majority-rule consensus tree of the post-burnin sample.

Table 4.1 Sample used in molecular analysis study: sample label, taxon name, haplotype number, sample collection locality and GenBank accession number

No	Specimen Code	Genus	Species	Haplotype	Sex	Locality	GenBank accession no.
1	LS013	*Liphistius*	*malayanus*	H_1	♀	Ampang Forest Reserve	KR017711
2	LS014	*Liphistius*	*malayanus*	H_1	♀	Ampang Forest Reserve	KR017712
3	LS020	*Liphistius*	*malayanus*	H_2	♀	Ulu Bendul Amenity Forest	KR028500
4	LS114	*Liphistius*	*malayanus*	H_2	♀	Ulu Bendul Amenity Forest	KR017713
5	LS012	*Liphistius*	*malayanus*	H_3	♀	Fraser Hill	KR028501
6	LS008	*Liphistius*	*malayanus*	H_4	♀	Fraser Hill	KR028502
7	LS009	*Liphistius*	*malayanus*	H_5	♂	Genting Highland	KR028504
8	LS011	*Liphistius*	*malayanus*	H_5	♀	Genting Highland	KR028503
9	LS010	*Liphistius*	*malayanus*	H_6	♀	Genting Highland	KR028505
10	LS00	*Liphistius*	*malayanus*	H_7	♀	Ulu Gombak Forest Reserve	KR028506
11	LS018	*Liphistius*	*malayanus*	H_7	♀	Ulu Gombak Forest Reserve	KR028507
12	LS019	*Liphistius*	*malayanus*	H_7	♀	Ulu Gombak Forest Reserve	KR028508
13	LM01	*Liphistius*	sp.	H_7	-	-	AB778257.1
14	LS098	*Liphistius*	*malayanus*	H_8	j	Kemensah Waterfall	KR028537
15	LS021	*Liphistius*	*desultor*	H_9	♀	Penang Botanic Garden	KR028514
16	LS034	*Liphistius*	*desultor*	H_9	♀	Penang Botanic Garden	KR028515
17	LS036	*Liphistius*	*desultor*	H_9	♀	Penang Botanic Garden	KR028516
18	LS053	*Liphistius*	*desultor*	H_10	♀	Telok Bahang Amenity Forest	KR028517
19	LS054	*Liphistius*	*desultor*	H_10	j	Telok Bahang Amenity Forest	KR028518

Table 4.2 Continued

No	Specimen Code	Genus	Species	Haplotype	Sex	Locality	GenBank accession no.
20	LS022	*Liphistius*	*murphyorum*	H_11	♂	Telok Bahang Amenity Forest	KR028519
21	LS035	*Liphistius*	*murphyorum*	H_11	♀	Telok Bahang Amenity Forest	KR028520
22	LS037	*Liphistius*	*murphyorum*	H_11	♀	Telok Bahang Amenity Forest	KR028521
23	LS093	*Liphistius*	*endau*	H_12	j	Endau Rompin National Park	KR028522
24	LS094	*Liphistius*	*endau*	H_12	♀	Endau Rompin National Park	KR028523
25	LS148	*Liphistius*	*endau*	H_13	♀	Endau Rompin National Park	KR028524
26	LS140	*Liphistius*	*endau*	H_14	♀	Kota Tinggi Waterfall	KR028525
27	LS141	*Liphistius*	*endau*	H_15	♀	Kota Tinggi Waterfall	KR028526
28	LS125	*Liphistius*	*laruticus*	H_16	♀	Maxwell Hill	KR028527
29	LS128	*Liphistius*	*laruticus*	H_16	♀	Maxwell Hill	KR028528
30	LS136	*Liphistius*	*laruticus*	H_17	♀	Maxwell Hill	KR028529
31	LS142	*Liphistius*	sp. 1	H_18	♀	Lake Kenyir	KR028530
32	LS143	*Liphistius*	sp. 1	H_18	♂	Lake Kenyir	KR028531
33	LS049	*Liphistius*	sp. 1	H_18	j	Pasir Raja Forest Reserve	KR028535
34	LS051	*Liphistius*	sp. 1	H_18	j	Pasir Raja Forest Reserve	KR028536
35	LS059	*Liphistius*	sp. 1	H_19	j	Felda Tekam	KR028538
36	LS067	*Liphistius*	sp. 1	H_19	j	Felda Tekam	KR028539
37	LS080	*Liphistius*	sp. 5	H_20	j	Ulu Kinta Amenity Forest	KR028532
38	LS081	*Liphistius*	sp. 5	H_20	♀	Ulu Kinta Amenity Forest	KR02853
39	LS082	*Liphistius*	sp. 5	H_20	♀	Ulu Kinta Amenity Forest	KR028534
40	-	*Ryuthela*	*ishigakiensis*	-	-	-	AB778250.1

4.4 Results

4.4.1 Mitochondrial region sequence analysis

Sequences for 552 bp of COI gene were obtained for 39 individual of *Liphistius* species. There were no stop codons identified in COI gene sequences indicating that PCR product were of mitochondrial origin. These COI sequences were aligned with COI sequences in GenBank database and display the absence of indels and in frame stop codons which show that the entire datasets were free from presence of nuclear mitochondrial pseudogenes (numts). Hence, it is assumed that our COI sequence data are functional mitochondria and therefore were suitable for subsequent analysis.

A total of 302 conserved sites and 229 parsimony informative sites were observed for *Liphistius* species. Among all the seven barcoded species, a total of 20 unique haplotypes were observed with no overlapping haplotype distribution between different species. The number of haplotype were eight in *L. malayanus* (n=13), four in *L. endau* (n=5), two in *L. desultor* (n=5), one in *L. murphyorum* (n=3), two in *L. laruticus* (n=3), two in *L.* sp. 1 (n=6) and one in *L.* sp. 5 (n=3). On average, the COI sequence was found to be AT rich (A=29.8%; C=21.5%; G=14.9%; T=33.7%).

Table 4.2 Evolutionary divergence over sequence pair between species and mean intraspecific distance based on COI gene (%). Analyses were conducted using the Kimura 2-parameter model.

	Species	1	2	3	4	5	6	Within species
1	*L. malayanus*							4.7
2	*L. desultor*	23.5						0.4
3	*L. murphyorum*	26.2	27.0					0.0
4	*L. endau*	22.2	22.5	24.1				4.9
5	*L. laruticus*	23.6	20.4	27.4	25.7			3.1
6	*L.* sp. 1	22.3	25.9	26.9	14.9	25.3		0.2
7	*L.* sp. 5	26.0	13.9	25.6	22.4	21.5	24.4	0.0

4.4.2 DNA barcoding analysis

Each of the seven species included in the NJ analysis tree possessed a distinct COI sequence with high support value (Figure 4.1). Mean intraspecific distances ranged from low (0%) in *L. murphyorum* and *L.* sp. 5 to high (4.9%) in *L. endau* (Table 4.2). By contrast, interspecific sequence divergence between the seven species ranged from low of 13.9% (*L. desultor – L.* sp. 5) to high of 27.4% (*L. laruticus – L. endau*). There was no overlap in the distribution of pairwise intra and interspecific distances; reflecting the utility of the DNA barcoding gap analysis. All the seven putative species are separated by >13% interspecific pairwise divergence, however, only two species pairs were under 20% pairwise divergence; *L. desultor – L.* sp. 5 (13.9%) and *L. endau – L.* sp. 1 (14.9%). Both of these species pairs consisted of species that are morphologically similar in poreplate shape with minor differences in receptacular cluster formation.

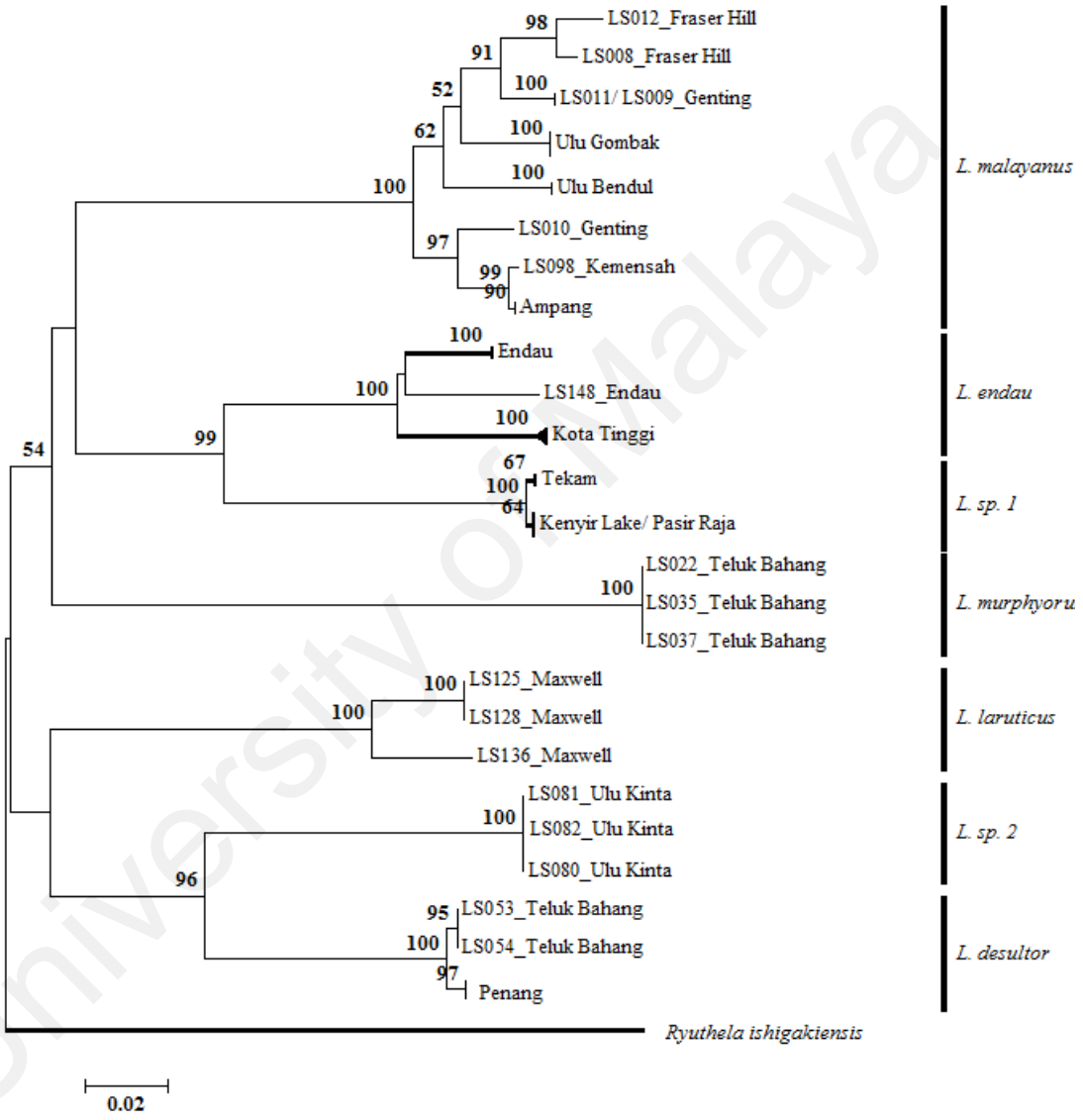

Figure 4.1 Neighbour-Joining (NJ) phylogenetic tree of COI sequence of haplotypes of species studied. Number on branches show bootstrap support >50

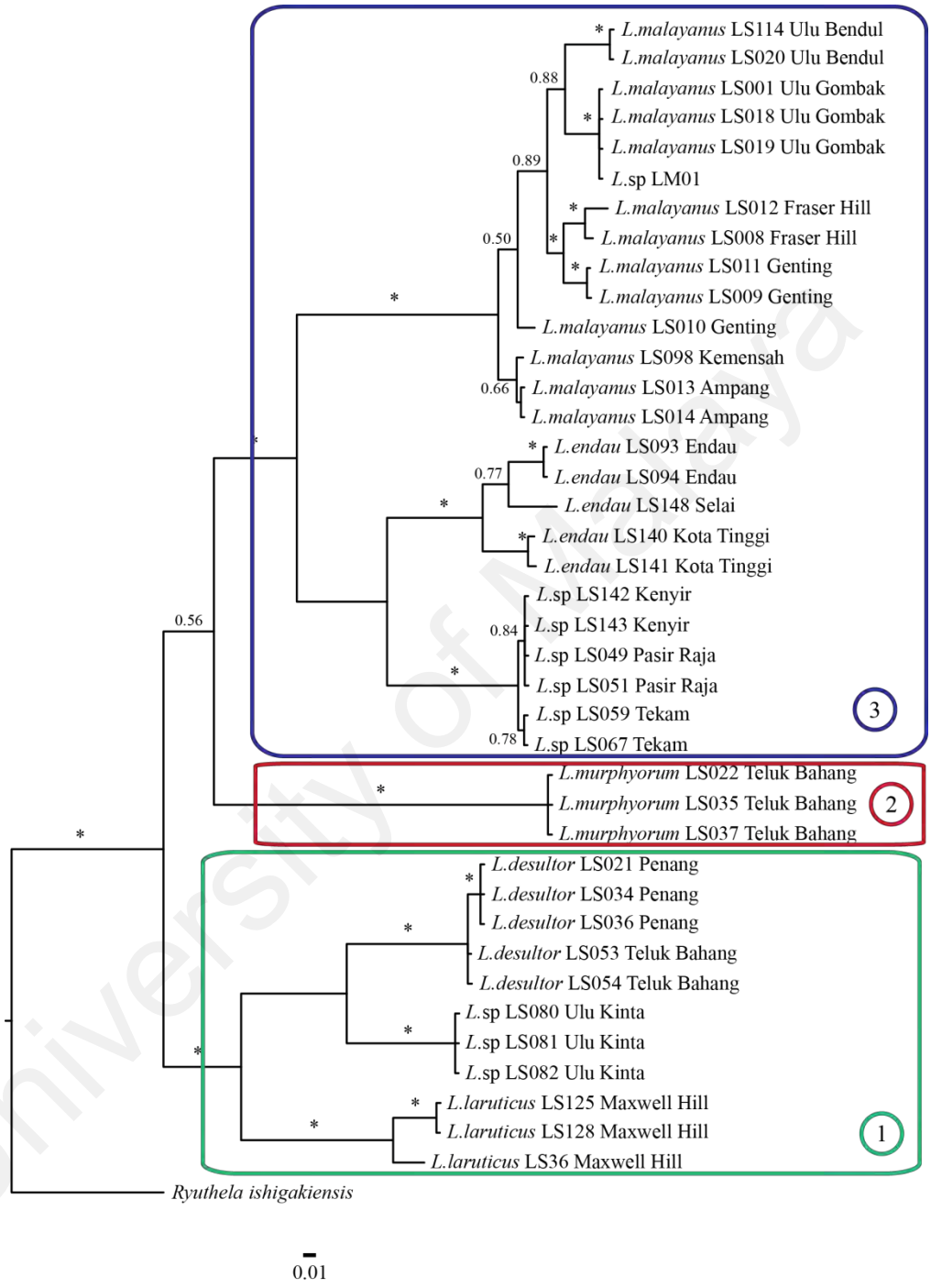

Figure 4.2 Phylogram of *Liphistius* spp. obtained from a Bayesian analysis using COI gene. Asterisk symbol (*) indicates Bayesian support value >0.95. All other values >0.50 are shown. Three species groups are indicated: species group 1,2 and 3.

4.4.3 Phylogenetic analysis

The monophyly of the genus *Liphistius* was supported by phylogenetic analysis of *COI* gene (see Figure 4.2). However, only BI tree supported the monophyletic of this genus with high BI posterior probabilities (1.00) but the degree of support for internal and inter-specific nodes were varied. Therefore, only BI tree was discussed here. *COI* Bayesian analysis produces the best resolution for relatively stronger supported species group.

Intra-specific nodes were not fully resolved (0.56) in the Bayesian analysis but allowed recognition of at least seven species of *Liphistius,* verifying prior species identification using traditional taxonomic characters (Chapter 3) and DNA barcoding analysis.

Three major clades corresponding to species group were identified in the Bayesian analysis, although relationships between these clades were not fully resolved due to the incomplete number of species in the genus (Figure 4.2). These included of species group 1, endemics to Northern of Peninsular Malaysia. This group consists of *L. laruticus*, *L. desultor* and *L*. sp. 5. *L. laruticus* were only collected on the higher elevation of Maxwell Hill, while *L. desultor* were collected in a wider distribution in Penang Island. Meanwhile *L.* sp. 5 was collected from a new population records from Ulu Kinta, Perak. This species group means sequences divergence ranging from 13.9%-21.5% between species.

Species group 2 was represented by a single species *L. murphyorum.* In this study *L. murphyorum* specimens were only collected in Teluk Bahang, Penang Island and were highly supported in the analysis. However, the relationship between *L. murphyorum* and other *Liphistius* in this study is not resolved. All the specimens collected shared only one haplotype.

Species group 3 was divided into two sub clade with high support value (0.96). The first sub clade was represented by the widely distributed *L. malayanus* while the second sub clade was divided into *L. endau* and an unknown species, *L.* sp. 1. This species group sequences divergence ranging from 14.9%-22.3% between species. Within this clade the monophyly of each species is supported by extremely high posterior probability of 0.99-1.00 and the relationship by all taxa are well resolved.

4.5 Discussions

In this study, by combining DNA barcoding and phylogenetic analysis, the status of seven *Liphistius* species is well supported. Results have shown that molecular technique can be used as an alternative species delimitation method when conventional methods were impossible to be applied. As previously mentioned, challenges in species identification and the rarity of adult specimens have caused *Liphistius* spp. not to be thoroughly studied.

It is commonly accepted that the use of COI gene sequences is reliable to identify species, and it is the most commonly used mitochondrial marker for assignation of barcodes for most animals (Hebert et al., 2003) including spiders (Barrett & Hebert, 2005). The results have shown that the interspecific divergences of *Liphistius* spp. (>13% between all the species studied) are consistent with their status as distinct.

For most taxa, the limit of 3% for genetic divergence is already a good indication to set separations between species (Sbordoni, 2010), however, the current study displayed a higher range. The results are equivalent to a study conducted on *Liphistius* sister genus, *Ganthela* which has found 4-12% gap (Xu et al., 2015a), and on the study of mygalomorph spider, *Aphonopelma hentzi* which has found a barcode limit of >6% (Hamilton et al., 2011). Therefore, the high interspecific genetic distances in our study

indicate that there were low levels of gene flow among populations, which is also usual in mygalomorphs (Hamilton et al., 2011; Hendrixson et al., 2013) and as serves as evidence of the endemicity of the species.

Conversely, we can also see there are higher intraspecific divergences found in *L. malayanus, L. endau* and *L. laruticus* (Table 4.2) which could indicate the presence of species complex (Barrett & Hebert, 2005). *L. malayanus* has a wide geographic distribution and inhabits different altitudinal zones that includes highland (Genting and Cameron in Pahang) and lowland. *L. endau* was only known from Johor, however no male specimen has been described so far. And for *L. laruticus*, the species was currently known from the higher elevation of Maxwell Hill. Thus, further research is needed to determine the reason for the existence of species complex within these *Liphistius* species.

The phylogenetic analysis presented here feature three main clades that correspond to the division of *Liphistius* into three species group. The findings presented in this chapter has been discussed in Chapter 3.

Further studies using more molecular markers could clarify the phylogeny of the *Liphistius* spp., taking into account that this study is lacking in the numbers of species representatives and limited to one gene marker only.

CHAPTER 5: GENERAL DISCUSSIONS AND CONCLUSIONS

5.1 Summary of results

In this dissertation, a study on the distribution and systematic of the genus *Liphistius* is presented, with a focus on Peninsular Malaysian species. This study aims to determine the species status of the genus, and to present a hypothesis for liphistiids phylogeny from this region. Nine species of Peninsular Malaysian *Liphistius* have been taxonomically studied. Furthermore, ten unidentified species from new population's locality was found. As a result of this research, the *Liphistius* spiders are now known to be widely distributed in the Peninsular Malaysia, and phylogenetic analysis of COI confirmed the monophyly of the genus *Liphistius*.

Analysis of the COI sequences presented in this study has shown that COI is a helpful tool for species identification, at least for the seven *Liphistius* species studied. This study also showed that it is possible to identify *Liphistius* using juveniles. The molecular data is compatible with identification based on internal genital morphological characters used in the original descriptions of these species and could be used to delimit between species in this genus. However, the relationships between species are still uncertain.

The molecular analyses also showed that *Liphistius* spp. is strongly endemic with high interspecific genetic distances. There is also the possibility of the existence of species complex due to high intra-species genetic distance found in *L. malayanus*, *L. endau* and *L. laruticus*. Next, this study had clarified *Liphistius* into three distinct subgroups, which eventually can be described based on their size and body coloration. Thus, this new

hypothesis will hopefully provide the foundation to the understanding of the relationship between all the existing *Liphistius* species in the future.

5.2 Limitations of the study

Small sample sizes are often an issue in ecological and genetic studies. Most samples used in this study were only identified from female and juvenile samples. Sample size for males were limited because of the rarity of the adult's specimens, mainly the male. The results of the phylogenetic analyses of *Liphistius* could be more comprehensive if more species were available. This could help define the evolutionary lineages of *Liphistius* and thus help in the effort to conserve the species. Apart from that, more genetic marker should be used to clarify the phylogenetic relationship among species. It is understood that the phylogeny concluded from a single marker gene only reflects the evolution of that particular gene. And the use of single marker can lead to interpretation problems as different genes may show different rate of evolutions. In addition, investigations of *Liphistius* are difficult as there is limited number of existing sequences in the GenBank database.

5.3 Further research

The conservation and management of species depends on a comprehensive understanding of its distributional patterns, population structure and species status of species. This study has helped to address several gaps in our knowledge about the distribution and evolution of *Liphistius* species. This study is the first to examine the genetic materials of *Liphistius* species which includes more species representative. Even though this study has given us important understanding into phylogenetic relationships of *Liphistius* species, further research is required to investigate some of the issue raised.

Clarification of the taxonomic issue raised in this study clearly awaits analysis of morphometric characters in combination with additional molecular data. Previous study had used characters that were subjective and difficult to be interpreted by other researchers. Genitalic differences are traditionally the most important feature in distinguishing species in this genus, however, very little is currently known about the genitalic variation within the same species. This issue has been raised previously (Schwendinger, 1995; Haupt, 2003), however, the lack of male samples remain a major obstacle. Work on other liphistiids spiders such as *Ryuthela* species had revealed a great intra-population variety in female receptacle, while much less variations in the shape of male palp (Tanikawa, 2013). Thus, the possibility for the same occurrence in *Liphistius* is not impossible. Indeed, more samples are needed to ensure this. Problems with species identification eventually mean that there is a need in more research in order to provide a better decision for conservation management.

Phylogeographic studies have been used to infer the evolutionary history of a species (Provan & Bennett, 2008), and to study the principles and process governing the geographic distribution of genealogical lineages, especially those within and among closely related species (Avise, 2000). Unraveling current and past demographic events and identifying key regions that should be prioritized for conservation have made clarifying the evolutionary history of biota to be crucial. (Lee et al., 2012). Although this study has revealed a wide distribution range of *Liphistius* species in Peninsular Malaysia, there is no record of the genus from Sabah and Sarawak. Thus, *Liphistius* provide an ideal candidate to examine the impact of past climate and environmental events across the region.

The genus *Liphistius* has been revealed to be strongly endemic with some species having a very limited range distribution. It is commonly known that limited range endemic species are vulnerable to extinction through habitat loss, and increased urban and rural development (Harvey, 2002) Therefore, it is important to include this spider genus in a well-managed conservation planning to help preserve biodiversity and natural heritage of Malaysia.

5.4 Conclusion

Based on the results of this study, nine *Liphistius* species were taxonomically identified, with ten unidentified species. Five known species and two unknown species has been further investigated in the molecular analyses and proven to be distinct. Apart from that, many new populations have been found, especially those from the reserved and protected forest. This study has opened a new chapter in the spider research in Malaysia.

This is the first molecular study that was carried out to test the phylogenetic relationship of the genus *Liphistius*. The molecular phylogenetic presented here used only the fast evolving genes, COI to preliminarily answer some taxonomic questions within the group. The result has further confirm the monophyly of *Liphistius* species with strong support in the Bayesian analysis. Relationship between species however, was less resolved.

This molecular study has led to a better understanding of the relationship among *Liphistius* species from Peninsular Malaysia. However, our taxonomic coverage is not comprehensive and lacks approximately 10 other species that has been found to be distributed throughout Peninsular Malaysia. Given these results, it is recommended that a revised morphological analysis be carried out to further investigate the taxonomic status of the *Liphistius* spider in Peninsular Malaysia.

Finally, this current study indicated that the COI gene is a reliable tool to investigate the genetic relationships among the existing species of *Liphistius* species from Peninsular Malaysia. This study could be improved by increasing taxon sampling and adding study regions from nuclear genes to gain more informative phylogenetic estimation. These results should be considered as an initial step toward understanding *Liphistius* relationships as they were only inferred from a single gene. This testimony of the *Liphistius* species will pave the way for future discussion on spider conservation strategy in Malaysia specifically and towards a better conservation assessment.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

Oral

A survey of the trap door spider genus *Liphistius* in Peninsular Malaysia, Symposium 'Spiders of the Greater Mekong Region', 12-15 November 2012, Pakse, Champasak, Laos, (International)

Phylogenetic relationship revealed by DNA barcoding of trap door spider genus *Liphistius* (Aranae: Liphistiidae) from Peninsular Malaysia, 2nd Annual Scientific Seminar, Zoological & Ecological Research in Progress (ZERP 2013), 27th December 2013, Institute of Biological Sciences, University of Malaya, (National)

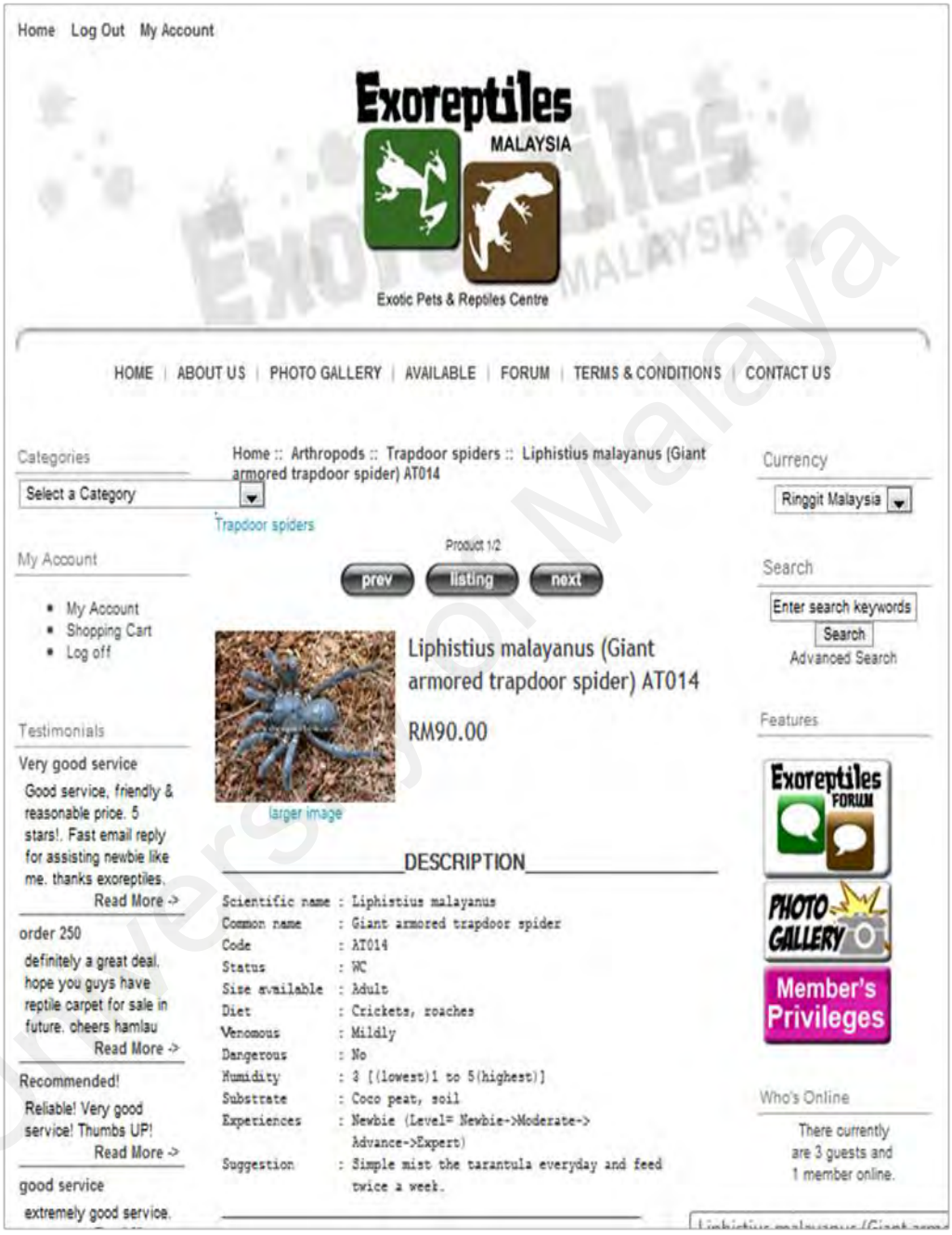
Phylogeny of the trap door spider genus *Liphistius* (Aranae: Mesothelae: Liphistiidae) in Peninsular Malaysia: An assessment based on partial cytochrome c oxidase subunit I sequences, 18th Biological Science Graduate Congress (BSGC), 6-8 January 2014, University of Malaya, Kuala Lumpur, (International)

Poster

Distribution of trap door spider genus *Liphistius* (Aranae: Liphistiidae) in Peninsular Malaysia- A preliminary survey, Malaysia International Biological Symposium (*i*-SIMBIOMAS 2012), 11-12 July 2012, Residence® Hotel at UNITEN, (International)

APPENDICES

Appendix 1 Online Website selling *Liphistius*



Appendix 2 The Star misleading headline

THE STAR ONLINE

Up 2 date on everything lifestyle

News Business Sport Metro Tech Lifestyle Travel Opinion Videos

Archives Home > Archives

REPLAY Ipoh • Kuching • Putrajaya

Tarantula under threat

Saturday April 2, 2005

Tarantula under threat

BY TEOH TEIK HOONG

PETALING JAYA: Demand from international spider collectors and local ignorance are killing a unique species of tarantula in Fraser's Hill.

A trap-door spider called *Liphistius malayanus*, which has remained ecologically unchanged since the dinosaur era, literally making it a living fossil, is found only at the hill resort and nowhere else in the world.

Its survival is now under threat.

"The trade in exotic Malaysian species is on the rise overseas with this particular tarantula being advertised for sale in the United States. Baby tarantulas are being offered for US$60 (RM228) each," said independent researcher Stephen Hogg, who has been studying the Fraser's Hill tarantula for almost two years.

"This species can only be found in Fraser's Hill. It is not difficult to work out where those being offered for sale in the United States came from," he told *The Star*.

Appendix 3 Wildlife Conservation Act

20 April 2012
20 April 2012
P.U. (A) 103

WARTA KERAJAAN PERSEKUTUAN

FEDERAL GOVERNMENT GAZETTE

PERINTAH PEMULIHARAAN HIDUPAN LIAR (PINDAAN JADUAL) 2012

WILDLIFE CONSERVATION (AMENDMENT OF SCHEDULE) ORDER 2012

DISIARKAN OLEH/
PUBLISHED BY
JABATAN PEGUAM NEGARA/
ATTORNEY GENERAL'S CHAMBERS

Appendix 4 Sequences nucleotide composition

	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	Pos #1	T-2	C-2	A-2	G-2	Pos #2	T-3	C-3	A-3	G-3	Pos #3
Liphistius_LS054_TelukBahang	36.4	21.0	28.1	14.5	552.0	22	23.4	31.5	23.4	184.0	46	25.5	13.6	15.2	184.0	42	14.1	39.1	4.9	184.0
Liphistius_LS059_Tekam	32.1	20.8	32.2	14.9	552.0	21	21.2	34.2	23.9	184.0	44	26.1	13.0	16.8	184.0	32	15.2	49.5	3.8	184.0
Liphistius_LS067_Tekam	32.1	20.8	32.2	14.9	552.0	21	21.2	34.2	23.9	184.0	44	26.1	13.0	16.8	184.0	32	15.2	49.5	3.8	184.0
Liphistius_LS080_UluKinta	36.6	21.0	27.5	14.9	552.0	22	22.3	32.1	23.4	184.0	46	25.5	13.6	15.2	184.0	42	15.2	37.0	6.0	184.0
Liphistius_LS081_UluKinta	36.6	21.0	27.5	14.9	552.0	22	22.3	32.1	23.4	184.0	46	25.5	13.6	15.2	184.0	42	15.2	37.0	6.0	184.0
Liphistius_LS082_UluKinta	36.6	21.0	27.5	14.9	552.0	22	22.3	32.1	23.4	184.0	46	25.5	13.6	15.2	184.0	42	15.2	37.0	6.0	184.0
Liphistius_LS093_Endau	33.5	20.5	30.8	15.2	552.0	22	20.1	33.7	23.9	184.0	44	26.1	13.0	16.8	184.0	34	15.2	45.7	4.9	184.0
Liphistius_LS098_Kemensah	33.3	21.9	29.9	14.9	552.0	21	21.2	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	35	19.0	42.4	3.8	184.0
Liphistius_LS140_KotaTinggi	34.2	20.7	29.5	15.6	552.0	23	19.6	33.7	23.9	184.0	45	25.5	13.0	16.8	184.0	35	16.8	41.8	6.0	184.0
Liphistius_LS141_KotaTinggi	33.9	20.8	29.7	15.6	552.0	23	19.6	33.7	23.9	184.0	45	25.5	13.0	16.8	184.0	34	17.4	42.4	6.0	184.0
Liphistius_LS142_Kenyir	32.4	20.7	32.1	14.9	552.0	21	20.7	34.2	23.9	184.0	45	26.1	12.5	16.8	184.0	32	15.2	49.5	3.8	184.0
Liphistius_LS143_Kenyir	32.4	20.7	32.1	14.9	552.0	21	20.7	34.2	23.9	184.0	45	26.1	12.5	16.8	184.0	32	15.2	49.5	3.8	184.0
Liphistius_sp._LM01	31.5	23.7	30.1	14.7	552.0	20	22.3	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	30	23.4	42.9	3.3	184.0
LS049_Pasir_Raja	32.4	20.7	32.1	14.9	552.0	21	20.7	34.2	23.9	184.0	45	26.1	12.5	16.8	184.0	32	15.2	49.5	3.8	184.0
LS051_Pasir_Raja	32.4	20.7	32.1	14.9	552.0	21	20.7	34.2	23.9	184.0	45	26.1	12.5	16.8	184.0	32	15.2	49.5	3.8	184.0
L_desultor_LS021_Penang	36.1	21.6	27.9	14.5	552.0	22	23.4	31.5	23.4	184.0	46	25.5	13.6	15.2	184.0	41	15.8	38.6	4.9	184.0
L_desultor_LS034_Penang	36.1	21.6	27.9	14.5	552.0	22	23.4	31.5	23.4	184.0	46	25.5	13.6	15.2	184.0	41	15.8	38.6	4.9	184.0
L_desultor_LS036_Penang	36.1	21.6	27.9	14.5	552.0	22	23.4	31.5	23.4	184.0	46	25.5	13.6	15.2	184.0	41	15.8	38.6	4.9	184.0
L_desultor_LS053_TelukBahang	36.4	21.0	28.1	14.5	552.0	22	23.4	31.5	23.4	184.0	46	25.5	13.6	15.2	184.0	42	14.1	39.1	4.9	184.0
L_endau_LS094_Endau	33.5	20.5	30.8	15.2	552.0	22	20.1	33.7	23.9	184.0	44	26.1	13.0	16.8	184.0	34	15.2	45.7	4.9	184.0
L_endau_LS148_Selai	33.7	20.5	30.4	15.4	552.0	23	19.0	33.7	23.9	184.0	44	26.1	13.0	16.8	184.0	34	16.3	44.6	5.4	184.0
L_laruticus_LS125_Bukit_Larut	34.8	22.3	27.0	15.9	552.0	21	23.4	31.0	24.5	184.0	45	26.1	13.6	15.2	184.0	38	17.4	36.4	8.2	184.0
L_laruticus_LS128_Bukit_Larut	34.8	22.3	27.0	15.9	552.0	21	23.4	31.0	24.5	184.0	45	26.1	13.6	15.2	184.0	38	17.4	36.4	8.2	184.0
L_laruticus_LS136_Bukit_Larut	34.1	22.3	27.4	16.3	552.0	21	23.4	31.0	24.5	184.0	45	26.1	13.0	15.8	184.0	36	17.4	38.0	8.7	184.0
L_malayanus_LS001_Genting	33.3	22.3	29.2	15.2	552.0	20	22.3	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	36	19.0	40.2	4.9	184.0
L_malayanus_LS001_Ulu_Gombak	31.5	23.7	30.1	14.7	552.0	20	22.3	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	30	23.4	42.9	3.3	184.0
L_malayanus_LS008_Fraser_Hill	33.0	22.5	29.3	15.2	552.0	20	22.3	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	35	19.6	40.8	4.9	184.0
L_malayanus_LS009_Genting	33.3	22.3	29.2	15.2	552.0	20	22.3	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	36	19.0	40.2	4.9	184.0
L_malayanus_LS010_Genting	32.4	23.0	30.1	14.5	552.0	18	23.4	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	34	20.1	42.9	2.7	184.0
L_malayanus_LS012_Fraser_Hill	33.5	21.9	29.0	15.6	552.0	20	22.3	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	36	17.9	39.7	6.0	184.0
L_malayanus_LS013_Ampang	33.2	22.1	30.1	14.7	552.0	20	21.7	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	35	19.0	42.9	3.3	184.0
L_malayanus_LS014_Ampang	33.2	22.1	30.1	14.7	552.0	20	21.7	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	35	19.0	42.9	3.3	184.0
L_malayanus_LS018_Ulu_Gombak	31.5	23.7	30.1	14.7	552.0	20	22.3	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	30	23.4	42.9	3.3	184.0
L_malayanus_LS019_Ulu_Gombak	31.5	23.7	30.1	14.7	552.0	20	22.3	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	30	23.4	42.9	3.3	184.0
L_malayanus_LS020_Ulu_Bendul	32.8	22.6	28.8	15.8	552.0	20	22.3	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	34	20.1	39.1	6.5	184.0
L_malayanus_LS114_Ulu_Bendul	32.8	22.6	28.8	15.8	552.0	20	22.3	34.2	23.9	184.0	45	25.5	13.0	16.8	184.0	34	20.1	39.1	6.5	184.0
L_murphyorum_LS022_TelukBahang	35.9	20.7	29.3	14.1	552.0	25	17.9	32.6	24.5	184.0	45	26.6	13.0	15.8	184.0	38	17.4	42.4	2.2	184.0
L_murphyorum_LS035_TelukBahang	35.9	20.7	29.3	14.1	552.0	25	17.9	32.6	24.5	184.0	45	26.6	13.0	15.8	184.0	38	17.4	42.4	2.2	184.0
L_murphyorum_LS037_TelukBahang	35.9	20.7	29.3	14.1	552.0	25	17.9	32.6	24.5	184.0	45	26.6	13.0	15.8	184.0	38	17.4	42.4	2.2	184.0
Ryuthela_ishigakiensis_IR11	35.9	20.1	27.4	16.7	552.0	23	19.6	31.5	25.5	184.0	45	26.1	13.0	16.3	184.0	40	14.7	37.5	8.2	184.0
Avg.	33.9	21.6	29.4	15.0	552.0	21	21.5	33.2	23.9	184.0	45	25.8	13.1	16.3	184.0	36	17.5	42.0	4.8	184.0

Appendix 5 Maximum parsimony phylogenetic tree; bootstrap value <50 was not shown