UNIVERSITI PUTRA MALAYSIA

INTEGRATIVE TAXONOMY AND MOLECULAR PHYLOGENETIC ANALYSIS OF BENT-WINGED FIREFLIES (COLEOPTERA: LAMPYRIDAE: Pteroptyx) IN PENINSULAR MALAYSIA AND SARAWAK

WAN FARIDAH AKMAL W. JUSOH

FP 2015 93

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By

WAN FARIDAH AKMAL W. JUSOH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

March 2015

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

INTEGRATIVE TAXONOMY AND MOLECULAR PHYLOGENETIC ANALYSIS OF BENT-WINGED FIREFLIES (COLEOPTERA: LAMPYRIDAE: *Pteroptyx*) IN PENINSULAR MALAYSIA AND SARAWAK

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Species delineation using integrative taxonomy has become popular since about a decade ago. Integrative taxonomy entails the use of multiple tools including DNA barcoding and morphological examination of specimens. Integrative taxonomy has reduced major problems in taxonomy such as the presence of cryptic species and the overabundance of synonymous names. In this study, the application of integrative taxonomy in Malaysian firefly taxonomy was presented. To do so, the diversity of fireflies in mangrove estuaries was explored by combining data of geographic distribution, morphology, DNA sequencing and phylogeny of fireflies. The geographic location was restricted to 11 mangrove sites in Malaysia, from which 756 specimens were studied. Morphological data was found to be compatible with molecular data and all species were monophyletic, i.e. both indicated 12 species including non-bent-winged firefly species. However, inclusion of females and larvae in DNA barcoding analysis increased the species count to 14 species. Using combined molecular datasets of three genes (mitochondrial DNA (mtDNA) cytochrome oxidase I (COI), mtDNA fragment containing the 3' end of COI gene, the adjacent complete of tRNAleu gene and the 5' end of the COII gene (COI-COII) and nuclear protein coding gene CAD)), the Bayesian analysis revealed that *Pteroptyx* was a monophyletic clade but it was clustered into two groups: A. (*Pteroptyx asymmetria + P. tener + P. bearni*)*;* B (*Pteroptyx gelasina, Pteroptyx malaccae* and *Pteroptyx valida*). The intraspecific variations in two bent-winged firefly species, *Pteroptyx bearni* Olivier and *Pteroptyx tener* Olivier arising from geographical isolation were confirmed. The analysis also supported the position of *P. gelasina* in the same clade with *P. malaccae* but with strong posterior probability to be distinguished as a distinct species. Consequently, a list of species and DNA barcode

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reference library of Malaysian fireflies were created for the first time. This study therefore supports the perspective that integrative taxonomy will speed up species discovery while maintaining species inventories. The application of such information for firefly and mangrove conservation is highly recommended.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

TAKSONOMI BERSEPADU DAN ANALISIS FILOGENI MOLEKUL BAGI KELIP-KELIP BERSAYAP LIPAT (COLEOPTERA: LAMPYRIDAE: *Pteroptyx*) DI SEMENANJUNG MALAYSIA DAN SARAWAK

Oleh

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Persempadanan spesies menggunakan taksonomi integratif telah menjadi popular sejak diperkenalkan kira-kira sedekad yang lalu. Taksonomi integratif melibatkan penggunaan pelbagai alat termasuk kod-bar DNA dan pemeriksaan morfologi specimen. Taksonomi integratif telah mengurangkan masalah seperti kehadiran spesies kripta dan kelimpahan nama-nama sinonim. Dalam kajian ini, penggunaan taksonomi integratif dalam taksonomi kelip-kelip Malaysia telah dibentangkan. Untuk berbuat demikian, kepelbagaian kelip-kelip di muara bakau telah diterokai dengan menggabungkan data taburan geografi, morfologi, penjujukan DNA dan filogeni kelip-kelip. Lokasi geografi terhad kepada 11 kawasan hutan bakau di Malaysia, yang mana sebanyak 756 spesimen telah dikaji. Data morfologi didapati serasi dengan data molekul dan semua kumpulan adalah monofiletik, iaitu 12 spesies dikenalpasti termasuk spesies kelip-kelip bukan bersayap lipat. Walau bagaimana pun, kemasukan specimen betina dan larva telah meningkatkan jumlah spesies kepada 14. Dengan menggunakan gabungan tiga set data molekul iaitu mitokondria DNA (mtDNA) cytochrome oxidase I (COI), pecahan mtDNA yang mengandungi 3’ terakhir gen COI, gen tRNAleu terhampir and 5’ terakhir gen COII (COI-COII) dan gen pengekodan protein nuclear, CAD, analisis Bayesian mendedahkan bahawa *Pteroptyx* adalah klad monophyletic tetapi ia berkelompok kepada dua kumpulan: A. (*Pteroptyx asymmetria* Ballantyne + *Pteroptyx tener* Olivier + *Pteroptyx bearni* Olivier); B (*Pteroptyx gelasina* Ballantyne*, Pteroptyx malaccae* Gorham dan *Pteroptyx valida* Olivier). Variasi intraspesies dalam dua spesies kelip-kelip bengkok bersayap, *P. bearni* dan *P. tener* yang disebabkan oleh isolasi geografi telah disahkan. Analisis ini juga menyokong kedudukan *P. gelasina* dalam klad yang sama dengan P. malaccae tetapi dibuktikan sebagai spesies berbeza oleh kebarangkalian posterior yang tinggi. Di akhir kajian ini, satu senarai spesies dan rujukan perpustakaan kod bar DNA kelip-kelip Malaysia telah diwujudkan buat kali pertama. Objektif utama kajian ini menyokong perspektif yang taksonomi integratif akan mempercepatkan penemuan spesies yang sama mengekalkan spesies inventori. Penggunaan maklumat tersebut untuk kelip-kelip dan pemuliharaan bakau amat disyorkan.

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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisory committee members: Associate Professor Dr. Nur Azura Adam, Professor Dr. Rita Muhamad and Professor Dr. Niklas Wahlberg who had been very helpful and supportive throughout my studies. I am grateful to Niklas for his constructive criticisms and thoughtful words which greatly improved my understanding in molecular systematics. I thank my former supervisors: Dr. Nor Rasidah Hashim, Associate Professor Dr. Zelina Zaiton Ibrahim and Dr. Law Yao Hua for sharing their expert advice in this study.

This study would not be possible without the generous funding from Ministry of Education (MyBrain15). DNA barcoding project was partially funded by Research University Grant Scheme, Malaysia (03-01-12-1602RU) and Nagao Natural Environment Foundation. I thank Dr. Jadranka Rota, Dr. Carlos *Peña* and Eero Vesterinen for lab assistance and Musa Musbah for sharing his material of Sarawak fireflies. Dr. Lesley Ballantyne (Charles Sturt University, Australia) and Dr. Ilari E. Sääksjärvi (Zoological Museum of University of Turku) provided valuable discussion on taxonomic studies.

I thank the individuals who helped me with my field sampling from 2011-2013: En. Ngatemin Saru (Rembau), Audi Jamaluddin, Neo, Raja Nadia, En. Fadhlullah Abd Aziz (UPM), Hiswani Mislan (Kota Tinggi), Pok Li (Chukai), En. Faizy Said (Merbok), Pak Min (Pekan) and Musa Musbah (Miri). I would also like to thank colleagues and friends at University of Turku for their encouragement and wonderful hospitality during my 32-months-mobility in Finland.

Last but not least, I thank my family and friends for their love and support.

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I certify that a Thesis Examination Committee has met on 30 March 2015 to conduct the final examination of Wan Faridah Akmal W. Jusoh on her thesis entitled "Integrative Taxonomy and Molecular Phylogenetic Analysis of Bent-Winged Fireflies (Coleoptera: Lampyridae: *Pteroptyx*) in Peninsular Malaysia and Sarawak" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

Common scientific usage

CAD	Cinnamyl alcohol dehydrogenase
COI	Cytochrome *c* Oxidase subunit I
COII	Cytochrome *c* Oxidase subunit II
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
UV	Ultra Violets

Morphological characters

A	Indication to a length from dorsal base of lateral lobes to median lobe, usually used to measure the dimension of aedeagus expressed as B/A
B	Indication to a length from dorsal base of lateral lobes to apex of lateral lobe, usually used to measure the dimension of aedeagus expressed as B/A
BL	Body length (=PL+EL)
BP	Basal piece
EL	Elytra length
FS	Flagellar segments of antenna
LL	Lateral lobes of aedeagus
LO	Light organ
MFC	Metafemoral comb
MPP	Median posterior projection
PLP	Posterolateral projection
PL	Pronotal length
T7, 8 etc.	Abdominal tergites
V6, 7 etc.	Abdominal ventrites

Specimen depository

AMNH	American Museum of Natural History
BBM	Bernice Bishop Museum, Hawaii
BORN	Borneensis, Sabah
LM	National Museum of Natural History Leiden
MNHN	Muséum National *d'*Histoire Naturelle*, Paris (France)*
MCSNG	Museo Civico di Storia Naturale Genoa (Italy)
NHML	Natural History Museum London
NMPC	National Museum Prague Czech Republic
FAUPM	Faculty of Agriculture, Universiti Putra Malaysia
TLC	The Linnean Collections, The Linnean Society of London (London)
UPOL	Collection of the Palacky University Olomouc Czech Republic
UQ	University of Queensland (Department of Entomology)

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USNM	United States National Museum, Washington (United States)
ZMMU	Zoological Museum of Moscow University, Moscow (Russia)
ZMUT	Zoological Museum of University of Turku, Finland

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Taxonomy is facing a crisis, either known as ‘taxonomic impediment’ (inability to identify species reliably) (Wheeler, 2004) or ‘taxonomic deficit’ (imbalanced proportion of expected taxa against named taxa) (Arribas et al., 2012). Historically, morphology has been used as the only approach to delimit species, however, the ‘old-fashioned’ typological approach with which taxonomists still describe the species is believed to be ‘the most over-looked aspects of the taxonomy crisis’ thus creating frustration among non-taxonomist biologists (Dayrat, 2005). The crisis has promoted the emergence of an integrative taxonomy which brings new theories and methods to describing the diversity of life (Dayrat, 2005; DeSalle et al., 2005; Padial et al., 2010).

The integrative taxonomy stands for an approach to use many different sources of data such as morphology, molecular, ecology and behaviour to delimit species (Padial et al., 2010; Yeates et al. 2011; Bergsten et al., 2012) in a stable and transparent manner (Fujita et al., 2012; Riedel et al., 2013). The term has been resounded in recent series of publications, mainly on successful discovery of putative new species using traditional morphology and DNA sequence data (Glaw et al., 2010; Heethoff et al., 2011; Reséndiz-Flores et al., 2014). Integrative taxonomy also paves the way for phylogenetic inference using molecular data (e.g. Wahlberg et al., 2014; Heethoff et al., 2011) or in some cases, using a combined dataset of several molecular markers and a morphological data matrix (Wahlberg et al., 2005). Although some studies are sceptical about the use of integrated data in taxonomy particularly DNA barcodes, they support the idea that taxonomy should be integrative (Will et al., 2005; Valdecasas et al., 2008; Wheeler, 2008). In this sense, it is apparent that taxonomy should be a ‘pluralistic quality’ to enhance species discovery and description (Padial et al., 2010).

DNA barcoding is a technique for sequencing a short standardized segment (~650 base pairs) of the 5’end of the mitochondrial cytochrome *c* oxidase I for biological species identification (Hebert et al., 2003). The COI gene region is proving highly effective in identifying many animal groups and it is also proven to identify variations among species (Hebert et al., 2004). DNA barcoding is not a replacement for traditional taxonomy in the delineation of species, instead it is a quick and informative tool that will greatly facilitate species identification (Ferri et al., 2009). Some have suggested that DNA barcoding will facilitate and complement taxonomic study (Hebert et al., 2003; Ferri et al., 2009; Valentini et al., 2009).

However, in order to overcome the taxonomic impediment in the study of fireflies, the suitability and efficacy of morphological approach and DNA barcoding concept needs to be addressed. In doing so, the following questions were asked in this study: How well morphology and DNA barcodes reveal true firefly species diversity? How quickly and accurately the species identified by DNA barcodes? Can integration of both techniques be a useful tool for detection of new species?

The use of DNA barcodes in the study of beetles are widely recognized (e.g. Arribas et al., 2012; Bergsten et al., 2012), but it was rarely reported from a group of mostly luminous beetles, Lampyridae. Lampyridae is known as fireflies, lightning bugs or glow-worms. The scientific studies of fireflies were started since two centuries ago (Buck, 1948), mostly on taxonomy. The firefly taxonomy that is based on the morphological characters could be traced back as early as 1800 (see Olivier, 1907). Today over 2000 species are recorded worldwide, of which less than ten percent were from Malaysia (McDermott, 1966). The present study uses an integrative approach to explore species boundaries of Malaysian fireflies, particularly the bent winged fireflies of the genus *Pteroptyx* Olivier by using morphology, DNA sequencing and geographic distribution data. Following successful species identification using integrative data, a species checklist and DNA barcode library of Malaysian firefly species are presented.

1.2 Problem statement

There is no doubt that the taxonomy of the bent-winged fireflies have been well revised since it was first introduced. However, identification and revision of the firefly species have been traditionally performed using morphological characters. Female firefly specimens have been neglected in taxonomic inventories because morphological descriptions relied only on firefly male specimens. In light of successful integrative taxonomy in many invertebrates, this study shows a necessity to integrate molecular data with morphological characters in current study. Since many of the *Pteroptyx* species inhabit mangrove forests, which are in many places threatened by development, the need to properly inventorise and to rapidly establish their distribution is urgent.

1.3 Objectives

The main objectives of this study are as follows:

1. To develop a checklist of Malaysian fireflies;

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2. To delineate firefly species using DNA barcode Cytochrome *c* Oxidase subunit I (COI) sequence;
3. To refine species identification based on genital examination as well as to establish distribution of the bent-winged fireflies in Malaysia and other flashing firefly species occurring in the same vicinity;
4. To infer the phylogenetic relationship among the bent-winged firefly species;

The main objectives above are addressed and covered in the specific objectives as laid out in Chapters 3-6.

1.4 Outline of thesis

This thesis is divided into seven chapters. Following this introductory chapter is Chapter 2 which presents some background for the research presented in this thesis. The literature addresses three important topics of fireflies: taxonomy, phylogeny and geographic distribution with a review of relevant studies on the bent-winged fireflies. In Chapter 3, a thorough review of Lampyridae catalogue to develop a first checklist of Malaysian fireflies are presented. In Chapter 4, the effectiveness of DNA barcodes to delineate and correct assignment of sexes to species are studied. This study determines the potential of barcoding method to identify female fireflies. In Chapter 5, morphology of fireflies are re-described using genital and bursa plate structures to refine species identification. Additionally, species' geographic distribution are established. In Chapter 6, molecular phylogenetic of the genus *Pteroptyx* inferred from molecular data is carried out to investigate the evolutionary relationships among species and to study possible morphological variation in the studied species. Finally, summary of this study and recommendations for future research are presented in Chapter 7.

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LIST OF PUBLICATIONS

Journal Articles (arised from this thesis)

1. **Wan F. A. Jusoh**, Nor Rasidah Hashim, Ilari E. Sääksjärvi, Nur Azura Adam and Niklas Wahlberg. 2014. Species Delineation of Malaysian Mangrove Fireflies (Coleoptera: Lampyridae) Using DNA Barcodes. *The Coleopterists Bulletin*. 68(4):703-711.

2. **Wan Faridah Akmal Jusoh**, Nor Rasidah Hashim & Nur Azura Adam. 2013. Distribution of the Synchronous Flashing Beetle *Pteroptyx tener* Olivier (Coleoptera: Lampyridae) in Malaysia. *The Coleopterists Bulletin*. 67 (4), 604-605. (ISI)

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