



**Diet of the wrinkle-lipped free-tailed bat (*Chaerephon plicatus*
Buchanan, 1800) in central Thailand**

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**A Thesis Submitted in Partial Fulfillment of the Requirements for
the Degree of Master of Science in Ecology (International Program)**

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Thesis Title Diet of the wrinkle-lipped free-tailed bat (*Chaerephon plicatus*
Buchanan, 1800) in central Thailand

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| ชื่อวิทยานิพนธ์ | อาหารของค้ำคาวปากย่น (<i>Chaerephon plicatus</i> Buchannan, 1800) ในภาคกลางของประเทศไทย |
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บทคัดย่อ

เพลี้ยกระโดดสีน้ำตาลเป็นแมลงศัตรูพืชที่สำคัญของนาข้าว ก่อให้เกิดความเสียหายอย่างมากกับผลผลิตข้าว งานวิจัยก่อนหน้านี้รายงานว่า ค้ำคาวปากย่นมีบทบาทสำคัญในการควบคุมปริมาณเพลี้ยกระโดดหลังขาวในนาข้าว แต่ยังไม่เคยมีรายงานบทบาทในการควบคุมเพลี้ยกระโดดสีน้ำตาล ดังนั้นวัตถุประสงค์ของงานวิจัยครั้งนี้เพื่อศึกษาอาหารของค้ำคาวปากย่นในพื้นที่ที่มีการระบาดของเพลี้ยกระโดดสีน้ำตาล โดยวิเคราะห์อาหารของค้ำคาวจาก 2 ถ้ำ ที่มีอัตราส่วนของพื้นที่นาข้าวรอบถ้ำต่างกัน (70% และ 22%) การเก็บข้อมูลดำเนินการเดือนละครั้ง ตั้งแต่เดือน ตุลาคม พ.ศ. 2558 ถึง เดือนกันยายน พ.ศ. 2559 วิเคราะห์กองมูลจำนวน 720 กอง จากไต้หวันอน ผลการศึกษาพบว่าค้ำคาวปากย่นกินแมลง 8 อันดับ คือ Coleoptera, Homoptera, Hemiptera, Diptera, Lepidoptera, Odonata, Hymenoptera และ Orthoptera โดยแมลงในอันดับ Homoptera มีเปอร์เซ็นต์ปริมาตรมากที่สุดในช่วงที่มีการทำนาข้าว ส่วนแมลงในอันดับ Coleoptera มีมากที่สุดในช่วงที่ไม่มีการทำนาข้าว แมลงในอันดับ Homoptera ส่วนใหญ่สามารถจัดจำแนกได้ว่าเป็นเพลี้ยกระโดดสีน้ำตาล ซึ่งเป็นศัตรูข้าวที่สำคัญในนาข้าว การนับจำนวนอวัยวะเพศผู้ (male genitalia) ของเพลี้ยกระโดดสีน้ำตาลพบว่ามีจำนวนมากที่สุดในช่วงที่มีการทำนามีค่าเฉลี่ย 4 ตัวต่อกองมูล การวิเคราะห์อาหารของค้ำคาวโดยใช้ทั้งสองวิธี คือ การประเมินเปอร์เซ็นต์ปริมาตร (percent volume) และ เปอร์เซ็นต์ความถี่ (percent frequency) พบว่าอาหารของค้ำคาวปากย่นไม่มีความแตกต่างกันอย่างมีนัยสำคัญระหว่างถ้ำทั้งสอง ($p > 0.05$) แม้ว่าสัดส่วนของนาข้าวรอบถ้ำจะแตกต่างกันการศึกษารุ่นนี้แสดงให้เห็นว่า ค้ำคาวปากย่นสามารถกินเพลี้ยกระโดดสีน้ำตาลได้อย่างน้อยสิบล้านตัวต่อคืน ซึ่งแสดงว่าค้ำคาวปากย่นเป็นตัวควบคุมทางชีวภาพที่สำคัญของเพลี้ยกระโดดสีน้ำตาลในนาข้าว

Thesis Title Diet of the Wrinkle-Lipped Free-Tailed Bat (*Chaerephon plicatus* Buchanan, 1800) in Central Thailand

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Abstract

Brown planthopper is one of the major insect pests of rice field. They have been widely acknowledged for significantly causing yield losses of rice production. Using wrinkle-lipped free-tailed bat (*Chaerephon plicatus* Buchanan, 1800) as a biological pest control agent for planthoppers were previously reported, however, it was unprecedented for brown planthoppers. The objective of this study to determine the diet of *C. plicatus* in the areas where brown planthopper is common. To accomplish this objective, we analyzed the diet of *C. plicatus* from two caves that differed in the percentage of surrounding land area occupied by rice fields (70% vs. 22%). A year round fecal samplings were carried out monthly. The total of 720 fecal pellets was collected and analyzed, the results revealed that *C. plicatus* fed on at least 8 insect orders belonging to Coleoptera, Homoptera, Hemiptera, Diptera, Lepidoptera, Odonata, Hymenoptera and Orthoptera. Surprisingly, Homoptera made up a greatest diet volume in period of active rice field where as Coleoptera was the most abundant in the diet during inactive rice period. Most Homoptera were identified as brown planthopper, an important economic pest in rice field. The number of male brown planthopper genitalia was counted and it showed the greatest number during rice planting period, an average of four males genitalia per pellet were recorded. Assessment of percent volume and percent frequency revealed that the diet of *C. plicatus* was not significantly different between the two study caves ($p > 0.05$), even though the proportion of surrounding active rice fields was different. According to the results, this study further suggests that at least ten millions individuals of brown planthopper are consumed by this bat colony each night. The highly importance of *C. plicatus* as a biological control against the brown planthoppers in rice fields is emphasized.

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CONTENTS

| | Page |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Approval page | ii |
| Certifications | iii |
| Abstract (Thai) | v |
| Abstract (English) | vi |
| Acknowledgements | vii |
| Contents | viii |
| List of figures | x |
| Chapter 1: General Introduction | 1 |
| 1.1 Introduction | 1 |
| 1.2 Literature reviews | 2 |
| 1.2.1 Taxonomy and Generalization | 2 |
| 1.2.2 Distribution, Population and Status | 3 |
| 1.2.3 Breeding | 4 |
| 1.2.4 Habitat and Ecology | 4 |
| 1.2.5 Diet | 4 |
| 1.2.6 Material available for food habits analysis | 5 |
| 1.2.6.1 Stomach contents and fecal analysis | 5 |
| 1.2.6.2 Culled parts | 6 |
| 1.2.6.3 Direct observations of feeding bats | 7 |
| 1.3 Objectives | 7 |
| Chapter 2: The wrinkle-lipped free-tailed bat (<i>Chaerephon plicatus</i> Buchanan, 1800) feeds mainly on brown planthoppers in rice fields of central Thailand. | 8 |
| 2.1 Introduction | 10 |
| 2.2 Materials and Methods | 12 |
| 2.2.1 Study Site | 12 |
| 2.2.2 Study Species | 13 |
| 2.2.3 Dietary analysis | 14 |

| | | |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|----|
| 2.2.4 | Insect Trapping | 15 |
| 2.2.5 | Data analysis | 15 |
| 2.3 | Results | 16 |
| 2.3.1 | Bat diet | 16 |
| 2.3.2 | Brown planthopper consumption | 18 |
| 2.3.3 | Modified light traps sampling of insects | 19 |
| 2.4 | Discussion | 20 |
| 2.4.1 | Diet | 20 |
| 2.4.2 | Proportion of active rice field to the bat diet | 22 |
| 2.5 | Conservation implications | 23 |
| 2.6 | Acknowledgements | 24 |
| 2.7 | Literature cited | 24 |
| Chapter 3 : Conclusion | | 31 |
| References | | 32 |
| Appendices | | 36 |
| Appendix 1 Figure of the wrinkle-lipped free-tailed bat (<i>Chaerephon plicatus</i> Buchannan, 1800). | | 37 |
| Appendix 2 Figures of part of insect in order Coleoptera | | 38 |
| Appendix 3 Figures of part of insect in order Diptera | | 39 |
| Appendix 4 Figures of part of insect in order Hemiptera | | 40 |
| Appendix 5 Figures of part of insect in order Homoptera | | 41 |
| Appendix 6 Figures of part of insect in order Hymenoptera, Lepidoptera, Odonata and Orthoptera | | 42 |
| Appendix 7 Map illustrating the distribution of the BPH and WBPH in Thailand (data from rice research stations throughout Thailand (www.ricethailand.go.th)). | | 43 |
| Vitae | | 44 |

LIST OF FIGURES

| Figure | | Page |
|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| 1 | A map of Thailand showing active rice fields (gray: newly planted rice, black: older rice; data from GISTDA, September 2016), and our two study caves housing <i>Chaerephon plicatus</i> bats at Khao Wongkot cave (KWC, 70% surrounding rice fields) and Khao Chakan cave (KCC, 22% surrounding rice fields) in central Thailand. The two circle insets show a 20-km radius (approximate bat foraging distance) around each cave. | 13 |
| 2 | Percent volume and percent frequency (mean \pm SE) of insect orders in the bat diet of <i>Chaerephon plicatus</i> at KWC and KCC in central Thailand during October 2015 to September 2016. [Other: Orthoptera and Hymenoptera combined.] | 16 |
| 3 | Monthly variation in the percent volume and percent frequency of insect orders in the bat diet of <i>Chaerephon plicatus</i> at KWC and KCC in central Thailand during October 2015 to September 2016. [Other: Orthoptera and Hymenoptera combined.] | 18 |
| 4 | The average monthly number of male brown planthopper genitalia per pellet (mean \pm SE) of insect orders in the bat diet of <i>Chaerephon plicatus</i> at KWC and KCC in central Thailand during October 2015 to September 2016. | 19 |
| 5 | Percent drymass of insects collected in suction traps at KWC and KCC in central Thailand during October 2015 to September 2016. [Others include Orthoptera, Trichoptera, Odonata, Blattellidae, Psocoptera, Plecoptera, Isoptera, Dermaptera, and Acari]. | 20 |

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Most bats are highly mobile predators of night-flying insects, many of which are significant pests in natural and agricultural ecosystems. Many insectivorous bats are generalist predators (Lee and McCracken, 2005; Clare et al., 2009) and bats often are cited as important agents for the suppression of agricultural pests (Boyles et al., 2011). Previous studies have shown that they prey on a number of major crop pests such as corn borers, plant hoppers, tobacco budworms, and oriental armyworms (Whitaker, 1993; Leelapaibul et al., 2005). The recent studies have confirmed the importance of bats in regulate insect abundance and herbivory in tropical coffee and cacao agroforests (Karp et al. 2013, Maas et al., 2013). Large colonies of insectivorous bats could help regulate pests in surrounding farmlands. *Chaerephon plicatus* normally form large colonies, from 10,000 to 2.6 million bats in Thailand (Hillman, 1999). Currently, 17 cave colonies of this species were registered in central Thailand, in which their populations were provisionally estimated to be eight million individuals (Boonkerd and Wanghonga, 2002). Insectivorous bats ingests around half (39-73%, Kunz et al., 1995) of their body mass in insects per night. It was estimated that 2.6 million individuals of 15.5g *C. plicatus* in Khao Chong Pran Cave, Ratchaburi could consume up to 17.5 tons of insects per night (Hillman, 1999).

Dietary studies of insectivorous bats are essential for understanding their feeding behavior, food preference and their role in the ecosystem. The diet composition and foraging behavior of bats are dependent on many factors, such as region, season, time of day, and size of prey (Whitaker et al., 1996; Verts et al., 1999; Leelapaibul et al., 2005; Zhang et al., 2005). Thus, even bats of the same species, but occurring in different regions, can have highly different diets (Kurta and Whitaker, 1998). In another study Srinivasulu and Srinivasulu (2005) compared the diet composition of *Taphozous melanopogon* bats in forests versus semi-urban habitats, and found that bat foraging was influenced by the diversity and availability of insect prey, as well as by roosting conditions.

While previous work has examined the annual diet of *C. plicatus* in western Thailand (Leelapaibul et al., 2005), where white-backed planthoppers are common, no studies have examined their diet in other regions of Thailand, where brown planthoppers are common (Vungsilabutr, 2001; www.ricethailand.go.th). It is therefore highly important to investigate the diet of *C. plicatus* in other areas, and to understand their importance in the biological control of the brown planthopper. To accomplish these objectives, we analyzed the diet of *C. plicatus* from two caves that differed in the percentage of surrounding land area occupied by rice fields (70% vs 22%). We hypothesized that the diet of *C. plicatus* would depend on the proportion of rice fields around the cave, in which the colony near abundant active rice fields would have a higher proportion of brown planthoppers in the diet.

1.2 LITERATURE REVIEWS

1.2.1 Taxonomy and Generalization

Molossidae are a widely distributed family of bats which comprises 13 genera and around 89 species (Corbet and Hill, 1992). Generalization of family according to Francis (2008), they are medium-small to large insectivorous bats distinguished by their stout tail, which protrudes conspicuously beyond the narrow interfemoral membrane. Lips often with a series of folds in the skin, appearing wrinkled. The ears are variable in form, usually freshly, sometimes joined across the forehead; the tragus of each ear is rudimentary and the antitragus is usually large. Wings long and narrow that bats are adapted for swift flight. Members of the subgenus *Chaerephon* (*C. plicata*) are distinguished by the premaxillae (in the skull) which are usually fused and have palatal branches isolating the two small palatal foramina.

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Chiroptera

Suborder: Microchiroptera

Family : Molossidae

Genus: *Tadarida*

Subgenus: *Chaerephon*

Species: *C. plicatus* (Buchanan, 1800)

The common name of *Chaerephon plicatus* (Buchanan, 1800) is the 'wrinkle lipped free-tailed Bat'. Bates and Harrison (1997) reported that it is the smallest species of *Tadarida* known from the region with an average forearm length of 46.3 mm (43.1-50.2 mm). It is superficially similar to *Tadarida aegyptiaca*. However, unlike this species and *Tadarida teniotis*, the fur is soft, dense and very short, on the dorsal surface, it is usually dark brown, underparts paler with grey tips to hairs (Francis, 2008). Upper lip heavily wrinkled, nostrils protruding slightly in front; ears moderate, thick and rounded, joined across front of head by flap of skin (Francis, 2008). Two upper premolars, the anterior quite small; posterior upper molar well developed, about half the area of the second molar (Francis, 2008).

1.2.2 Distribution, Population and Status

This very widely distributed species is found throughout much of Southeast Asia: Myanmar, Thailand, Laos, Vietnam, Cambodia and Peninsular Malaysia. Also Sri Lanka, India, China, Hainan, Sumatra, Java, Borneo, Lesser Sunda Island and Philippines (Francis, 2008). This is a widespread but localized species which occurs in large colonies usually in caves (Molur et al. 2002). The total population in Thailand is around eight million, with the largest population consisting of over two million individuals (Boonkerd and Wanghongsa, 2002). Although still abundant in many areas, with some large colonies well protected, its tendency to roost in large colonies makes it vulnerable to hunting or disturbance; all large colonies have disappeared in the Philippines, and some large colonies have been lost in Cambodia and Laos (Francis, 2008).

IUCN Red List Assessment

Red List Category

LC = Least Concern

Justification; this species is listed as “Least Concern” because of its wide distribution, large population, occurrence in a number of protected areas, and because it is unlikely to be declining at the rate required to qualify for listing in a threatened category.

1.2.3 Breeding

According to Hillman (1998), *C. plicatus* breeds twice a year, with births occurring in March, April and October. The time interval between these two breeding seasons and the frequency of observations are not enough to be certain. It has been shown that rainfall is the most important factor influencing the breeding of tropical insectivorous bats due to the connection to abundance of prey (Racey, 1982; Hillman, 1998). During the dry season, there is probably less food available and the young’s development may be delayed because of reduced foraging success of the females. Therefore, it is likely that the usual period between breeding seasons is six months.

1.2.4 Habitat and Ecology

Roots in caves in large densely packed colonies, but can also be found in crevices in rocks and old disused buildings (Molur et al. 2002). This species can form large colonies of thousands or millions of individuals (Francis, 2008). Often flies out before darkness in dense flocks to forage high above ground (Francis, 2008). Populations generally forage close to roost sites, and have been recorded hunting in forested areas and over rice fields (Utathamachai, 2008). It is a high and fast flyer that feeds on insects and other invertebrates (Leelapaibul et al., 2005).

1.2.5 Diet

Stomach content analysis of female *Tadarida brasiliensis* revealed that the diet, expressed as percent volume, consists largely of lepidopterans, coleopterans, hymenopterans, and dipterans, in decreasing order of importance (Kunz et al., 1995). Individual bats produced an average of 2-3.6 insects/pellet and indicates that at least five pellets are needed to establish the number of insect taxa (families) consumed by a bat. Lee and Mcracken (2005) examined food habits of Brazilian free-tailed bats (*T. brasiliensis*) in central Texas. Fecal samples collected contained remains of 12 orders

and 35 families of insects. Daily and seasonal patterns of insect consumption were closely correlated to patterns of emergence, migration, and availability of adult populations of noctuid moths, major crop pests (Lee and Mccracken, 2005). Leelapaibul et al. (2005) studied of the diets of the Wrinkle-lipped free-tailed bats (*T.plicata* (syn.)) in Ratchaburi province reveal Homoptera, Lepidoptera, Hemiptera, Coleoptera, Diptera, Hymenoptera, Odonata, Orthoptera and Psocoptera. Homoptera had the highest frequency percentage by which most of were white-backed planthopper (*Sogatella* sp.). This study suggests that crop pests comprise a substantial portion of the bats' diet and that bats provide valuable natural pest control services.

1.2.6 Material available for food habits analysis (according to Whitaker et al., 2009)

The fragmented remains of insects and small vertebrates are usually difficult to identify; the habit of many bats to leave the harder, and often the only estimated, parts of their prey (Whitaker et al., 2009). Gardner (1977) mentions to several ways by which information on the food habits of bats can be got either directly or indirectly: stomach or fecal analysis, culled items and direct observation.

1.2.6.1 Stomach contents and fecal analysis

There are many published researches diet of insectivorous bats is from stomach contents or fecal analysis. Stomach contents analysis provides verification of the bats 'last meal', but digestion tends to destroy soft and small insect parts, resulting in a bias on less digestible parts of insect in fecal analysis (Whitaker et al., 2009).

For stomach contents analysis, bats should be killed suddenly upon capture to minimize digestion. This approach put forwards legal and ethical questions, especially when endangered or threatened species are involved. Typically, stomach content analysis is no longer preceded except the bats are available from another study, such as bats that were submitted for rabies testing

Fecal analysis, permits nondestructive sampling (McAney et al., 1991). Furthermore, problems implicated with differential digestion are not serious in insectivorous bats because most insects have hard exoskeletons composed mostly of

protein and chitin which it was long believed to be indigestible by vertebrate enzymes (Snodgrass, 1935). Feces can be collected from individually captured bats and also can be collected beneath roosts, assuming that identity of the bats is known and that there is no contamination from bats of other species. When sampling pellets from beneath a roost, it is necessary to assume that all are from different bats or times. Weakness of fecal analysis include uncertainty of the time period over which the food was eaten especially the feces collected from beneath a roost.

Whitaker et al. (1981) found good agreement between results from stomach and fecal analysis of bats, and Kunz and Whitaker (1983) found that analysis of feces allowed a relatively precise picture of insect food fed by *Myotis lucifugus* as compared to stomach analysis.

1.2.6.2 Culled parts

Analysis of culled parts can be too useful in food habits analysis. For this method to yeild reliable results, it is imperative that most or all foods be regularly culled. LaVal and LaVal (1980) indicated some biases in this method, along with the fact that insects eaten whole will not show as cullings, and insect larvae will most likely be underrepresented, if present at all (Jones, 1990).

Bell (1982) estimated that nearly 30% of microchiropterans are gleaners. Various gleaners fed rather large items that almost usually need to be culled (Fenton et al., 1983). Roosts are often used night after night by the same individual bats (Belwood and Fullard, 1984), although one should be aware that sometimes more than one species of bat will use the same roost.

Important usefulness of analyzing culled parts is greater ease and preciseness in identification of prey, because individual items often are large and diagnostic (e.g., wings, head parts etc.). However, this approach does not allow estimates of relative volumes of various foods, or of percentage of bats eating the food.

1.2.6.3 Direct observations of feeding bats

Racey and Swift (1985) used reflective tape and chemiluminescent tags to study foraging behavior of *Pipistrellus pipistrellus*. Vaughan (1976) used a night-viewing device equipped with a 135-mm f-1.8 lens to observe the foraging behavior of *Cardioderma cor* (Megadermatidae). During the dry season, this bat fed primarily by flying rapidly to the ground from a low roost to capture prey (mainly large beetles and centipedes). During the rainy season, beetles remained important, but a greater variety of items was eaten, including moths, locusts, katydids, and other relatively large insects.

1.3 OBJECTIVES

1. To examine the diets of *C. plicatus* in the central Thailand
2. To determine how many brown planthopper contribute to the diet of this bat in the central Thailand, where they are common rice pests.
3. To investigate whether the difference in the proportion of active rice field around caves affects the bat diets

CHAPTER 2

THE WRINKLE-LIPPED FREE-TAIL BAT (*Chaerephon plicatus* BUCHANNAN, 1800) FEEDS MAINLY ON BROWN PLANTHOPPERS IN RICE FIELDS OF CENTRAL THAILAND

This work was accepted to the Acta Chiropterologica

The wrinkle-lipped free-tailed bat (*Chaerephon plicatus* Buchanan, 1800) feeds mainly on brown planthoppers in rice fields of central Thailand

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Abstract

The brown planthopper (*Nilaparvata lugens* (Stal, 1854)) is one of the major insect pests of rice fields in Southeast Asia. They have been widely acknowledged for causing significant rice yield losses. However, the wrinkle-lipped free-tailed bat (*Chaerephon plicatus* Buchanan, 1800) is known biological pest suppression for white-backed planthoppers, and may also suppress brown planthopper populations. Hence, it is highly important to investigate the diet of *C. plicatus* in areas where brown planthoppers are common to determine whether these bats feed on brown planthoppers. To accomplish this objective, we analyzed the diet of *C. plicatus* from two caves that differed in the percentage of surrounding land area occupied by rice fields (70% vs. 22%). Bat fecal pellets were collected monthly for a year. A total of 720 fecal pellets were analyzed, and the results revealed that *C. plicatus* feeds on at least 8 insect orders, including Coleoptera, Homoptera, Hemiptera, Diptera, Lepidoptera, Odonata, Hymenoptera and Orthoptera. Specifically, homopterans comprised the greatest diet volume in the rice field growing season, where as coleopterans were most abundant in the diet when rice fields were inactive. Moreover, most homopterans were identified as brown planthoppers. To estimate the relative numbers of brown planthoppers consumed during each month, the number of genitalia of male brown planthoppers was counted. We recorded the greatest numbers of genitalia during the rice planting period, with an average of four genitalia per pellet. Examining both the percent volume and percent frequency of each insect order in the diet of *C. plicatus* revealed that the two study caves were no significantly different,

even though the proportion of surrounding active rice fields was different. Our results, suggest that tens of millions of brown planthoppers are consumed by this bat species each night. The similar diets of the two study colonies may be due to their high altitude foraging and preference for migratory insects. Our results indicate that the wrinkle-lipped free-tailed bat is an important biological suppression agent of brown planthoppers in rice fields.

Key words: Biological pest suppression, economic pest, insectivory, diet, high altitude foraging, Homoptera, percent volume, percent frequency

2.1 INTRODUCTION

Rice is a staple food of approximately 3.5 billion people worldwide. Rice production and consumption are among the highest in Asian populations (Muthayya *et al.*, 2014). In Thailand, rice production changed greatly during the Green Revolution in the 1960's, when the irrigation system was extensively developed, particularly in the Central Plains region. This advance in agricultural irrigation has increased rice production up to two to three crops per year (Srisompun and Isvilanonda, 2012). However, insect pests play a crucial role in limiting rice yields. Planthoppers are the major insect pests of rice and cause significant yield losses (Heong *et al.*, 2013). In Asia, two planthoppers of economic importance are recognized, the brown planthopper, *Nilaparvata lugens* (Stal, 1854), and the white-backed planthopper, *Sogatella furcifera* (Horvath, 1899) of the family Delphacidae (Catindig *et al.*, 2009). Planthopper outbreaks have occurred commonly in Thailand over the past 10 years due to constant food availability (from continuous rice planting) as well as misuse of insecticides (Bottrell and Schoenly, 2012). In 2010, the outbreaks caused losses worth 52 million USD, or equal to approximately 173,000 tons of rice. The brown planthopper is especially problematic, and has also caused losses in a number of major rice-producing countries in East and Southeast Asia over the past decade (Heong *et al.*, 2013).

Most insectivorous bats are highly-mobile, generalist predators of night-flying insects, many of which are significant pests in natural and agricultural ecosystems (Whitaker, 1993). Bats are often cited as important agents for the suppression of agricultural pests (Boyles *et al.*, 2011). Previous studies have confirmed the

importance of bats in regulating insect abundance and herbivory in tropical coffee and cacao agroforests (Maas *et al.*, 2013). In corn agriculture, bats suppressed crop pest numbers, crop damage and also indirectly suppressed both of pest-associated fungus and a toxic produced by the fungus (Maine and Boyles, 2015). Large colonies of insectivorous bats can therefore help regulate pests in surrounding farmlands. Until now, most studies on pest suppression have focused on a single species, *Tadarida brasiliensis*. These studies have examined the foraging behavior and prey consumption patterns of *T. brasiliensis*, together with an assessment of the ecosystem services these bats provide (Lee and McCracken, 2002; Lee and McCracken, 2005; Cleveland *et al.*, 2006; Horn and Kunz, 2008; McCracken *et al.*, 2008).

In Southeast Asia, *Chaerephon plicatus*, a close relative of *T. brasiliensis*, normally forms large colonies ranging from 10,000 to several million individuals (Hillman, 1999; Molur *et al.*, 2002). Currently, 17 cave colonies of *C. plicatus* have been registered in central Thailand, in which their populations were provisionally estimated to number around eight million individuals total (Boonkerd and Wanghongsa, 2002). Given that insectivorous bats can ingest around half of their body mass in insects per night (39-73%, Kunz *et al.*, 1995), this bat species can potentially consume a large number of insects each night in central Thailand (Leelapaibul *et al.*, 2005).

Dietary studies of insectivorous bats are essential for understanding their feeding behavior, food preference and their role in the ecosystem. The diet composition and foraging behavior of bats are dependent on many factors, such as region, season, time of day, and size of prey (Whitaker *et al.*, 1996; Verts *et al.*, 1999; Leelapaibul *et al.*, 2005; Zhang *et al.*, 2005). Thus, even bats of the same species, but occurring in different regions, can have highly different diets (Kurta and Whitaker, 1998). In another study Srinivasulu and Srinivasulu (2005) compared the diet composition of *Taphozous melanopogon* bats in forests versus semi-urban habitats, and found that bat foraging was influenced by the diversity and availability of insect prey, as well as by roosting conditions.

While previous work has examined the annual diet of *C. plicatus* in western Thailand (Leelapaibul *et al.* 2005), where white-backed planthoppers are common, no studies have examined their diet in other regions of Thailand, where brown

planthoppers are common (Vungsilabutr , 2001; www.ricethailand.go.th). It is therefore important to investigate the diet of *C. plicatus* in other areas, and to understand their importance in the biological suppression of the brown planthopper. To accomplish these objectives, we analyzed the diet of *C. plicatus* from two caves that differed in the percentage of surrounding land area occupied by rice fields (70% vs 22%). We hypothesized that the diet of *C. plicatus* would depend on the proportion of rice fields around the cave, and that the colony near abundant active rice fields would have a higher proportion of brown planthoppers in the diet.

2.2 MATERIALS AND METHODS

2.2.1 Study Site

The present study was conducted in the central flood plain of Thailand, where 17 cave colonies of the wrinkle-lipped free-tailed bat (*C. plicatus*) are registered (Boonkerd and Wanghonga, 2002). This study focused on two cave colonies of *C. plicatus* that were surrounded by different proportions of rice fields (GISTDA, 2016, Fig.1). Rice farming in both areas is usually active throughout most of the year due to irrigation. Generally, there are three seasons: hot (March–May), rainy (June–November) and the cool dry season (December–February) (Inoue *et al.*, 2016). However, a drought during our study period (October 2015–September 2016) prevented year-round farming, and rice was only grown during October–November 2015 and August–September 2016.

One of our study colonies was located at Khao Wongkot cave (KWC; 15°01'06.04'N, 100°32'42.81'E). This cave is on a 100-ha limestone outcrop and bat guano was harvested once a week by local villagers. Annual rainfall is 1,058 mm per year (Tukaew *et al.*, 2016). The surrounding land use within a 20-km radius of this cave includes rice fields (70%), sugarcane plantations (8%), corn and cassava plantations (2%), and human settlements (20%) (GISTDA, 2016; Fig.1).

The other study colony was situated at Khao Chakan cave (KCC; 13°39'44.86'N, 102°05'25.50'E), which is 265 km away from the first cave, and located on a prominent karst outcrop (750 × 1,250 m). Local villagers collect bat guano from this cave once a month. Annual rainfall in this area is 1,039 mm per year

(Noichaisin, 2016). Land use within a 20-km radius around the cave includes rice fields (22%), sugarcane plantations (24%), cassava plantations (26%), rubber plantations (3%), and human settlements (25%) (GISTDA, 2016; Fig.1).

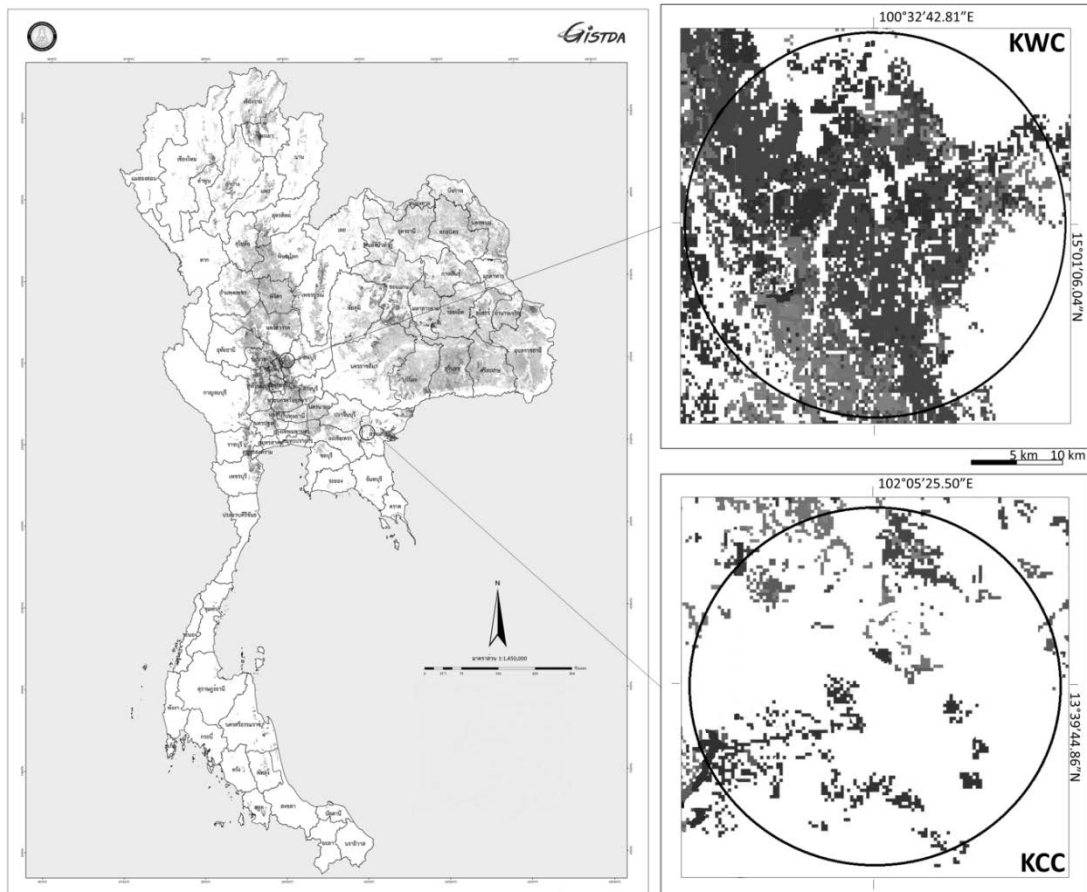


FIG. 1. A map of Thailand showing active rice fields (gray: newly planted rice, black: older rice; data from GISTDA, September 2016), and our two study caves housing *Chaerephon plicatus* bats at Khao Wongkot cave (KWC, 70% surrounding rice fields) and Khao Chakan cave (KCC, 22% surrounding rice fields) in central Thailand. The two circle insets show a 20-km radius (approximate bat foraging distance) around each cave.

2.2.2 Study Species

The wrinkle-lipped free-tailed bat (*Chaerephon plicatus* Buchanan 1800) is a free-tailed species, which was originally described as *Vespertilio plicatus* (Buchanan 1800). Subsequently, the species was reclassified as *Tadarida (Chaerephon) plicata*, *Chaerephon plicata* and *Chaerephon plicatus* (Thong, 2014). *Chaerephon plicatus* is

the smallest species in its genus with a forearm length of 46.24–49.32 mm (Thong, 2014), and an average weight of 15.5 g (Leelapaibul *et al.*, 2005). It typically roosts in caves, but can also be found in crevices in rocks and old disused buildings. It has a narrow wing shape, indicating that it is a fast-flying, open-space bat (Leelapaibul *et al.*, 2005). Generally, this insectivorous bat forages close to roost sites, and has been recorded hunting in forested areas, villages, and rice fields (Utthammachai *et al.*, 2008). There are at least 17 caves in Thailand that support a total of eight million *C. plicatus* bats, most of which are in central Thailand (Yenbutra and Felten, 1986; Boonkerd and Wanghongsa, 2002). A few colonies were reported to be temporarily absent during the early part of the cool dry season during our study period (S. Binlasoi pers.comm.). *Chaerephon plicatus* has two breeding periods, and the majority of late pregnant females are found in February-March and August-September (Hillman, 1999).

2.2.3 Dietary analysis

Fecal samples were collected monthly between October 2015 and September 2016. A single fecal pellet was collected from each of 30 small baskets (10 cm diameter) spaced 1–2 m apart (along a horizontal plane) underneath *C. plicatus* roosts in order to maximize the probability that samples came from different bats. Baskets were left to collect fecal pellets for 12 hours (18.00 to 06.00 h). Fecal pellets from each basket were collected in the morning and transferred to labeled Eppendorf tubes with silica gel. In the laboratory, one randomly selected fecal pellet per basket was soaked in 50% alcohol for 15 minutes, where they were allowed to soften undisturbed to prevent insect parts in feces from being damaged (Leelapaibul *et al.*, 2005). Insect parts were viewed with a digital microscope (CMOS Digital Microscope) and identified to order following Whitaker (1988), Triplehorn and Johnson (2005), Wilson and Claridge (1991), and our reference insect collections obtained from suction traps. Additionally, homopterans were identified to genus when possible, and the relative number of ingested brown planthoppers per pellet was counted from the number of male genitalia which is the most consistent and reliable indicator (Wilson, 2005) of brown planthopper presence in fecal samples. San San Win *et al.* (2011) indicated that the male to female sex ratio of brown planthoppers is 0.512:0.488.

2.2.4 Insect Trapping

Modified light traps were used to capture insects from the natural habitat in order to evaluate the insect prey available to *C. plicatus*. This trap was designed to collect insects throughout the night in rice fields without electricity. Three traps per study cave were set in fixed locations at a height of 5 m in surrounding rice fields, and all traps were activated at night. The foraging range of *C. plicatus* is ambiguous, but we placed traps within 15 km of the study caves, as William et al. (1973) reported that the closely related *T. brasiliensis* most commonly forages 15–20 km from the cave. Suction trap stations were spaced at least 1 km apart. There were no streetlights in nearby villages. Insects were trapped for 12 hours (18.00 to 06.00 h) on the same nights that fecal samples were collected. Insects were stored in 70% alcohol and identified to order using a digital microscope (CMOS Digital Microscope) following Triplehorn and Johnson (2005).

2.2.5 Data analysis

To assess the abundance of each prey item found in fecal samples, we estimated the percent frequency (the number of occurrences of a particular insect order divided by total occurrences for all orders, multiplied by 100) and the percent volume (the average percentage by volume of each insect order in the total sample; volumes were approximated visually) following Whitaker et al. (2009).

To determine the abundance of each insect order in the environment, the drymass of specimens collected from suction traps was estimated as: $W = 0.0305L^{2.62}$, where W is dry mass (mg) and L is body length (mm) (Rogers et al., 1976).

A Chi-square contingency test was used to determine whether the percent volume and percent frequency of insect orders differed between the two study caves (which were surrounded by different proportions of rice fields). Spearman's correlation test was also applied to investigate the relationship between the number of ingested brown planthoppers and percent volume of Homoptera in each month. Data were analyzed by R (version 3.4.0).

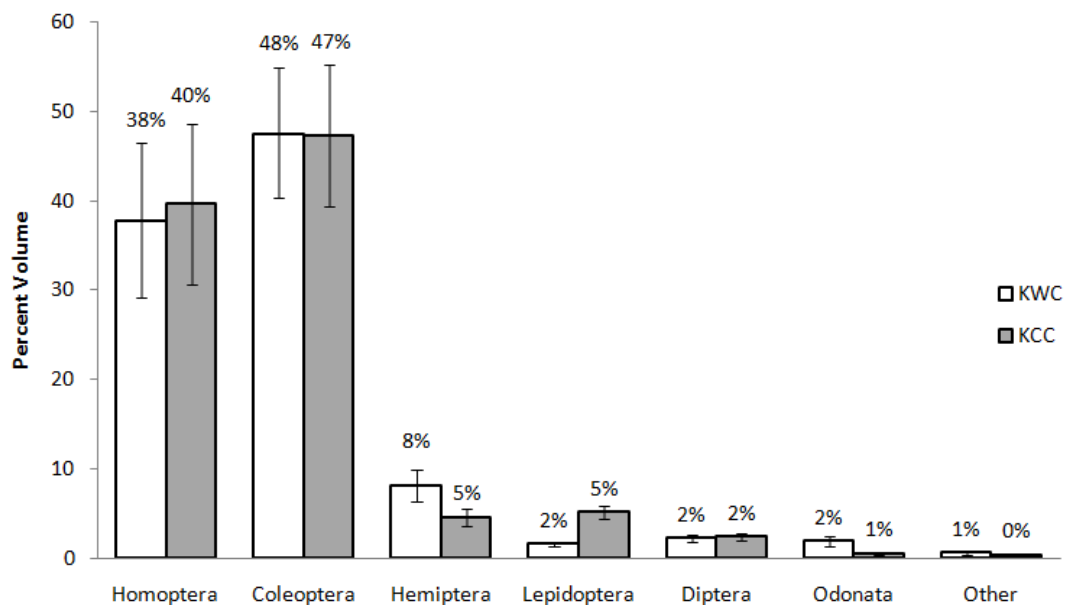
2.3 RESULTS

2.3.1 Bat diet

We analyzed 720 fecal pellets from Khao Wongkot Cave (KWC; n = 360 pellets) and Khao Chakan Cave (KCC; n = 360 pellets) collected between October 2015 and September 2016. Overall, we identified insects from at least eight orders: Coleoptera (beetles), Homoptera (hoppers), Hemiptera (true bugs), Diptera (flies), Lepidoptera (moths), Odonata (dragonflies and damselflies), Hymenoptera (wasps), and Orthoptera (grasshoppers and crickets) (Fig. 2).

Based on the percent volume of insects examined from fecal samples, Coleoptera (47 - 48%) and Homoptera (38 - 40%) were the most important diet items for bat in both caves, accounting for more than 80% (86 - 87%) of the diet combined. They were followed by Hemiptera, Lepidoptera, Diptera, Odonata, Hymenoptera, and Orthoptera (Fig. 2).

Analysis by percent frequency showed that five orders were major components in the diet: Coleoptera (23-24%), Homoptera (20%), Hemiptera (12-15%), Lepidoptera (14-19%), and Diptera (17%). In contrast, Odonata, Hymenoptera, and Orthoptera were found in smaller proportions (Fig. 2).



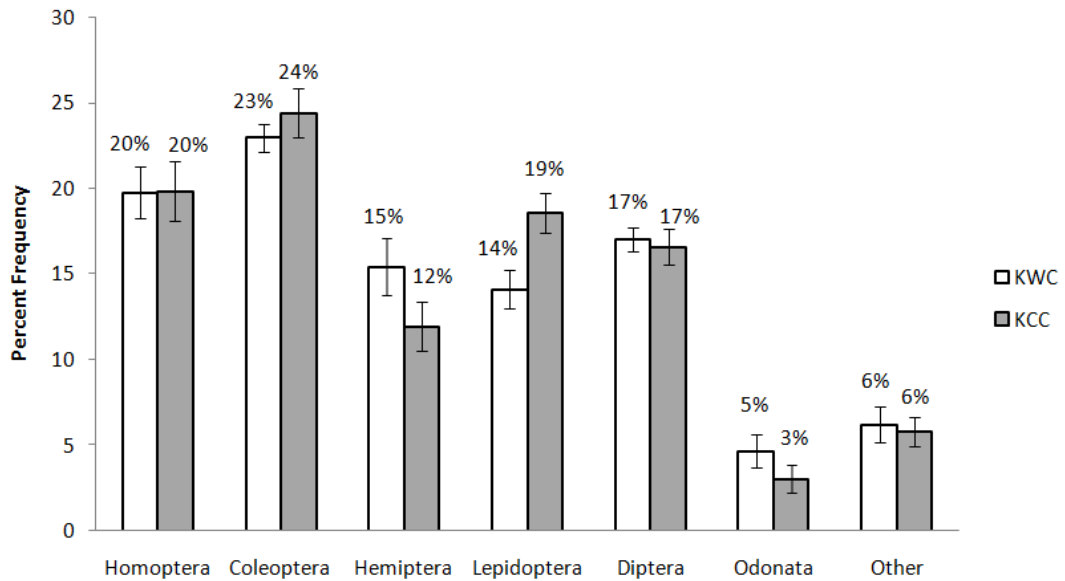


FIG. 2. Percent volume and percent frequency (mean \pm SE) of insect orders found in fecal samples of the wrinkle-lipped free-tailed bat (*Chaerephon plicatus*) collected monthly from October 2015 to September 2016 at Khao Wongkot cave (KWC, 70% surrounding rice fields) and Khao Chakan cave (KCC, 22% surrounding rice fields) in central Thailand. [Other: Orthoptera and Hymenoptera combined]

Examining both percent volume and percent frequency revealed that the diet of *C. plicatus* was not significantly different between the two study caves (percent volume: $\chi^2 = 3.37$, $df = 6$, $P > 0.05$; percent frequency: $\chi^2 = 1.60$, $df = 6$, $P > 0.05$), even though the proportion of surrounding active rice fields was different.

Monthly variation in dietary composition was observed for both sites and appeared to have similar patterns. At KWC, Homoptera comprised the greatest diet volume during October and November 2015 and between August and September 2016. During December 2015 through July 2016, bat diets were dominated by Coleoptera. Similarly, at KCC, Homoptera accounted for greatest volume of prey in October and November 2015, and in February, August and September 2016, while diets from December 2015 through July 2016 (except February) consisted mostly of Coleoptera (Fig. 3).

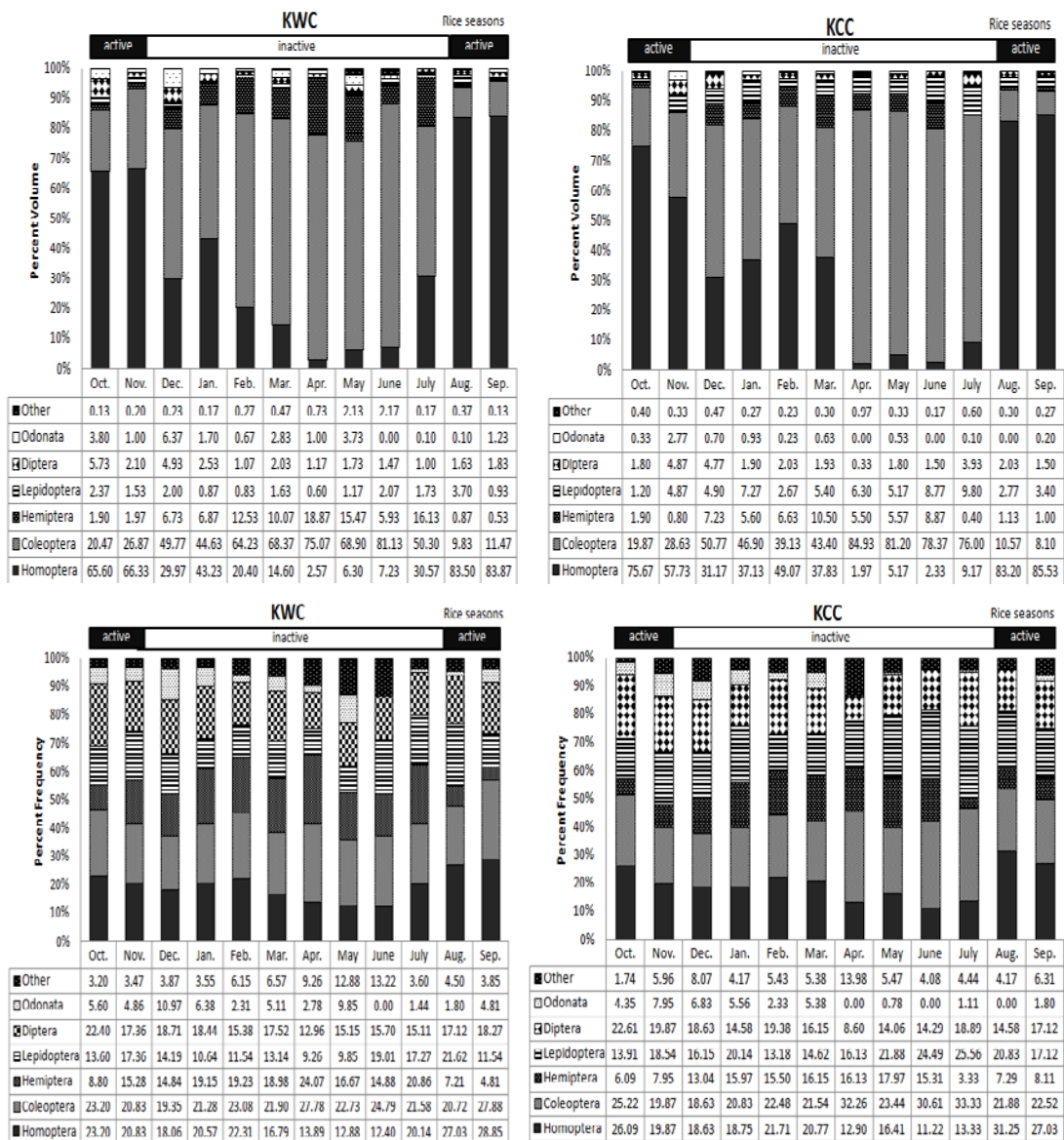


FIG. 3. Monthly variation in the percent volume and percent frequency of insect prey in fecal samples of the wrinkle-lipped free-tailed bat (*C. plicatus*) collected from October 2015 to September 2016 at Khao Wongkot cave (KWC, 70% surrounding rice fields) and Khao Chakan cave (KCC, 22% surrounding rice fields) in central Thailand. [Other: Orthoptera and Hymenoptera combined.]

2.3.2 Brown planthopper consumption

Most homopterans observed in fecal samples were brown planthoppers. The number of ingested brown planthoppers was estimated from the number of male genitalia per pellet. The number of male genitalia was greatest in October, November,

August, and September (Fig. 4). This pattern corresponds to the percent volume of Homoptera in each month (Spearman's correlation test, $r = 0.908$, $p < 0.001$). In general, the average number of male genitalia per pellet was similar in both colonies during the rice growing season (KWC = 3.87 ± 0.12 , KCC = 3.96 ± 0.41). The annual average number of genitalia per pellet was 2.16 ± 1.55 (mean \pm SE) for KWC and 2.55 ± 1.68 for KCC. However, in rice inactive period, male genitalia also high in several months such as during December to March.

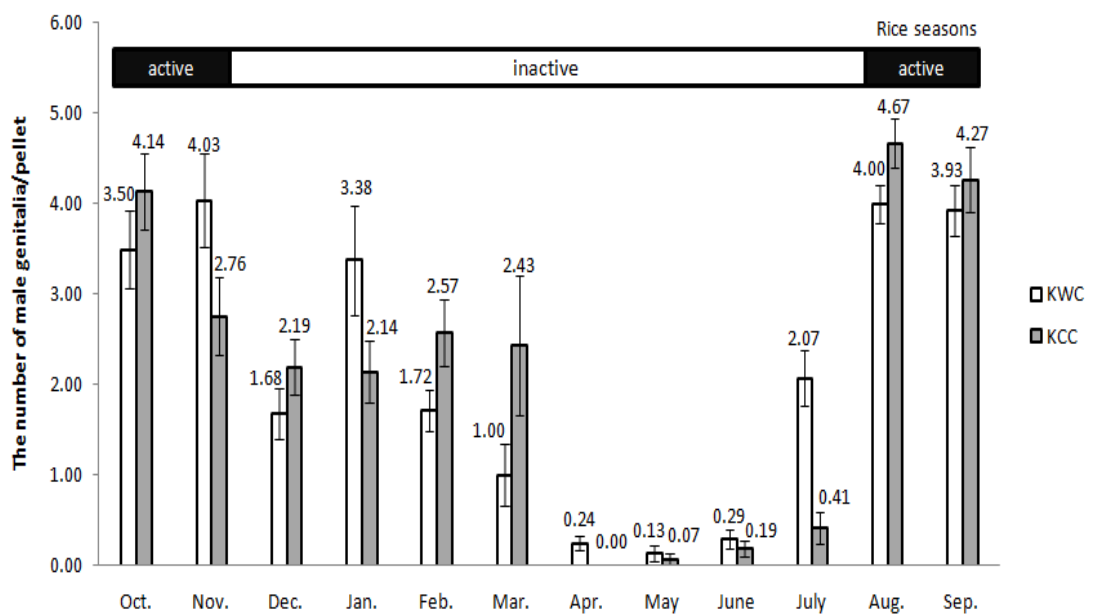


FIG. 4. The average monthly number of male brown planthopper genitalia per pellet (mean \pm SE) in the diet of *Chaerephon plicatus* at two caves: Khao Wongkot Cave (KWC) and Khao Chakan Cave (KCC) in central Thailand (2015 – 2016), as determined by fecal analysis. The yearly average number of genitalia per pellet was 2.16 ± 1.55 (mean \pm SE) for KWC and 2.55 ± 1.68 for KCC.

2.3.3 Modified light traps sampling of insects

Monthly variation in dry insect mass in the suction traps at KWC was mostly comprised of Coleoptera in October, November, February, June and August, Lepidoptera in December, January, March, and April and Diptera in May, July and September while Homoptera, Hymenoptera, Hemiptera and other orders were relatively rare (Fig. 5). At KCC was mostly comprised of Coleoptera during October to December, February, April, August, and September. Diptera in March, May, June

and July and other order in January while Lepidoptera, Homoptera, Hymenoptera and Hemiptera were relatively rare (Fig. 5)

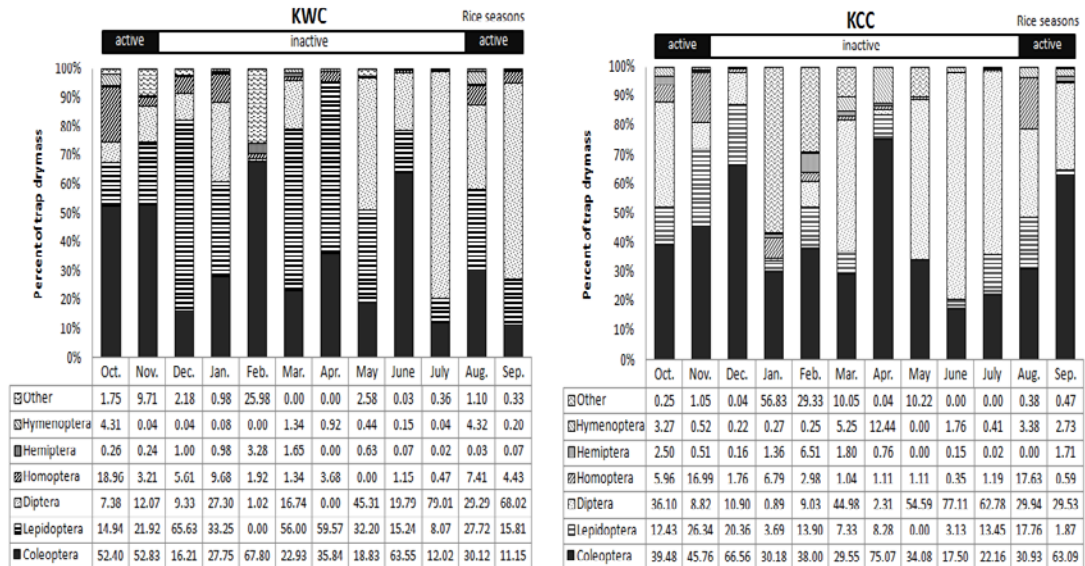


FIG. 5. Percent drymass of insects collected in Modified light traps during October 2015 to September 2016 at Khao Wongkot Cave (KWC) and Khao Chakan Cave (KCC) in central of Thailand. [Others include Orthoptera, Trichoptera, Odonata, Blattodea, Psocoptera, Plecoptera, Isoptera, Dermaptera, and Acari].

2.4 DISCUSSION

2.4.1 Diet

The diet of *C. plicatus* in our study was comprised mainly of Coleoptera and Homoptera, followed by Hemiptera, Diptera, and Lepidoptera, respectively. This is consistent with other descriptions of this bat's diet in Thailand. Previous studies found that *C. plicatus* feeds mainly on Homoptera, Coleoptera, Lepidoptera, Hemiptera, and Diptera (Leelapaibul *et al.*, 2005; Boonkird *et al.*, 2009), although the relative order varies between studies. In the present study, diet composition differed between our two estimation methods (percent volume and percent frequency). Percent frequency is more suitable for small bodied, soft-bodied and easily digested insects such as Lepidoptera, Diptera, Odonata, Orthoptera, and Hymenoptera (Kaspari and Joern, 1993; Lease and Wolf, 2010). These prey items comprised a low volume in the fecal pellet, but were still counted at a relatively high frequency. For example, small and

easily-digested Lepidoptera constitute a small volume of the total fecal pellet, but moths have numerous scales that can remain in the gut and be detectable for several days (Whitaker *et al.*, 2009) which is the disadvantage of percent frequency method. In contrast, Coleoptera, Hemiptera and Homoptera dominated the diet based on percent volume. They were also the most chitinous arthropods (Coleoptera 44% chitin, Homoptera 42% chitin, and Hemiptera 28% chitin; Kaspari and Joern, 1993). As such, percent volume method is more applicable given the focus on brown planthoppers in order Homoptera. Fragments of head, wing and hind leg are important hard parts to distinguish planthopper from other insects.

When examining temporal variation in the diet, Homoptera accounted for the greatest diet volume during the rice growing season (50% to 86%), while Coleoptera were abundant in the diet of this bat when rice fields were inactive. The dominance of Coleoptera is likely due to the wide diversity of this order, as they are found in all major habitats (Banerjee, 2014), including inactive rice fields. Our data support this conjecture, since insect dry mass in our suction traps was mostly comprised of Coleoptera. However, during the rice growing season, homopterans were more abundant in our suction traps. Most homopterans in this study were identified as brown planthoppers (Delphacidae), which are common in central Thailand (Vungsilabutr, 2001; APPENDIX 1) where our study was located. A parallel diet study by molecular analysis confirmed that *C. plicatus* feeds on brown planthoppers (K.Thongjued pers. comm.). In addition, other pests of rice were found such as white-backed planthoppers and zigzag leafhoppers etc. but in a very relatively low percentage. Our findings corroborate a previous study in western Thailand, which found that local *C. plicatus* mainly consumed white-backed planthoppers (Leelapaibul *et al.*, 2005), which are abundant in that region (Vungsilabutr, 2001).

Estimating planthopper consumption based on male genitalia revealed that consumption was greatest during the active rice growing season. We found an average of four male genitalia per pellet during the rice growing season, which also corresponds with the percent volume of Homoptera in the diet. The male to female sex ratio of brown planthoppers is 0.512:0.488 (San San Win *et al.*, 2011), which suggests an average of eight brown planthoppers per pellet including females. The populations of *C. plicatus* from two study caves were estimated to be around 1.3

million individuals (Boonkerd and Wanghongsa, 2002). This population size implies that up to tens of millions of planthoppers are consumed by this bat each night, and supports the role of *C. plicatus* in pest suppression in the rice field ecosystem. Wanger *et al.* (2014) reported that this bat prevents an annual loss of 2,297 tons of rice in Thailand (more than 1,340,000 USD per year) based on the number of consumed white-backed planthoppers. Similar studies should be conducted to estimate the pest suppression service by *C. plicatus* in other parts of central Thailand where the brown planthopper is common.

2.4.2 Proportion of active rice field to the bat diet

Our results reveal that the surrounding proportion of active rice field (70% versus 22%) does not affect bat diet. One possible explanation is that different colonies of *C. plicatus* may forage from the same pool of insect prey as this bat species may forages on migratory insects at high altitudes. Insects such as moth, beetle as well as planthopper are known to migrate at high altitude in Asia (Feng *et al.*, 2004; Otuka *et al.* 2005; Zhang *et al.*, 2008). Brown planthoppers are known to migrate at altitudes between 300 and 1000 m (Riley *et al.*, 1990). Macropterous adults of brown planthopper take off for flight mostly around sunset (Riley *et al.* 1991). Vungsilabutr (1996) suggested that population of brown planthopper locally migrate within central Thailand as active rice fields always occur in this area. In the Chao Phraya river basin, irrigated system allows rice farmers to cultivate rice year round even in dry season during January to April (APPENDIX 2). During that period, the southerly wind could bring planthopper from Chao Phraya river delta to upper central Thailand as indicated by insect traps (Vungsilabutr, 1996; Sintusek and Saengkaew, 1996). This could be the reason that the brown planthoppers were found in bat diets in both colonies even though no active rice fields occurred in such area in that time. *Chaerephon plicatus* may forage at high altitudes similar to other molossid, which forage at altitudes up to 3.1 km (William *et al.*, 1973; McCracken, 1996; Fenton and Griffin, 1997). A previous study on the closely-related *T. brasiliensis* also found that these bats foraged at higher altitudes when moths migrated at high altitudes; by following food resources in time and space, even distant colonies had similar diets (Krauel, 2014; Krauel *et al.*, 2015).

The second possible explanation for the similar diets is that *C. plicatus* may move within a network of roosting caves each night, and so fecal samples from one cave may be the result of foraging from habitats around a different cave, consequently, diet of two study colonies are similar. This hypothesis is supported by a population study of *C. plicatus* in Thailand, in which the population of every colony fluctuates by 30-40% on consecutive nights (S. Binlasoi pers. comm.). Rhodes (2007) reported that *Tadarida australis* employs a fission-fusion pattern based on individual movements to and from a single communal site. He also argued that the roost network of one communal roost and many satellite roosts may be regarded as a single interconnected unit. In our study area, there are several colonies of *C. plicatus* and the closest ones are 15-40 km from our study colonies. However, bats are more sedentary during the breeding period, and diet should be more different during such period. This was not supported by the diet during breeding periods in this study that were largely similar between the two colonies. Further intensive studies on individual foraging behavior are needed to verify whether this bat demonstrates high altitude foraging and show inter-cave movement within a night.

2.5 CONSERVATION IMPLICATIONS

Our study provides some insight into the diet of *C. plicatus* in central Thailand, where rice fields are common and scattered throughout the area. The results of this study suggest that this colonial cave bat helps significantly regulate pest insect in rice fields, in particular the brown planthopper (*Nilaparvata lugens*). This study highlights the need to establish conservation management plans for protecting roosting caves and bat populations. In addition to the current ecological engineering approach, in which farmers grow nectar-rich flowering plants to attract natural insect predators for pest suppression (Gurr *et al.* 2012), promoting natural pest suppression agent populations can limit potential crop pests (Naylor and Ehrlich, 1997). For example, *Pipistrellus pygmaeus* in northeastern Iberia is used to suppress the rice borer moth, which is a major pest of rice around the world, and pest levels have declined since the establishment of bat boxes for *P. pygmaeus* in rice fields (Puig-Montserrat *et al.*, 2015). In our study area, several insectivorous bat species in addition to *C. plicatus* were observed, such as *Taphozous*, *Scotophilus*, *Myotis*, and

Rhinolophus species (P. Suksai, pers. comm.). Future conservation plans should also include these bat species, as they may also contribute to pest regulation throughout the landscape. The results from this research can help rice farmers to design integrated pest management schemes, especially in rice fields adjacent to colonies of bats.

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CHAPTER CONCLUSION

Wrinkle-lipped free-tailed bat (*Chaerephon plicatus* Buchannan, 1800) feeds on at least 8 insect orders belonging to Coleoptera, Homoptera, Hemiptera, Diptera, Lepidoptera, Odonata, Hymenoptera and Orthoptera. Surprisingly, Homoptera made up a greatest diet volume in period of active rice field whereas Coleoptera was the most abundant in the diet during inactive rice period. Most Homoptera were identified as brown planthopper (*Nilaparvata lugens*), an important economic pest in rice field in South East Asia. The number of male brown planthopper genitalia was counted for the first time and it showed the greatest number during rice planting period, an average of four males genitalia per pellet were recorded. According to the results, this study further suggests that at least ten millions individuals of brown planthopper are consumed by this bat colony each night. *C. plicatus* is undoubtedly an agent of biological pest control against the brown planthoppers in the central part of Thailand. However, bats are in the face of growing anthropic disturbance and habitat degradation which may have impact on bat population and can affect population recruitment and major role in brown planthopper pest control.

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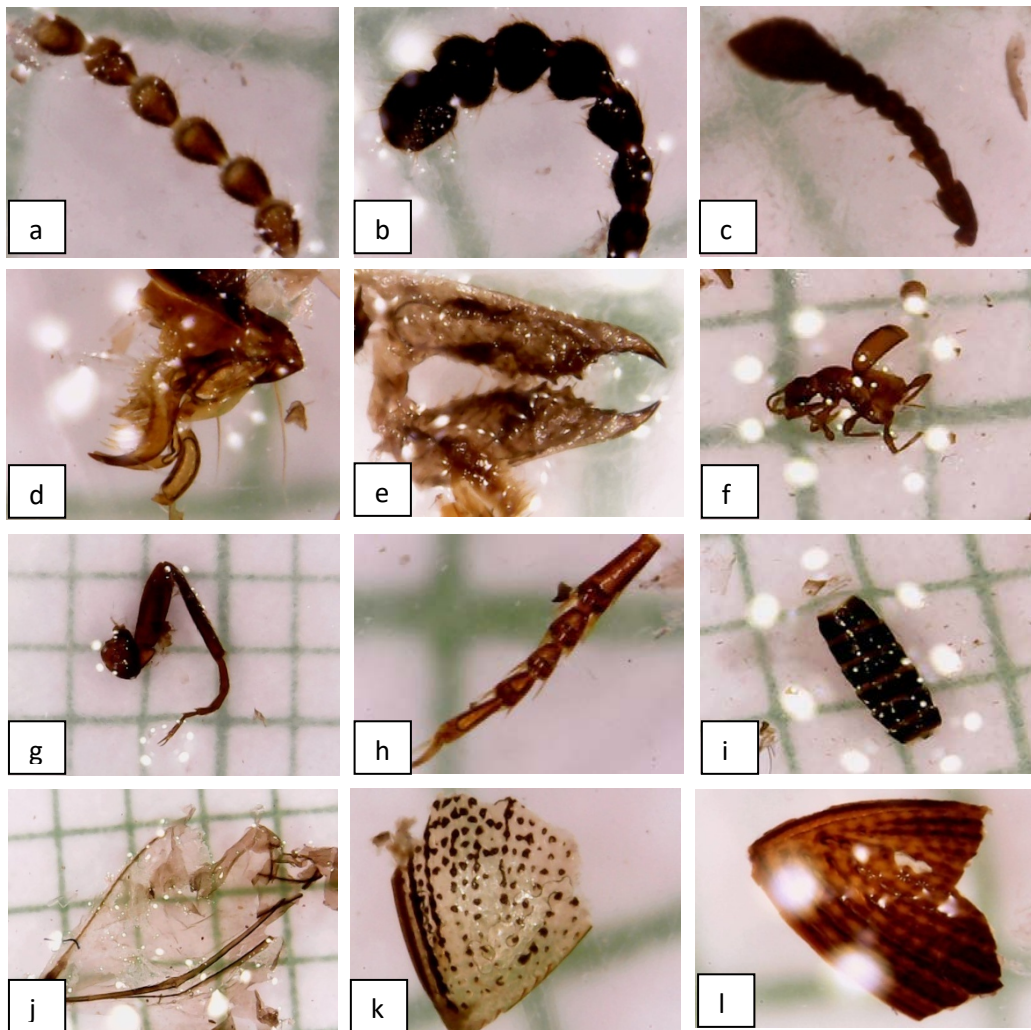
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APPENDICES



Appendix 1 Figure of the wrinkle-lipped free-tailed bat (*Chaerephon plicatus* Buchanan, 1800).



Appendix 2 Figures of part of insect in order Coleoptera

a-c parts of antenna in order Coleoptera

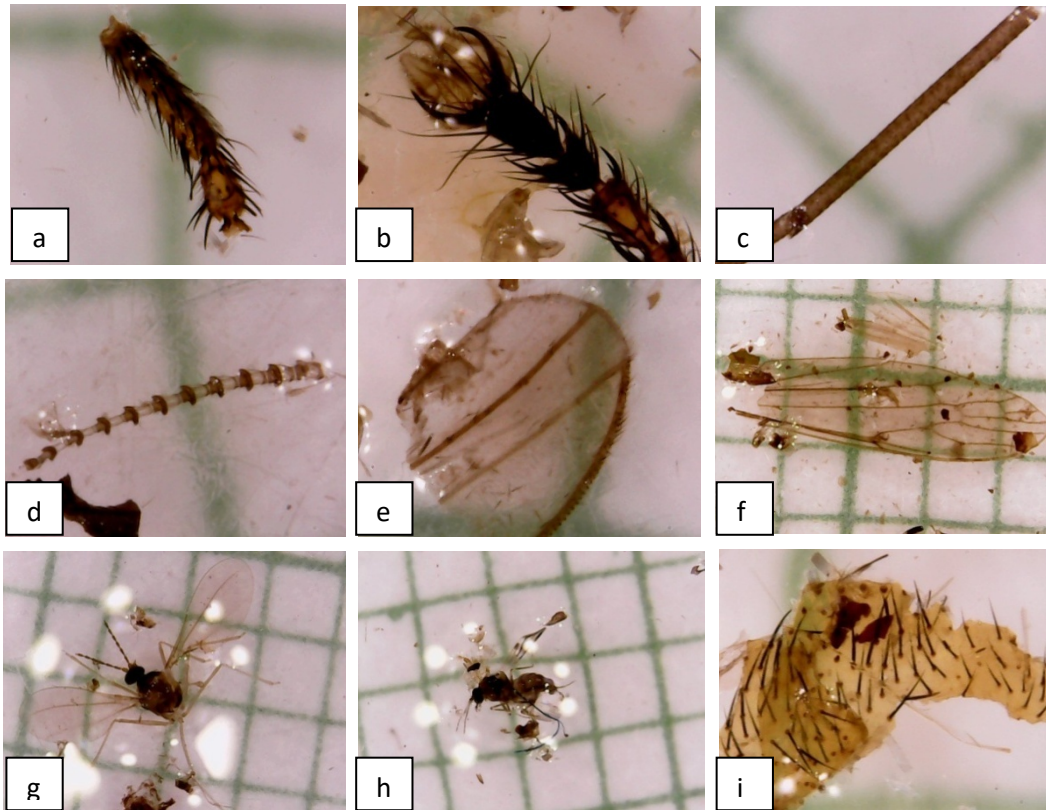
d-e parts of mandible in order Coleoptera

f part of head, pronotum and wing in order Coleoptera

g-h parts of leg in order Coleoptera

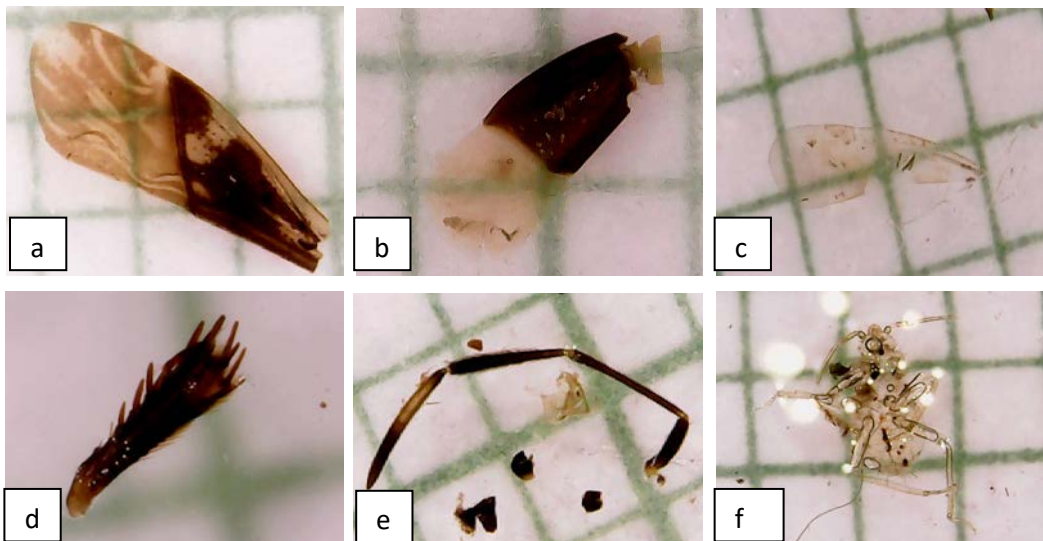
I part of abdomen in order Coleoptera

j-l parts of wing in order Coleoptera



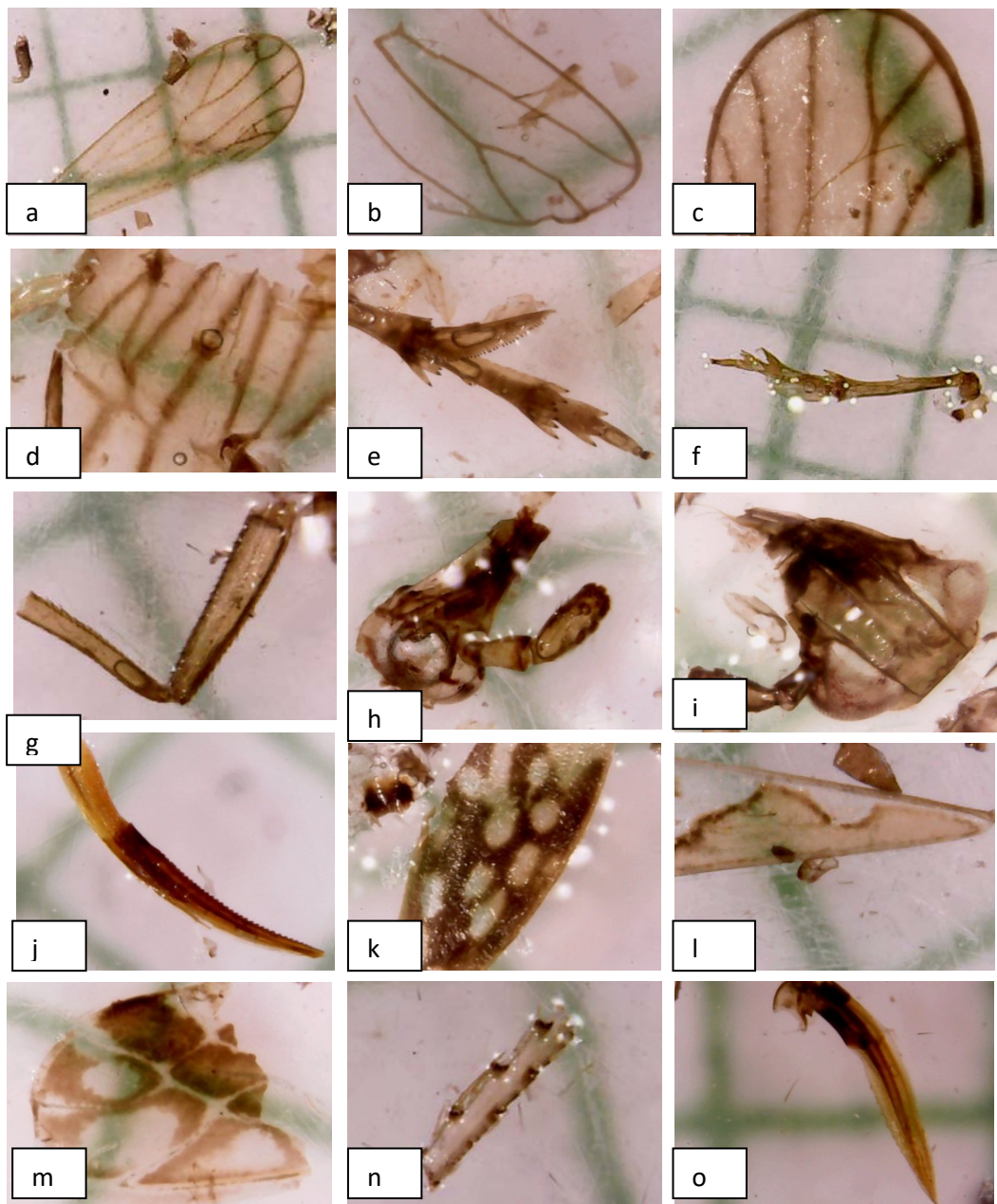
Appendix 3 Figures of part of insect in order Diptera

- a-c parts of leg in order Diptera
- d part of antenna in order Diptera
- e-f parts of wing in order Diptera
- g parts of antenna, head and wing in order Diptera
- h Whole body in order Diptera
- i part of thorax in order Diptera



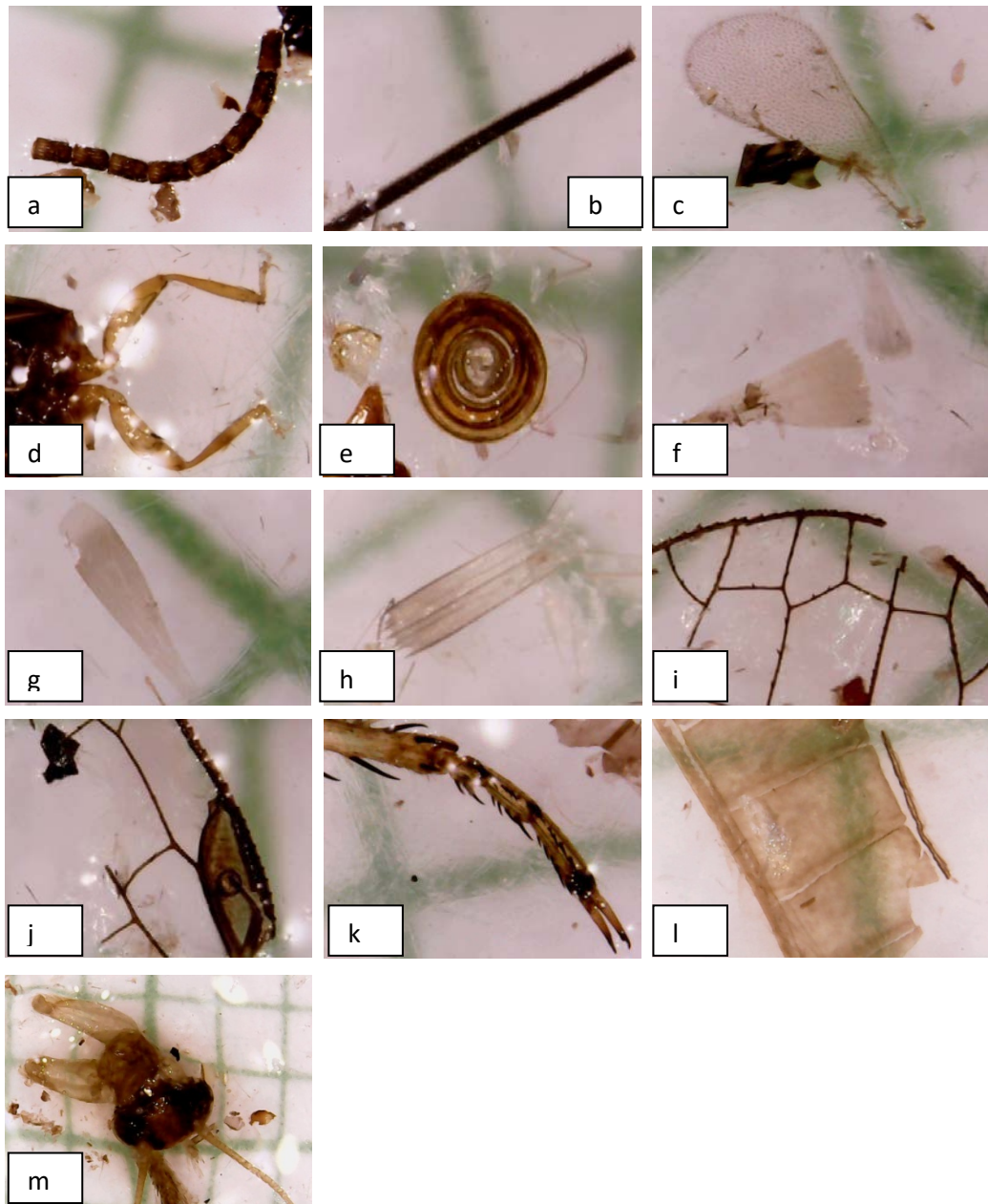
Appendix 4 Figures of part of insect in order Hemiptera

- a-c parts of wing in order Hemiptera
- d part of leg in order Hemiptera
- e part of antenna in order Hemiptera
- f whole body in order Hemiptera



Appendix 5 Figures of part of insect in order Homoptera

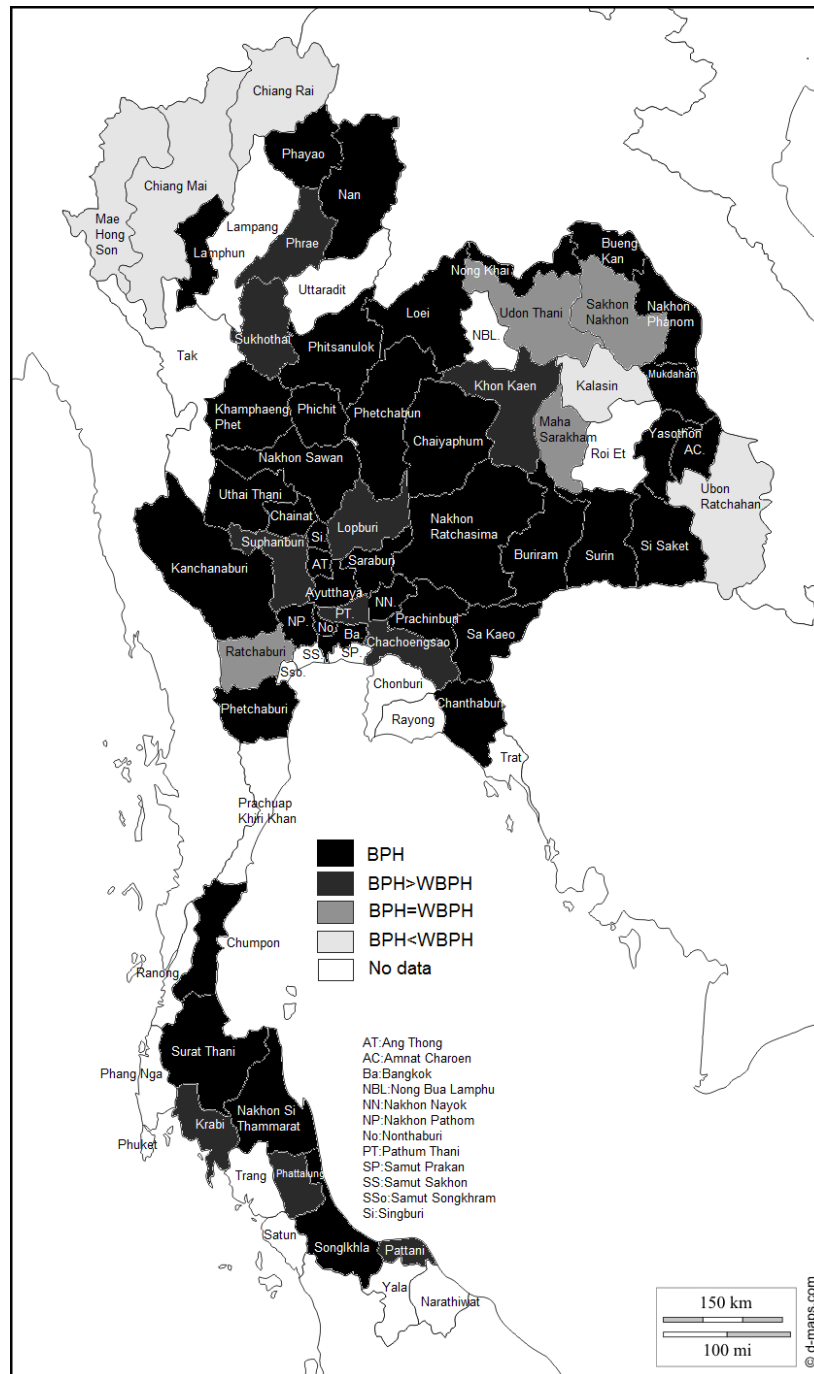
- a-d parts of wing in family Delphacidae order Homoptera
- e-g parts of leg in family Delphacidae order Homoptera
- h-i parts of head in family Delphacidae order Homoptera
- j part of male genitalia in family Delphacidae order Homoptera
- k-m parts of wing in family Cicadellidae order Homoptera
- n part of leg in family Cicadellidae order Homoptera
- o part of male genitalia in family Cicadellidae order Homoptera



Appendix 6 Figures of part of insect in order Hymenoptera, Lepidoptera, Odonata and Orthoptera

- a part of antenna in order Hymenoptera
- b part of ovipositor in order Hymenoptera
- c part of wing in order Hymenoptera
- d part of leg in order Hymenoptera
- e part of proboscis in order Lepidoptera
- f-h scales of wing in order Lepidoptera

- i-j parts of wing in order Odonata
 k part of leg in order Odonata
 l part of wing in order Orthoptera
 m part of head and leg in order Orthoptera



APPENDIX 7 Map illustrating the distribution of the BPH and WBPH in Thailand (data from rice research stations throughout Thailand (www.ricethailand.go.th)).

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List of Publication and Proceeding

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