ไฟโลจีโอกราฟีของนกอีแอ่นรังขาว *Aerodramus fuciphagus* (Thunberg, 1812) ในประเทศไทย

นางสาวอัญชลี เอาผล

PHYLOGEOGRAPHY OF THE WHITE-NEST SWIFTLET

Aerodramus fuciphagus (Thunberg, 1812) IN THAILAND

Miss Anchalee Aowphol

A Dissertation Submitted in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy Program in Biological Sciences

Faculty of Science

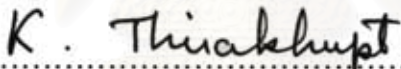Chulalongkorn University

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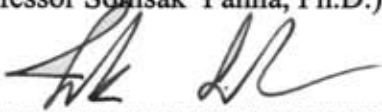
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อัญชลี เอาผล : ไฟโลจีโอกราฟีของนกอีแอ่นรังขาว *Aerodramus fuciphagus* (Thunberg, 1812) ในประเทศไทย (PHYLOGEOGRAPHY OF THE WHITE-NEST SWIFTLET *Aerodramus fuciphagus* (Thunberg, 1812) IN THAILAND) อ. ที่ปรึกษา : รศ. ดร. กำธร ธีรคุปต์, อ. ที่ปรึกษาร่วม : Dr. Harold K. Voris, ผศ. ดร. พงชัย หาญยุทธนากร. 121 หน้า.

ในธรรมชาติ นกอีแอ่นรังขาว *Aerodramus fuciphagus* (Thunberg, 1812) มักพบอาศัยอยู่รวมกันเป็นกลุ่มขนาดใหญ่ตามถ้ำบนเกาะในทะเล แต่ปัจจุบันนกชนิดนี้ได้เข้ามาอาศัยในสิ่งปลูกสร้างประเภทต่างๆของมนุษย์เช่นโบสถ์และบ้านนก การศึกษาครั้งนี้พบว่ากลุ่มประชากรนกอีแอ่นรังขาวมีการกระจายในบริเวณกว้างตลอดแนวชายฝั่งทะเลของประเทศไทย ตั้งแต่ภาคตะวันออกถึงภาคใต้ของอ่าวไทยและชายฝั่งทะเลอันดามันโดยเข้ามาอาศัยอยู่ในบ้านนกที่ถูกสร้างขึ้นเป็นจำนวนมากตามบริเวณชายฝั่งทะเลบนแผ่นดินใหญ่ โดยเฉพาะบริเวณที่อยู่ตรงข้ามกับถ้ำธรรมชาติบนเกาะ บริเวณปากแม่น้ำ และเขตชุมชน นอกจากนี้ยังมีแนวโน้มว่าจำนวนบ้านนกจะเพิ่มขึ้นอย่างรวดเร็วในอีกไม่กี่ปีข้างหน้าเนื่องจากปัจจุบันยังไม่มีกฎหมายที่ใช้บังคับควบคุมการก่อสร้างบ้านนกซึ่งอาจก่อให้เกิดผลกระทบในเชิงลบต่อมนุษย์และระบบนิเวศต่อไปในอนาคต ดังนั้นจึงมีความจำเป็นอย่างเร่งด่วนที่ต้องทำการศึกษาผลกระทบทางนิเวศวิทยาและการประเมินความเสี่ยงด้านสุขภาพที่อาจเกิดขึ้นเพื่อวางมาตรการในการจัดการที่เหมาะสมต่อไป

การศึกษาความสัมพันธ์ทางพันธุกรรมภายในประชากรและระหว่างประชากรนกอีแอ่นรังขาวที่เข้ามาอาศัยในสิ่งปลูกสร้างของมนุษย์ ได้ศึกษาจากยีนในไมโทคอนเดรียคือยีน cyt-*b* และยีน ND2 และไมโครแซทเทลไลท์ จำนวน 8 ตำแหน่งและทำการเก็บตัวอย่างนกอีแอ่นรังขาวจากประชากร 10 ประชากรที่อาศัยอยู่ในสิ่งปลูกสร้างของมนุษย์บริเวณแนวชายฝั่งทะเลอ่าวไทยและชายฝั่งทะเลอันดามันในประเทศไทย จากผลการศึกษาพบว่าความหลากหลายทางพันธุกรรมของไมโทคอนเดรีย ดีเอ็น เอมีค่าต่ำมาก ส่วนความหลากหลายทางพันธุกรรมของไมโครแซทเทลไลท์มีค่าสูง เมื่อวิเคราะห์ค่าความแตกต่างทางพันธุกรรมระหว่างคู่ประชากรนกอีแอ่นรังขาวซึ่งได้แก่ค่า Φ_{ST} (ไมโทคอนเดรียล ดีเอ็น เอ) และค่า F_{ST} (ไมโครแซทเทลไลท์) พบว่ามีค่าต่ำมากและบางค่าเป็นลบซึ่งแสดงถึงความผันแปรของพันธุกรรมภายในประชากรนกอีแอ่นรังขาวมีสูงกว่าระหว่างประชากร จากการศึกษารูปแบบความสัมพันธ์ระหว่างแฮพโพไทป์ของไมโทคอนเดรียล ดีเอ็น เอ และข้อมูลไมโครแซทเทลไลท์ของประชากรนกอีแอ่นรังขาว ไม่พบลักษณะโครงสร้างทางพันธุกรรมของประชากรนกอีแอ่นรังขาวในประเทศไทย นอกจากนี้เมื่อวิเคราะห์ข้อมูลไมโครแซทเทลไลท์ด้วยโปรแกรม สตรัคเจอร์ พบว่าประชากรของนกอีแอ่นรังขาวมีเพียง 1 ประชากร (K = 1) การที่ไม่พบความแตกต่างทางพันธุกรรมของประชากรนกอีแอ่นรังขาวที่อาศัยในสิ่งปลูกสร้างของมนุษย์ น่าจะเป็นผลมาจากการแลกเปลี่ยนยีนระหว่างประชากรในปริมาณสูงและประชากรนกอีแอ่นรังขาวมีขนาดใหญ่ ดังนั้นผลการศึกษาครั้งนี้สามารถสรุปได้ว่าประชากรของนกอีแอ่นรังขาว *A. fuciphagus* ที่อาศัยอยู่ในสิ่งปลูกสร้างของมนุษย์เป็นประชากรขนาดใหญ่เพียงประชากรเดียว

สาขาวิชา ....วิทยาศาสตร์ชีวภาพ......... ลายมือชื่อนิสิต......อัญชลี เอาผล......

ปีการศึกษา...........2550........... ลายมือชื่ออาจารย์ที่ปรึกษา.....กำธร ธีรคุปต์.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....H. Voris.....

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KEY WORDS: *Aerodramus fuciphagus* / WHITE-NEST SWIFTLET / GENETIC SRTUCTURE / MICROSATELLITES / PANMICTIC

ANCHALEE AOWPHOL : PHYLOGEOGRAPHY OF THE WHITE-NEST SWIFTLET *Aerodramus fuciphagus* (Thunberg, 1812) IN THAILAND. THESIS ADVISOR: ASSOC. PROF. KUMTHORN THIRAKHUPT, Ph.D., THESIS CO-ADVISOR: HAROLD K. VORIS, Ph.D., ASST. PROF. PONGCHAI HARNYUTTANAKORN, Ph.D. 121 pp.

Originally the white-nest swiftlet, *Aerodramus fuciphagus,* lived in large colonies in natural caves on coastal islands, but now it also occurs in man-made buildings such as sacred buildings and swiftlet houses. The observations in this study found that colonies of *A. fuciphagus* in swiftlet houses have been widely expanded along the coastlines, ranging from the East through the South of the Gulf of Thailand and Andaman Sea. The majority of swiftlet houses are located on the mainland opposite the natural cave habitats on islands, at the river mouth areas and in urban areas. Due to the fact that the number of swiftlet buildings has been enormously increased in the past several years and that to date there is no official regulation or law for controlling the construction of swiftlet houses, negative impacts to human and natural ecosystems could occur in the future. Therefore, the studies on ecological impact and health risk assessment for setting the proper management plan are urgently needed.

The genetic relationship of *A. fuciphagus* colonies was investigated using two mitochondrial DNA genes (cyt-*b* and ND2) and eight microsatellite loci among and within swiftlet colonies from across recently established man-made colonies in Thailand. Ten white-nest swiftlet colonies were sampled along the coast of the Gulf of Thailand and the Andaman Sea in Thailand. Genetic diversity of mtDNA was very low whereas that of microsatellite loci was high. Estimates of genetic differentiation, Φ_{ST} (mtDNA) and F_{ST} (microsatellites) values were very low and some negative values were found, indicating that genetic variability within colonies was higher than between colonies. Analyses of mtDNA haplotype relationships and microsatellite data did not detect genetic structure across the sampled distribution. Microsatellites were also analyzed using STRUCTURE and it was found that the number of subpopulations of white-nest swiftlets in sampled colonies was one (K = 1). The lack of genetic differentiation among swiftlet house colonies could be a result of high gene flow between colonies and large population sizes. The results suggest that *A. fuciphagus* living in recently established man-made houses in Thailand should be considered members of a single panmictic population.

Field of study... Biological Sciences...... Student's signature. *Anchalee Aowphol*

Academic year............2007............... Advisor's signature. *K. Thirakhupt*

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Co-advisor's signature....

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

BSA	Bovine serum albumin
CP	Chumphon
Cyt-*b*	Cytochrome-*b*
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DTT	Dithiothreitol
EtOH	Ethyl alcohol
KB	Krabi
min	minute
mtDNA	mitochondrial DNA
NaOAc	Sodium Acetate
NK	Nakohon Si Thammarat
ND2	NADH Dehydrogenase Subunit 2
NW	Narathiwat
PCR	Polymerase Chain Reaction
PKK	Prachuap Khiri Khan
PN	Pattani
PNG	Phang-Nga
RN	Ranong
s	second
SK	Songkla

SP   Samut Prakarn

SRT   Surat Thani

SS   Samutsakhon

ST   Satun

TR   Trang

TT   Trat

CHAPTER I

INTRODUCTION

1.1 Rationale

Aerodramus swiftlets (Family Apodidae) are a group of small insectivorous birds with wide distribution across South Asia, Southeast Asia, Northern Australia and the South Pacific (Medway, 1966; Chantler and Driessens, 2000). Nests of several swiftlet species such as *Aerodramus fuciphagus*, *A. maximus* and *A. unicolor* are harvested by local people for Chinese cuisine and pharmaceutical purposes. Due to high morphological similarity among swiftlet species, the taxonomic classification has a history of debate. Most authors recognize three genera of swiftlets: *Aerodramus*, *Collocalia* and *Hydrochous*. However, the within-group classification is still in flux.

The white-nest swiftlet, *Aerodramus fuciphagus*, has long been well-known for its commercially valuable nest, but only a few studies on its biology have been conducted. This colonial species lives in large colonies and is distributed throughout Southeast Asia, Hainan, Andaman and Nicobar Islands (Medway, 1966; Chantler and Driessens, 2000), and has become popular for commercial husbandry by local people in several countries such as Indonesia, Malaysia and Thailand. The natural nesting sites of this species are limestone caves on islands adjacent to the sea coast, but birds now use abandoned houses and even buildings constructed for them to roost and nest. The number of caves of appropriate size and location on the coastal islands in the region are finite and probably limited the number of colonies in pre-historical times. Natural cave populations of *A. fuciphagus* have been reported for declining in some areas such as the Andaman and Nicobar Islands in India and Sabah and Sarawak,

Malaysia (Lau and Melville, 1994; Chantler and Driessens, 2000; Sankaran, 2001). However, overall numbers of this bird across its geographic range appear to be dramatically increasing due to the colonization of many new man-made structures provided for them over the past decades. In Thailand, the first opportunity likely occurred several hundreds of years ago on a relatively small scale when swiftlets began to colonize temples or shrines built along the coasts. This early expansion has been followed by an enormous increase in the past few decades with the construction of numerous large buildings with the express purpose of housing large colonies of these economically valuable birds. At present, *A. fuciphagus* has increased in both the number of individuals and the number of colonies due to high reproductive ability and increased habitat through man-made buildings. The source of birds for this rapid expansion was initially from natural caves to man-made buildings, but in recent time, birds that colonize new buildings may come from other established colonies in buildings. Such a rapid population expansion would likely affect genetic variation within the species over a long term period and has conservation consequences.

1.2 Objectives

1. To investigate the taxonomic status and the distribution of the white-nest swiftlet in Thailand.

2. To assess genetic variation within and between recently established man-made colonies of the white-nest swiftlet in Thailand using molecular markers with differing inheritance patterns; maternally inherited mitochondrial DNA cytochrome-*b* (cyt-*b*) and NADH dehydrogenase subunit 2 (ND2) genes and biparentally inherited nuclear DNA (microsatellites).

1.3 Anticipated benefit

The results provide basic knowledge on the distribution and genetic relationship within and among the white-nest swiftlet populations living in man-made buildings in Thailand and the additional information on genetic diversity of birds as a result of human activities. Furthermore, the results on the distribution and rapid expansion of swiftlet colonies in the cities indicate that the ecological study on *Aerodramus fuciphagus* is urgently needed for the impact assessment and for setting the proper management plan in the near future.
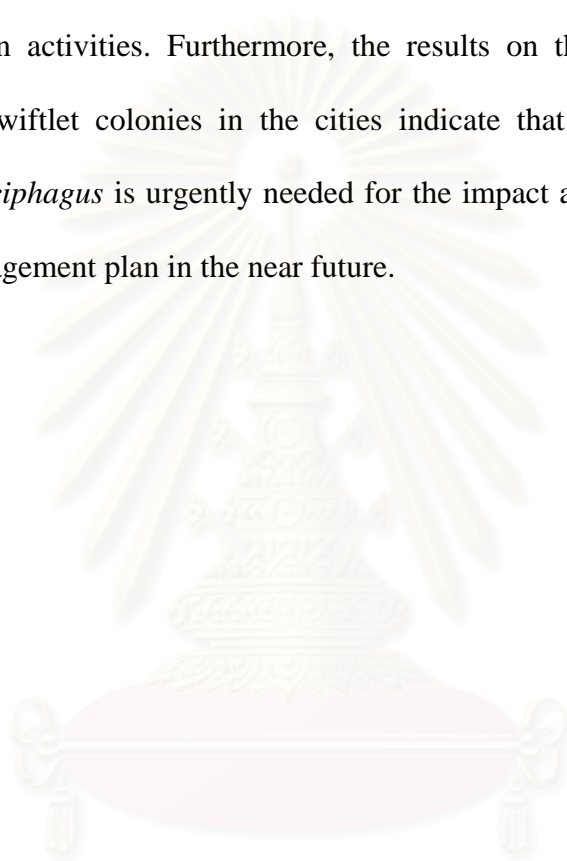
CHAPTER II

LITERATURE REVIEW

2.1. Taxonomy and phylogeny of Family Apodidae

The basic classification of swiftlets has been historically based on morphology, nest characters and presence or absence of echolocating ability. Swiftlets are known for high morphological similarity among species (Fig 2.1), causing much debate among taxonomists. Swiftlets are, together with the South American oilbird, *Steatornis caripensis*, unique among birds in their echolocating ability (Novick, 1959; Pye, 1980; Suther and Hector, 1982, 1985). Unlike insectivorous bats, swiftlets do not apparently have the echolocating acuity for capturing insect prey (Medway 1967; Griffin and Suther, 1970; Fenton, 1975; Griffin and Thompson, 1982). Echolocation appears to provide advantages for these birds by permitting them to roost and nest in dark cave sites, which free from visually orienting predators and competitors (Fenton, 1975; Del Hoyo *et al*., 1999). However, not all swiftlet species are able to echolocate. The dichotomy in echolocating ability within swiftlets provided basic information for classification of these birds (Medway, 1966).

Swiftlets are classified into Order Apodiformes, Family Apodidae, Subfamily Apodinae, Tribe Collocaliini (Sibly and Monroe, 1990; Del Hoyo *et al*., 1999; Chantler and Driessens, 2000). They have been placed into one, two, or three genera for several times. The allocation of swiftlet genera has been a subject of some debate. Originally swiftlets were classified into genus, *Collocalia* (Peter, 1940; Medway, 1966). Brooke (1970) divided swiftlets into three subgenera i.e.

Aerodramus, consisting of echolocating species, *Collocalia*, comprising of all non-echolocating species, and *Hydrochous*, a monotypic genus containing *Hydrochous gigas*. Subsequently, Brooke (1972) proposed these three subgenera to be genera. Sibley and Monroe (1990) grouped the birds into two genera, *Collocalia* (Brooke's *Collocalia* and *Aerodramus*), and *Hydrochous*. Del Hoyo *et al.* (1999) followed Brooke's classification, but grouped *Aerodramus* and *Schoutedenapus* within the Tribe Collocaliini. Chantler and Driessens (1995, 2000) returned to the original classification of a single genus, *Collocalia*. To date twenty-six swiftlet species are recognized in the Family Apodidae, Subfamily Apodinae, Tribe Collocaliini (Chantler and Driessens, 2000).

Figure 2.1. Swiftlet specimens at the Raffle Museum of Biodiversity Research, National University of Singapore showing high morphological similarity in color and size among species.
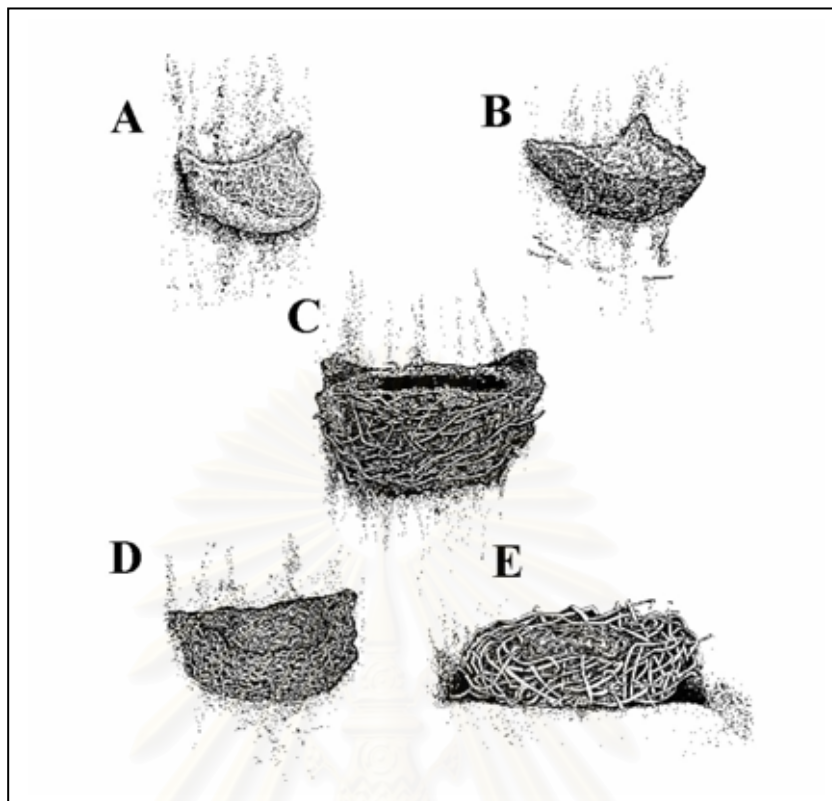
Figure 2.2. Swiftlet nests showing species-specific variation in characters used for the basic classification (Lee *et al*., 1996). A) *Aerodramus fuciphagus* (pure saliva) B) *Aerodramus maximus* (saliva and feather) C) *Aerodramus brevirostris* (saliva, feather and vegetation) D) *Aerodramus spodiopygius* (saliva and vegetation) and E) *Aerodramus sawtelli* (saliva and vegetation).

Obviously, morphological characters do not contain enough information to be able to produce reliable phylogenetic relationships (Thomassen, 2005). Therefore, phylogenetic studies have been developed based on nest characters (Fig. 2.2), molecular data, and echolocating ability. In the view of phylogenetic study, swiftlets are divided into three genera; *Aerodramus*, *Collocalia* and *Hydrochous* (Lee *et al*., 1996; Thomassen *et al*., 2003; Price, Johnson and Clayton, 2004). Lee *et al*. (1996) reported that nest characters were not phylogenetically informative. Later on several phylogenetic studies examined phylogenetic relationship among swiftlets and found that swiftlets are a monophyletic group based on DNA sequences of mtDNA genes (Thomassen *et al*., 2003; Price *et al*., 2004; Price *et al*., 2005; Thomassen *et al*., 2005). Based on complete cytochrome-*b* sequences, *Collocalia* is a sister-group of *Hydrochous*+*Aerodramus* in the monophyletic group of swiftlets (Thomassen *et al*., 2003). However, the status of *H. gigas* in Thomassen *et al.* (2003) was unresolved because it was either placed within *Aerodramus* or as a sister group of *Aerodramus*. Subsequently, Price *et al*. (2004) included more species in the phylogenetic analyses of swifts and swiftlets and could resolve the relationship within genera with more precision based on a part of cytochrome-*b* (cyt-*b*) gene and a part of dehydrogenase subunit 2 (ND2) gene. However, Price *et al*. (2004) did not include *H. gigas* in the analyses. Thomassen *et al*. (2005) included two additional regions of more conservative DNA i.e. 12S rRNA and β-fibrinogen intron 7 nuclear DNA in order to increase resolving power and found that *H. gigas* was placed as the sister group of *Aerodramus* with high support. Meanwhile, Price *et al*. (2005) investigated molecular phylogenetic relationship of the Papuan swiftlet, *Aerodramus papuensis* and other swiftlets. They found that *A. papuensis* and *Hydrochous gigas* are sister taxa and
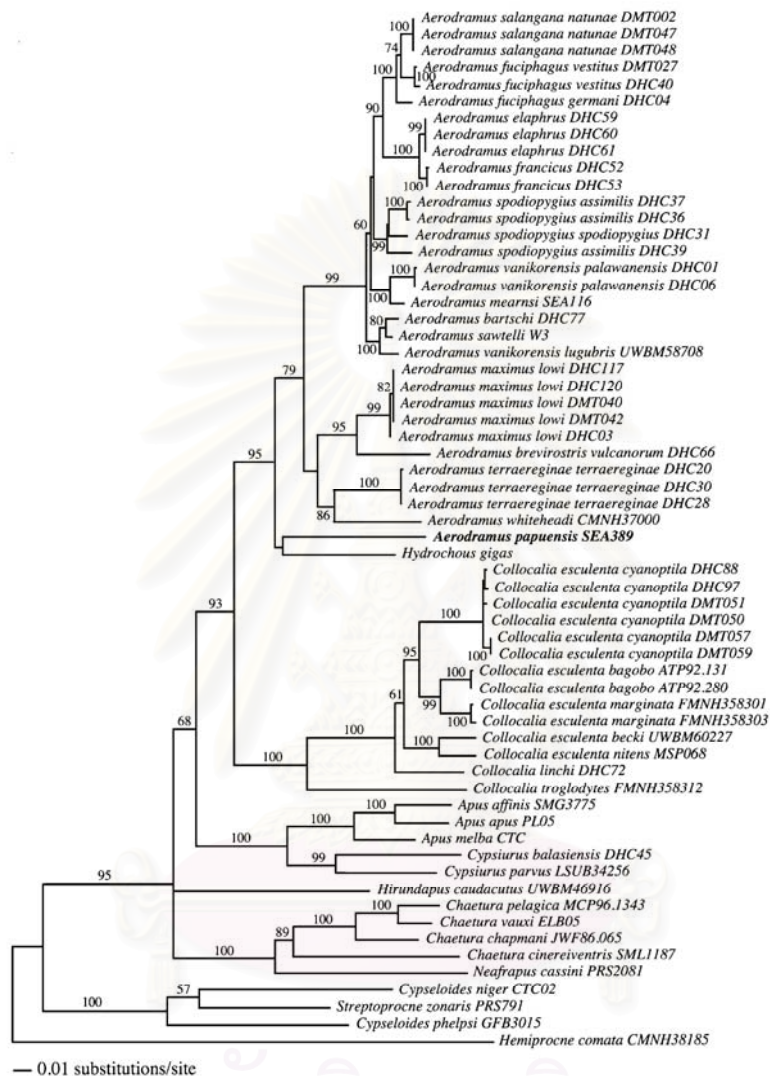
Figure 2.3. Phylogenetic tree showing monophyly of swiftlet species and paraphly of genus *Aerodramus* (Price *et al.*, 2005).

strongly supported that the relationship of *A. papuensis* and other *Aerodramus* taxa showed the paraphyly of the genus *Aerodramus* (Fig. 2.3). Even though phylogenetic relationship among swifts and swiftlets could be resolved using molecular data, the taxonomic nomenclature within swiftlet group has been under investigation. One has proposed that swiflets may possibly return to a single genus (Price *et al*., 2004). Moreover, the results of phylogenetic analyses provided a firm base to continue studying the evolution of echolocation and what morphological adaptations to echolocation have evolved (Thomassen, 2005).

As a result of phylogenetic studies, Price *et al*. (2004) suggested that the presence of echolocation of swiftlets is not a useful character for distinguishing genera *Aerodramus* and *Collocalia* since the ability of echolocation has been found in *Collocalia troglodytes*. This finding did not support the previous idea that echolocation evolved only once in the ancestor of *Aerodramus* clade. Price *et al*. (2004) and Price *et al*. (2005) proposed that echolocation has either risen independently in *Aerodramus* and *Collocalia* clades or evolved only once at the base of swiftlet clade and subsequently lost in some taxa (Fig. 2.4). Moreover, Thomassen and Povel (2006) reported that echo clicks and social vocalizations were species-specific, but these characters revealed no consistent phylogenetic patterns when mapped these characters on a DNA-based tree.
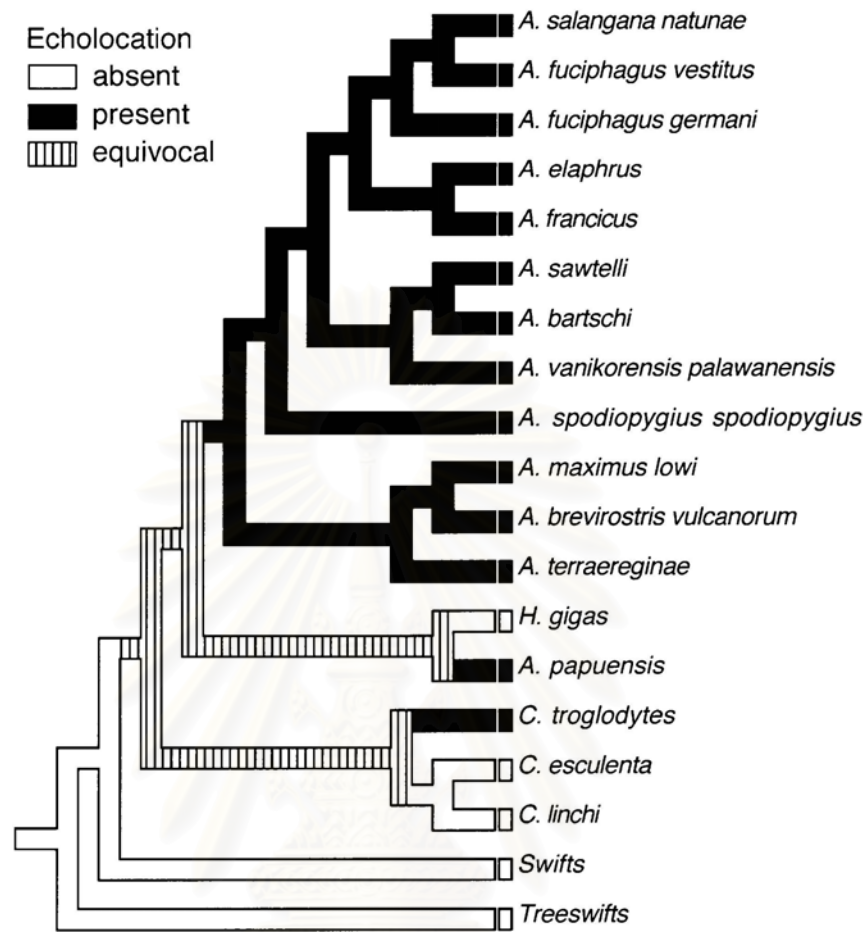
Figure 2.4. Echolocation reconstructed onto the maximum likelihood phylogeny of the swiftlet (Price *et al*., 2005).

2.2. Study organism; white-nest swiftlet, *Aerodramus fuciphagus* (Thunberg, 1812)

The white-nest swiftlet, *Aerodramus fuciphagus* is a medium-sized swiftlet. Males and females have high similarity in color and body size (Fig. 2.5), causing trouble for sex identification. Its coloration is dark brownish or blackish, sometimes glossy and the rump of some forms is distinctly paler than the back. This swiftlet has a very shallow tail-fork. Wing length ranges from 106-125 mm which is shorter than Himalayan swiftlet, *Aerodramus brevirostris* (116-141 mm) and the black-nest swiftlet, *A. maximus* (over 125 mm). Tarsus lightly feathered or unfeathered differing from the black-nest swiftlet, *A. maximus* (Medway; 1966; Chantler and Driessens, 2000).

Several forms of the white-nest swiftlet were united under the name of *Aerodramus fuciphagus* (or *Collocalia fuciphagus*) (Medway, 1966; Chantler and Driessens, 2000; Nguyen Quang, Vo Quang and Voisin, 2002). This species has been proposed that it is composed of eight subspecies, *Aerodramus fuciphagus fuciphagus*, *A. f. germani*, *A. f. inexpextatus*, *A. f. amechanus*, *A. f. vestitus*, *A. f. micans*, *A. f. dammermani*, *A. f. perplexus*. These subspecies have been reported for their geographic distribution and very slightly morphological differences such as upperpart, underpart and rump coloration which have been used for field identification (Medway, 1966; Chantler and Driessen, 2000; Nguyen Quang *et al*., 2002). However, the taxonomic status of these subspecies is uncertain. In Thailand, Lekagul and Round (1991) mentioned that the white-nest swiftlet, *A. fuciphagus* in Thailand possibly consists of two subspecies; *A. fuciphagus germani* and *A. f. amechanus*.

Figure 2.5. A pair of white-nest swiftlet living in a man-made building.

2.3 Distribution and habitat

2.3.1 Distribution and habitat of swiftlets

Swiftlets are widely distributed from the Indian Ocean, through South and South East Asia, to North Australia and the Pacific Islands, but most swiftlet species are found in South East Asia (Chantler and Driessens, 1995, 2000). They live in wide variety of habitats, but usually not far from water and are found from lowlands to high altitude over both forest and more open wooded country (Chantler and Driessens, 2000). Some swiftlet species such as *H. gigas* live in unusual habitats. Habitat of *H. gigas* is primarily associated with waterfalls in mountainous rainforest region in

Malay Penninsula and found to roost at both behind, and on cliffs next to, the waterfall. Some species such as *Collocalia esculenta* and *C. linchi* become established in man-made structures in Indonesia (Lim and Cranbrooke, 2000).

2.3.2. Distribution and habitat of the white-nest swiftlet

The white-nest swiftlet, *A. fuciphagus*, is distributed extensively, from Hainan throughout Southeast Asia, Andaman and Nicobar Islands (Welis, 1999; Chantler and Driessens, 2000). This species originally lives in caves along coastal areas and is resident throughout its range. In Thailand, it is distributed along coastal areas of the Gulf of Thailand and Thai Andaman Sea and on some islands such as See-Ha islands, Moo Ko Chumphon, Phi Phi Le (Dunlap, 1907; Quate, 1952, Boswell and Kanwanish, 1978, Lekagul and Round, 1991; Welis, 1999; Viruhpintu, 2002; Pothieng, 2004). *Aerodramus fuciphagus* subspecies have been reported by some authors and their geographic distribution has been initially proposed by Medway (1966) (Fig. 2.6).
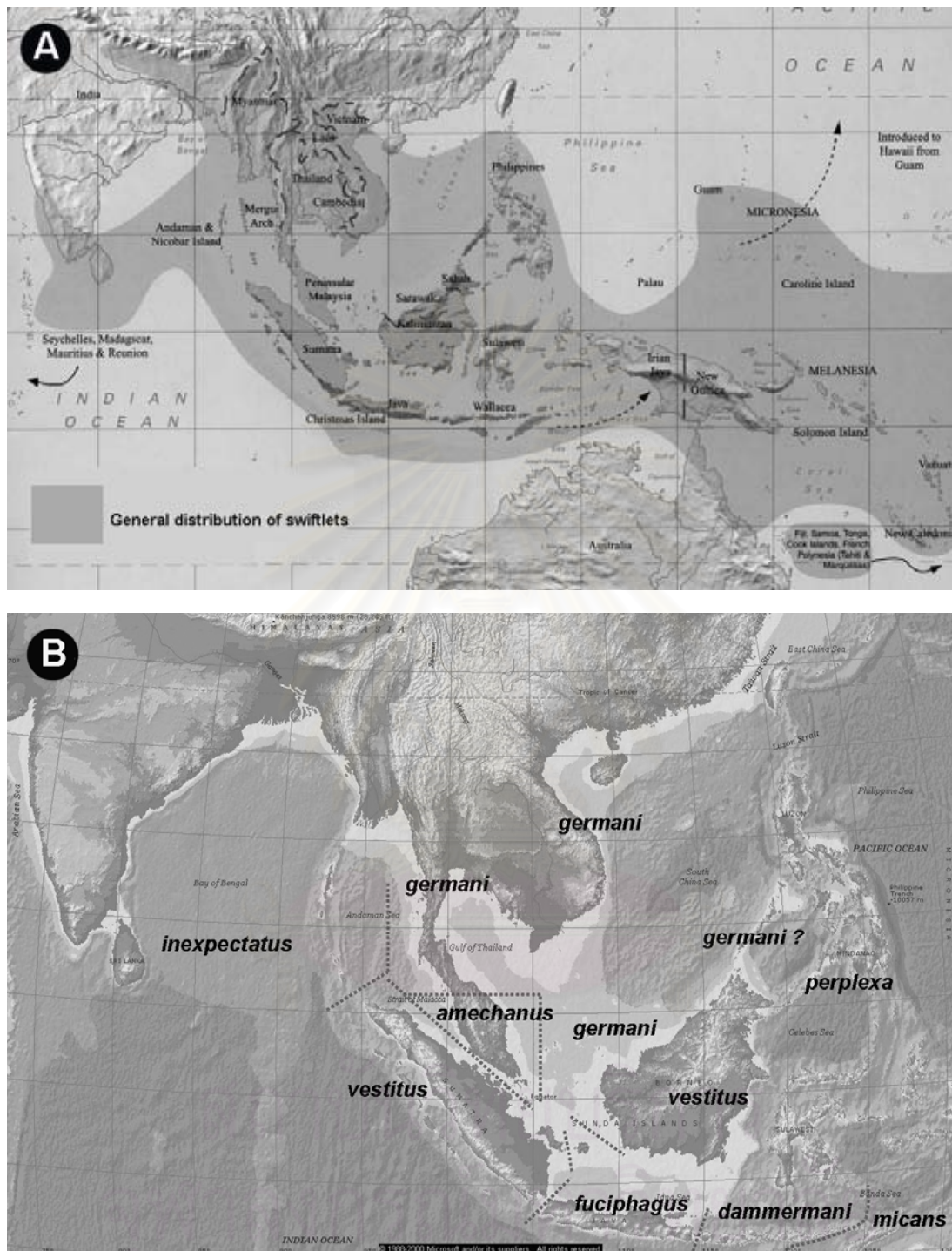
Figure 2.6. Distribution maps of (A) all swiftlet species (from Lim and Cranbrook, 2002) (B) white-nest swiftlet *A. fuciphagus* subspecies (data from Medway, 1966; Chanlter and Driessens, 2000; map from © 1988 - 2000 Microsoft Corp. and/or its suppliers).

2.3.2.1. Natural habitat

Original habitat of the white-nest swiftlet is caves on islands or mainland and this species sometimes shares caves with other animals such as black-nest swiftlet, *A. maximus* and/or bats e.g. black-bearded Tomb bat, *Taphozous melanopogon* and naked bat, *Cheiromeles torquatus* (Ponak, 2004; Clements *et al.*, 2006). *Aerodramus fuciphagus* roosts and nests in caves (Fig. 2.7). This bird uses echolocation to navigate in darkness, but this ability is not used for locating aerial insect prey and echo click of swiftlets are within human range of hearing. Considering congeners living in the cave, Viruhpintu *et al.* (2002) found that *A. fuciphagus* and *A. maximus* use different areas of the cave wall for nesting. This indicated that these two species avoid interspecific competition. Several studies reported that some climatic factors e.g. temperature, relative humidity and rainfall can determine breeding season of the white-nest swiftlet. Within caves, temperature is usually between 27-32˚C and relative humidity is over 70% (Viruhpintu *et al*., 2002; Ponak, 2004). Moreover, Nguyen Quang (1994) mentioned that orientation of cave openings and wind direction could also affect the breeding (nesting) period of white-nest swiftlets in Vietnam.
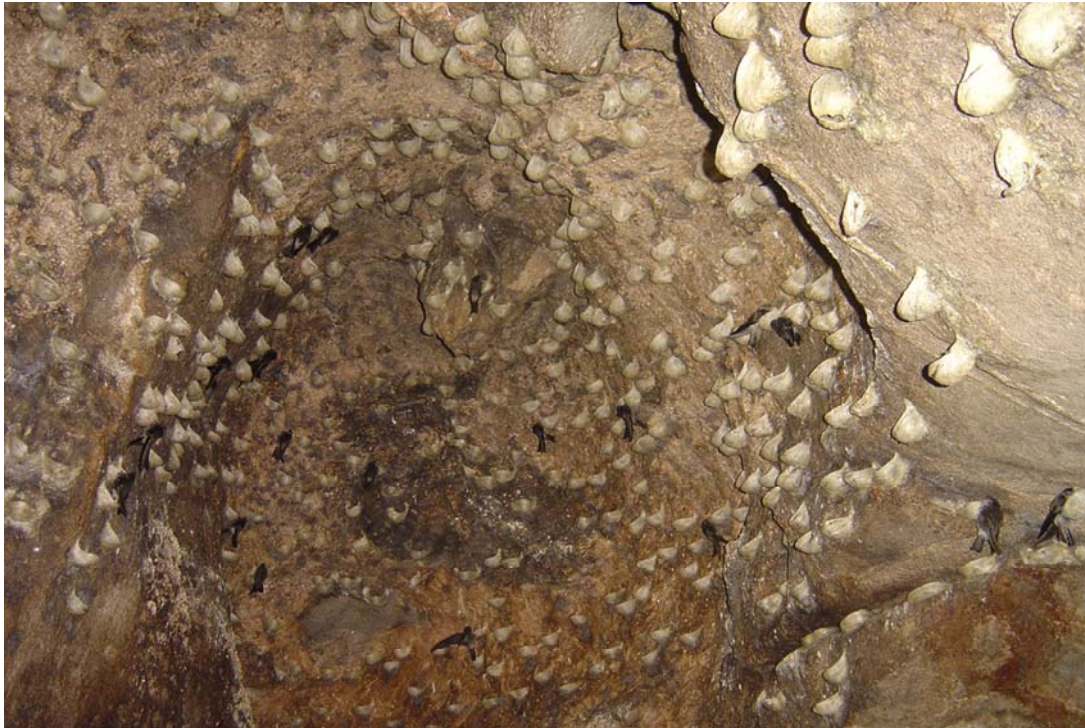
Figure 2.7. Natural habitat of the white-nest swiftlet *A. fuciphagus* at Ko Chan, Prachuab Khiri Khan Province (21 August, 2005).

2.3.2.2. Man-made habitat

Over past centuries, *A. fuciphagus* from natural colonies have established in man-made buildings such as abandon buildings, sacred buildings and old houses in Southeast Asia (Fig. 2.8).   In Thailand white-nest swiftlet was reported that it primarily colonized in houses in Pak Panang, Nakhon Si Thammarat Province (Quate, 1952).   Due to high commercial value of nests and limit of accessibility to natural caves, the idea of the construction of buildings for swiftlets has originally begun in

some areas of Indonesia (Nugroho and Whendrato, 1996) and spread rapidly to other areas in Southeast Asian countries over decades.

Unlike traditional zoo or captive breeding program, swiftlets are free to roam and forage anywhere outside, only returning to roost at night (Lim and Cranbrook, 2000). Knowledge on swiftlet farming has been developed and a few methods for attracting swiftlets to new colonies have been used. Environmental condition within houses is improved as cave-like condition for old houses whereas that of new buildings is designed since the construction has been planned. Two techniques for attracting birds have been used; swiftlet song playback and foster parenting. Swiftlet song has been automatically played everyday. Foster parenting technique has been used in Java, Indonesia. The eggs of *C. esculenta* or *C. linchi*, which tend to more easily colonize in buildings, are substitute for those of white-nest swiftlets taken from another colony and the foster parents are able to incubate and raise the young successfully. After fledging, white-nest swiftlets leave the house, but return to it once they have reach sexual maturity and build nests in darker area of the house due to its echolocating ability which is not found in *C. esculenta* and *C. linchi* (Lau and Melville, 1996; Nguyen Quang *et al*., 2002). However, only a number of swiftlet houses were occupied successfully by the white-nest swiftlet.

Figure 2.8. White-nest swiftlet colony in man-made structure.

2.4. Breeding biology

Biology of white-nest swiftlet *Aerodramus fuciphagus* has been studied in both natural caves, and man-made buildings. *Aerodramus fuciphagus* is monogamous bird. It is faithful to its mate, and has high nest site fidelity (Lim, and Cranbrook, 2002; Viruhpintu, 2002). Breeding season, and period of breeding activities such as nest building, egg-laying, egg incubation, and young rearing of *A. fuciphagus* are varied, depending on locations. The variation could be due to effects of some environmental factors such as climatic condition, and food availability (Langham, 1980; Nguyen Quang, 1994). *Aerodramus fuciphagus* feeds in flight on insects

during the day and its breeding season coincides with seasonal insect abundance (Langham, 1980). Both male and female white-nest swiftlets participate in most breeding activities.

In general, one breeding cycle of this swiftlet is 90-120 days (Lim and Cranbrook, 2002). They spend approximately 30 days for nest building, and then the female lays eggs (Langham, 1980; Viruhpintu, 2002). Two eggs per clutch is the common clutch size for this bird, although the clutch size may decrease in the second or third breeding cycle in that year, depending on food availability (Langham, 1980; Nguyen Quang and Voisin, 2002). Incubation period for this bird ranges from 17-31 Days (Langham, 1980; Viruhpintu, 2002). After hatching, parents rear and feed the nestlings about 40 days. Periods of nestling feeding are not much different across its distribution line (Langham, 1980; Kang *et al*., 1991; Kang and Lee, 1991; Nguyen Quang, 1994).

2.5. Molecular approaches for studying genetics of populations

The term "Phylogeography" was initially used in 1987 by John Avise and defined as a field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages, especially those within and among closely related species (Avise, 2004). Molecular analyses at the level of population have been conducted for hundreds of species at a variety of temporal and spatial scales. Studies on population genetics were primarily analyzed allozymes to investigate genetic structure or differentiation between populations, but its information has detected little geographical structure in some birds, comparing to other vertebrates (Zink, 1997). To date the fast-evolving molecular markers such as mtDNA and microsatellites which have higher levels of genetic differentiation are the most widely used alternative to allozymes for population genetic studies (Zink, 1997; Crochet, 2000).

2.5.1. Mitochondrial DNA

Mitochondrial DNA has been extensively used over past few decades as a tool for inferring the evolutionary and demographic history of populations and species (Randi, 2000). It has become a standard molecular tool. This could be because protocols of mtDNA studies have been developed for a wide range of taxa (Kocher *et al*., 1989) and it is practically easy for enzymatic amplification. Moreover, mitochondrial phylogeographic studies often discover significant geographic and behavioral information (e.g. Bowie *et al*., 2004; Godoy *et al*. 2004; Yang *et al*., 2006b).

As a molecular marker, mtDNA has many advantages to address a variety of ecological and evolutionary questions. Mitochondrial DNA sequences evolve, on average 5-10 times, faster than nuclear genes (Wilson *et al*., 1985; Avise, 2004). Mitochondrial DNA divergence rate in vertebrate is high which is about 1-2% per million years (Wilson *et al*., 1985), but relative and absolute rates can vary widely among lineages and genes. Mitochondrial DNA represents maternal mode of inheritance. From phylogenetic view, these features mean that mtDNA usually has a single genealogical history through maternal lineages.
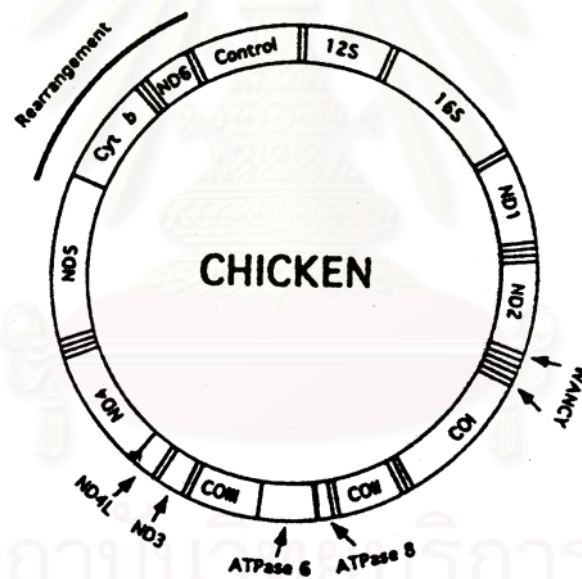
Figure 2.9. Schematic diagram of the gene content and gene order of the chicken mitochondrial DNA genome (Desjardins and Morais, 1990).

Vertebrate mitochondrial DNA (mtDNA) is a small (15-20 kb) circular molecule. Typically, each mtDNA genome consists of 37 functionally distinct genes. These loci encode 22 different tranfer RNAs, two ribosomal RNAs and 13 messenger RNAs specifying polypeptide subunits of proteins involved in electron transport and

oxidative phosphorylation that take place on the mitochondrion's inner membrane (Baker, 2000). Mitochondrial gene order is mostly conserved within, but varies between different phyla or order (Baker, 2000). Closely related mtDNA sequences differ mainly in transition (Ti) rather than tranversion (Tv) and value of Ti:Tv ratios can be 10-20 or higher in intraspecific comparison. However, Tv accumulate with time and Ti:Tv become lower than 1, indicating mutational saturation and increasing occurrence of multiple Ti at the same site. Among avian species, some mtDNA regions (Fig. 2.9) such as cytochrome-*b*, dehydrogenase subunit 2 (ND2) and control region have been extensively investigated for phylogenetic studies (Mindell, 1997) and demonstrated to be useful phylogenetic markers at population level (e.g. Pavlova *et al*., 2005; Nyári, 2007). Most studies on population history, phylogeographic or genetic structure have usually been conducted across species' distribution ranges or over interesting areas and these results sometimes can be used for conservation management.

Yang *et al*. (2006b) studied phylogeographic structure of the ground tit, *Psuedopodoces humilis* across the Tibetan Plateau of which an endemic area for this species using control region sequences of mtDNA. This genetic structure could be explained by climatic and paleogeographic changes following the uplift of the Tibetan Plateau and might be restricted gene flow between populations.

Seki *et al*. (2007) reported genetic structure of Japanese wood pigeon *Columba janthina* which is endemic to islands of East Asia based on mtDNA control region. Analyses of genetic differentiation indicated that Japanese wood pigeon, comprised of three population groups; northern (Okinawa, Tokara, Goto, Setouchi, Oki and Izu islands), southeastern (Ogasawara Islands) and southwestern (Sakishima

Islands) groups across their distribution range. This study suggested that these three population groups should be considered as independent management groups and the southeastern group has conservation priority due to small population size and its narrow distribution.

Kahindo *et al*. (2007) reviewed recent genetic studies on African birds in Afro-montane regions. Several species, for example, montane greenbul (*Andropadus*) species (cyt-*b* and ND2 sequences), and African highland sunbird (*Nectarinia*) species (NADH3 and control region sequences), showed genetic structure in the montane forest regions. This indicated little or no gene flow between their closet populations due to the geographic isolation, causing high levels of genetic differentiation among isolated populations or taxonomically divergent populations.

For highly mobile species such as birds and marine animals, genetic differentiation of subpopulations is generally exhibited low levels and sometimes found lack of genetic structure. However, information from mtDNA could be inferred historical demographic events of a species.

Burns and Barhoum (2006) reported that population history of the wrentit, *Chamaea fasciata* was investigated across the California Floristic Province using several mtDNA regions i.e. cyt-*b*, ATPase6, ATPase6, tRNA-Lys and small portions of COII and COIII. This species is sedentary and native to scrub and chaparral habitat in this region. Population analyses suggested that *C. fasciata* was isolated into southern refugia during Pliestocene and has undergone to a recent range expansion.

Yang *et al*. (2006a) analyzed mtDNA control region segment of the white-rumped snowfinch, *Onychostruthus taczanowskii* populations through its Tibetan Plateau distribution range. They found very low genetic diversity and no genetic

differentiation between geographic groups. This may be because of a combination of ecological and historical factors such as high gene flow, lack of geographic barrier and rapid population expansion.

2.5.2. Microsatellites

Microsatellites are often used in biological studies in addressing questions at a variety of scales, ranging from individual-specific level such as determining gender, genetic relatedness or paternity (e.g. Longmire *et al*., 1993; Griffith *et al*., 1999; Feldheim *et al*., 2004; Rudnick *et al*., 2005) or at population level such as population structure, subspecies evolution and classification (e.g. Grant *et al*., 2000; Chan and Arcese, 2003; Carreras-Carbonell *et al*., 2006) and up to higher order systematics and taxonomy (e.g. Petren *et al*., 1999; Burg and Croxall, 2004).

Microsatellites or simple sequence loci constitute part of a group of loci known as variable number of tandem repeat (VNTR) loci. Microsatellite repeats typically comprise 10-50 copies of short repeat motif (1-10 base pairs (bp), usually 2-5 bp). The other well-known class of VNTR loci is minisatellites which differ from microsatellites in size (10-100 bp) and the number of tandem repeats. Microsatellite loci can be classified on the basis of the repeat motif length i.e. dinucleotide, trinucleotide, tetranucleotide, etc. Characteristics of the repeat motif i.e. type, length appear to affect the rate of mutation and levels of allelic variation. Loci with longer repeats are generally more polymorphic than loci which are composed of short motif. The predominant mutation mechanism which is used to explain high mutation rates of microsatellites is slipped strand mispairing of complementary bases at the site of a short repeat during DNA replication (Ashley and Dow, 1994). Slippage, follow by

replication or repair would lead to insertion or deletion of one or more repeat units (Ashley and Dow, 1994). Longer microsatellite loci may be more susceptible to slipped strand mispairing, whereas point mutations may interrupt repeat sequences and thereby decrease microsatellite length and reduce slippage (Karhu, 2001). Mutation rates of microsatellites vary considerable among microsatellite loci and may also differ between sexes (Primmer et al. 1998) and among individuals (Brohede, Møll and Ellegren, 2004). Mutation rate is often about 10^{-3} or 10^{-4} per locus per gamete per generation (Xu et al., 2000). The method of assaying and analyzing microsatllites loci has several advantages over other molecular techniques currently in use. It relies on polymerase chain reaction (PCR) rather than Southern blotting and hybridization with labeled probe and requires much less DNA (Ashley and Dow, 1994). Codominant alleles at single microsatellites loci can be scored by size. Typically, the genotypic data provided by microsatellites show higher variation than other molecular techniques such as allozyme. To date, a number of studies have used microsatellite loci, sometimes in conjunction with other genetic markers, to examine genetic differentiation of bird populations. Population structure can be detected using this highly variable molecular marker at both macro- and microgeographical scales.

Chan and Arcese (2003) investigated genetic variation of song sparrow, *Melospiza melodia* at microgeographic scale. They studied morphological and microsatellite variation of five subspecies of song sparrow living in San Francisco Bay area. High morphological differentiation was found between subspecies whereas genetic variation at microsatellite loci was low. Low concordance of morphological and genetic estimates of divergence suggests that selection or phenotypic plasticity in morphology has caused morphological differentiation among the subspecies.

Rocha and Lama (2004) compared genetic variability and structuring of wood stork, *Mycteria americana*, from Brazilian Pantanal subpopulations with a North American population using allozyme and microsatellite data. This study showed no significant of genetic differentiation in Pantanal subpopulations and low differentiation between Panatal and North American population. Lack of differentiation among Panatal subpopulations may be due to high gene flow and low natal philopatry. Low differentiation between North and South American populations could be a result of occupying in neighboring regions by these populations during late glaciation or continuous of gene flow between them via Central American or northern South American populations.

Funk *et al*. (2007) examined the genetic structure of snowvy plover, *Charadrius alexandrinus*, within and among breeding areas in the Western Hemisphere which includes North America, the Carribbean, and the west coast of South America using mitochondrial control region and microsatellites. Results indicated that Puerto Rican breeding group is genetically divergent from sites in the continental US and high levels of genetic differentiation were found between sites from North America and South America. Therefore, three subspecies should be recognized; *C. a. nivosus* in continental US including Florida, *C. a. tenuirostris* in Puerto Rico and *C. a. occidentalis* in South America.

2.6. Genetic analyses at the level of populations

2.6.1. Measures of genetic variation

In order to document the amount of genetic variation in a standardized way, some measures have been conducted to quantify this information for populations.

2.6.1.1. Genetic variation of mitochondrial DNA

Nucleotide diversity

Nucleotide diversity (π) is defined as the average number of nucleotide differences per site between sequences.

$$\pi = \frac{n}{(n-1)\sum x_i x_j \pi_{ij}}$$ (Nei, 1987, equation 10.5)

or

$$\pi = \frac{\sum x_{ij}}{n_c}$$ (Nei, 1987, equation 10.6)

Where n is the number of sequences examined, x_i is the frequency of the ith type of DNA sequence in the sample and n_c is the total number of the sequence comparison.

Haplotype diversity

A haplotype is a unique sequence of linked genetic marker, in this case, nucleotide (Halliburton, 2004). The haplotype diversity is described as the number and frequency of different haplotypes in the sample (Nei, 1987).

$$Hd = (n/n-1)\left(1 - \sum f_i^2\right)$$

where

f_i^2 is the frequency of the *i*th haplotype

n is the number of individuals sampled.

2.6.1.2. Genetic variation of microsatellites

Heterozygosity

The most widespread measure of genetic variation in populations is the amount of heterozygosity (Hendrick, 2004). Individuals in diploid species can be heterozygous or homozygous at a locus and the parameter "heterozygosity" is a biological useful quantity. Generally, heterozygosity values range from "0" to "1". High heterozygosity means high genetic variability whereas low heterozygosity means little genetic variability which could be a result of some forces such as inbreeding.

The expected Hardy-Weinberg heterozygosity of a population for a particular locus with *n* alleles can be calculated as

$$H_E = 1 - \sum_{i=1}^{n} p_i^2$$

which is one minus the Hardy-Weinberg homoygosity. Nei (1987) called this measure as gene diversity and suggested that it is particularly useful because it is applicable for genes of different ploidy levels and in organisms with different reproductive system.

Using the estimated allele frequencies, an unbiased estimate of the expected heterozygosity at a locus, using small sample size correction, is

$$\hat{H}_E = \frac{2N}{N-1}(1 - \sum_{i=1}^{k} \hat{p}_i^2)$$

In most outbreeding populaions, the observed heterozygisity is quite close to the theoretical heterozygosity. However, for populations in which genotype frequencies may not be close to Hardy-Weinberg proportions, the observed heterozygosity may be calculated as

$$\hat{H}_O = \sum_{i<j}^{n} P_{ij}$$

where $\hat{P}_{ij}$ is the estimated frequency of genotype ij and $\hat{H}_O$ is the summation over the frequencies of all heterozygotes.

2.6.2. Demographic history

DNA polymorphisms are powerful sources of information for studying the evolution of populations and several methods (e.g. mismatch distribution and test of neutrality) have been used to investigate demographic history of populations. "Mismatch distribution" or the distribution of the number of pairwise differences between DNA sequences was presented by Roger and Harpending (1992). Under the demographic equilibrium or population of constant size, mismatch distribution is expected to be a multimodal distribution. Conversely, populations that have sudden demographic expansion show a unimodal distribution.

Another approach which is used to investigate population history is test of neutrality. Several statistics, for example, Tajima's D (Tajima, 1989), F (Fu and Li, 1993) and F_s (Fu, 1997), have been developed for testing the hypothesis that all mutations are selectively neutral (Kimura, 1983). Tajima's D (Tajima, 1989) is probably the most classic test and the resulting statistic is often used to test selective neutrality of DNA sequences under an "infinite-site" model (Watterson, 1975; Avise,

2004), but popular outcome can also be affected by historical demographic event such as dramatic expansion in population size (Avise, 2004). Tajima's D test is based on the differences between the number of segregating sites and the average number of nucleotide differences. The D test statistic can be computed as

$$D = \frac{d}{\sqrt{V(d)}} = \frac{k - \dfrac{S}{a_1}}{\sqrt{e_1 S + e_2 S(S-1)}}$$

where

        S, is the total number of segregating sites.

        k, is the average number of nucleotide differences between pairs of sequences

$$a_1 = \sum_{i=1}^{n-1} \frac{1}{i} \qquad \text{where } n, \text{ the number of nucleotide sequences}$$

$$e_1 = \frac{1}{a_1}\left(\frac{n+1}{3(n-1)} - \frac{1}{a_1}\right) \quad e_2 = \frac{c_2}{a_1^2 + a_2}$$

where $a_2 = \sum_{i=1}^{n-1} \frac{1}{i^2}$ and $c = \dfrac{2(n^2 + n + 3)}{9n(n-1)} - \dfrac{n+2}{a_1 n} + \dfrac{a_2}{a_1^2}$

A positive value of Tajima's D indicates that there has been 'balancing selection' and the data will show a few divergent haploypes, whereas a negative value suggests that 'purifying selection' may have occurred. In the latter instance, the data will reveal an excess of singletons.

For addressing historical population growth explicitly, Fu (1997) introduced Fu's *Fs* test that distinguishes excesses of low-frequency alleles in an expanding population as compared with the number expected in a static population.

$$F_S = \ln\left(\frac{S'}{1 - S'}\right)$$

The F_S test is sensitive to demographic expansion and genetic hitchhiking. Negative F_S value indicates that there is an excess of rare alleles with an excess of recent mutations as would occur with a selective sweep or population expansion.

2.6.3. Deviation from Hardy-Weinberg equilibrium

The Hardy-Weinberg principle provides the information for many theoretical investigations in population genetics (Harlt, 2000). Main assumptions of the Hardy-Weinberg model are:

- Random mating

- Large population size

- No migration

- No mutation

- No selection

- Nonoverlapping generation

- Sexual reproduction

- Diploid organism

The first five of these assumptions relate directly to the five major forces that drive evolutionary change. Under the Hardy-Weinberg condition, allele frequencies and genotype frequencies will remain constant in a population from generation to generation (Hartl, 2000; Halliburton, 2004). Therefore, the Hardy-Weinberg expected genotype frequencies for two alleles, A and a, are in the proportion, p^2 (AA), q^2 (aa), $2pq$ (Aa)

$$(p+q)^2 = p^2 + 2pq + q^2$$

This is often called "the Hardy-Weinberg equilibrium (HWE)" (Hartl, 2000). To determine whether studied population deviates from Hardy-Weinberg expectation, the chi-square test (χ^2) is commonly used and can be calculated as

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

where O and E are the observed and expected numbers for each class (genotype) and the summation is overall classes (genotypes). The calculated value is compared to a critical value obtained from a chi-square table. If the observed values differ significantly from expectation values, then null hypothesis is rejected (Halliburton, 2004; Hendrick, 2005). Deviation from HWE indicates the effect of at least one evolutionary process. However, if deviation from HWE is not detected, it can not be concluded that no evolutionary process is operating (Halliburton, 2004).

2.6.4. Linkage disequilibrium

Under the assumptions for HWE, the alleles present at one locus are in random association with one another (Harlt, 2000). Linkage disequilibrium (LD) is defined as the nonrandom association of alleles at different genetic loci in a population (Lewontin and Kojima, 1960; Mueller, 2004). Synonymous terms are "allelic association" or "gametic phrase disequilibrium (Crow and Kimura, 1970)". LD measures the allelic association in the same gamete or same chromosome. Some evolutionary force such as genetic drift and mutation appear to be major factors generating disequilibrium (Halliburton, 2004). LD has been useful in gene mapping (Jorde, 1995; Greenspan and Geiger, 2004; Morton, 2005) and describing

demographic and evolutionary processes in plant and animal populations (Mueller, 2004), for example, natural selection (Cannon, 1963), population subdivision (Kitada and Kishino, 2003).

The simplest measure of linkage disequilibrium for two biallelic loci, D, is the difference between the observed and expected frequencies of a gamete (haplotype) as defined by

$$D = x_{11} - p_1 q_1$$

where A_1 and A_2 are alleles at locus A and B_1 and B_2 are alleles at locus B

x_{ij} values are the frequencies of the four possible gametes (or haplotypes)

p_1 and q_1 are the frequencies of alleles A_1 and B_1

In order to compare LD quantities among different pairs of loci with differing allele frequencies, one way of standardization has been proposed:

$$D' = \frac{D}{D_{max}}$$

D' was proposed that has the same maximum, 1 or -1, for all combinations of allele frequencies where D_{max} is the maximum disequilibrium possible for the given allele frequencies. When D > 0 then D_{max} is the lesser of $p_1 q_2$ and $p_2 q_1$ and when D < 0 then D_{max} is the lesser of $p_1 q_1$ and $p_2 q_2$.

Significant testing for the LD coefficient D follows testing for independence in a 2×2 contingency table. The usual methods; a chi-square test, a likelihood test or Fisher's exact test can be employed (Mueller, 2004).

2.6.5. Population genetic structure and gene flow

Populations of most species usually exhibit at least some degree of genetic differentiation across their geographic distribution. Population subdivision may take place on several different spatial or geographic scales and could cause by geographical, ecological or behavioral factors. When a population is subdivided, the amount of genetic connectedness among the parts of the population can differ (Hendrick, 2004). In animals, genetic differentiation usually correlates with ability of mobility. For example, insects e.g. Dawson's burrowing bee (Beveridge and Simmons, 2006) and birds often show less genetic differentiation than do relative sedentary animals such as some amphibians e.g. eastern red-backed salamander *Plethodon cinereus* (Noël *et al.*, 2007) and reptiles e.g. black caiman *Melanosuchus niger* (Thoisy *et al.*, 2006), sharp-snouted pitviper *Deinagkistrodon acutus* (Huang *et al.*, 2007).

Several approaches have been used to investigate the amount of genetic differentiation the subdivisions of a population (Hendrick, 2004). The commonly used statistics is "*F*-statistics" which was originally introduced by Wright (1951). This statistics measures loss of heterozygosity at three levels of hierarchical population structure: within subpopulations, between subpopulations, and total population (Conner and Hendrick, 2004). It consists of three different F coefficients; F_{IS}, F_{ST} and F_{IT}. F_{IS} measures the proportional reduction in heterozygote within subpopulation due to inbreeding. F_{ST} measures the proportional reduction in the heterozygosity of the total population due to differentiation among subpopulations, relative to expectation with no population subdivision. F_{IT} measures the reduction in heterozygosity in an individual (I) relative to the total population (T) due to both

nonrandom mating within subpopulations and genetic drift among subpopulations. The three statistics are related as follows:

$$(1 - F_{IT}) = (1 - F_{ST})(1 - F_{IS})$$

F_{ST} is often called fixation index because it increases as more subpopulations become fixed for one allele (Conner and Hendrick, 2004). Theoretically, values of F_{ST} are positive and can range from 0.0 (for subpopulations genetically identical) to 1.0 (for subpopulations fixed for different alleles). However, some computer programs calculate F_{ST} as negative values. This can result from greater genetic variability within subpopulations than between subpopulations.

Gene flow is defined as all mechanisms resulting in the movement of genes from one population to another (Slatkin, 1985). Some mechanisms such as migration and dispersal are often used to explain how gene flow can occur. When the amount of gene flow between populations is high, gene flow has the effect of homogenizing genetic variation over the populations (Hendrick, 2004). When gene flow is low, genetic drift selection, and even mutation in the separate populations may lead to genetic differentiation (Hendrick, 2004). "Migration" refers to the movement of individuals among subpopulations (Harlt, 2000). "Dispersal" is defined as the movement of organisms from their natal sites (natal dispersal) or of adults between breeding attempts (breeding dispersal) (Greenwood and Harvey, 1977). Some animal groups such as birds, insects and marine animals have migratory behavior (e.g. seasonal migratory) or high dispersal capability. These mechanisms are a sort of genetic glue that holds subpopulations together and set a limit to how much genetic divergence can occur (Harlt, 2000).

Estimating of the amount gene flow in most situations is difficult. Both direct and indirect methods can be employed. Direct method of measuring gene flow can be obtained in the organism that can be identified individually using marking approach such as toe clipping in amphibians, leg banding in birds (Hendrick, 2004). Gene flow can be inferred indirectly from genetic data by statistical analysis approach. Usually gene flow is expressed as a migration rate, m, defined as the proportion of alleles in a population that are migrants in each generation (Avise, 2004). Nm parameter is interpreted as a mean-per generation estimate of absolute number of migrants exchanged among populations. A few indirect methods for estimating levels of gene flow have been often used and can be calculated as follow (Slatkin and Barton, 1989; Avise, 2004):

1. From F_{ST}. Wright (1951) showed the calculation of Nm in the island model.

$$Nm \cong \frac{(1 - F_{ST})}{4F_{ST}}$$

2. From private alleles. Slatkin (1985) showed the calculation of Nm from natural logarithm of the average frequency of private alleles relating to the natural logarithm of Nm:

$$Nm = e^{-[(\ln p(1) + 2.44)/0.505]}$$

Outcomes of Nm greater than about 1-4 indicate that the homogenizing influence of gene flow over time has overridden the diversifying effects of genetic drift, whereas values of $Nm < 1$ suggest the converse (Birky et al., 1983).

CHAPTER III

MATERIALS AND METHODS

3.1 Current distribution of *Aerodramus fuciphagus* populations on mainland in Thailand

Field surveys were conducted from 2003 to 2006 along the coastline of the Gulf of Thailand, ranging from Trat Province to Narathiwat Province, and the coastline of Thai Andaman Sea, ranging from Ranong Province to Satun Province. The coastlines of Gulf of Thailand and Andaman Sea are 1,972.5 km and 1,037.5 km, respectively (Royal Institute, 2002). The distribution of white-nest swiftlet was analyzed based on information from the survey in this study and historical distribution from literature review. The current distribution of the white-nest swiftlet along the coastlines of Thailand was plotted on the map.

3.2 Sampling

Due to the extremely high monetary value placed on the nests of the white-nest swiftlet, all natural caves and man-made buildings containing colonies are closely managed and protected. As visitors to swiflet colonies are not welcome, I was only able to sample some man-made colonies and sampling birds from any naturally-occurring cave colonies was not possible. Samples in this study came from swiftlet houses that had been established for 8 to 10 years.

Tissues or feathers from 160 white-nest swiftlets were collected between 2003 and 2006. Muscle tissues were obtained from newly dead or injured chicks dropped from their nests at their breeding colonies during nest harvesting by the house owners

and stored in 95% ethanol. To avoid sampling the same individual twice, feathers were plucked from individuals that were caught from their nests. Feathers were maintained dry using silica gel. Ten colonies were sampled along coastal areas, ranging from the east and west coasts of the Gulf of Thailand to southern Thailand and the Thai Andaman Sea coast (Fig. 3.1 and Table 3.1). The Chumphon samples (CP) include individuals from three swiftlet houses (CP1, CP2 and CP3) and the Songkla colony (SK) includes individuals from two swiftlet houses (SK1 and SK2). Samples from other colonies represent single swiftlet houses. Voucher specimens have been deposited at the Natural History Museum of Chulalongkorn University (CUMZ.AV.079.2006.1-CUMZ.AV.079.2006.78; Appendix I).
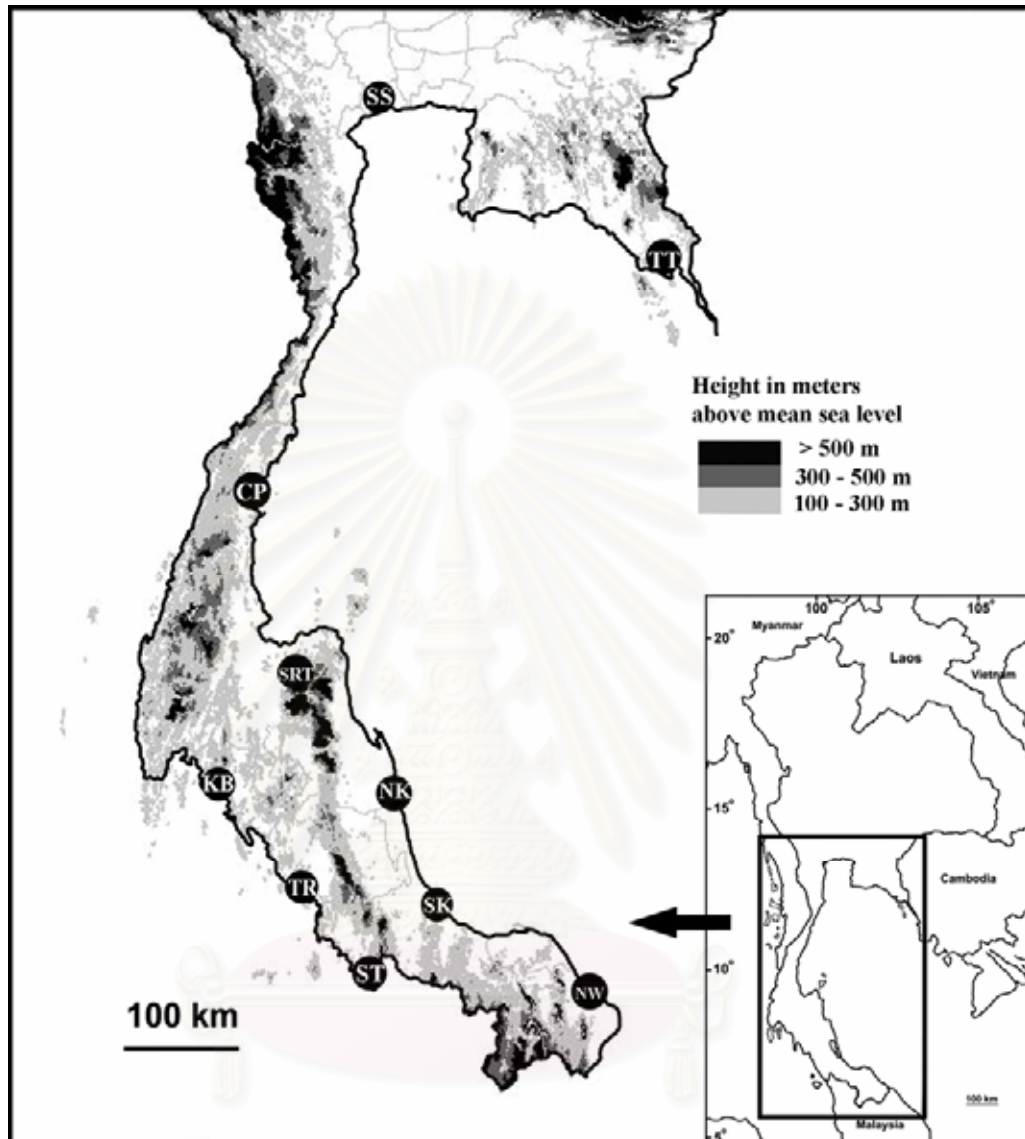
Fig. 3.1. Locations of sampled colonies of white-nest swiftlet (*A. fuciphagus*). Abbreviations for locations are; Trat (TT), Samut Sakhon (SS), Chumphon (CP), Surat Thani (SRT), Nakhon Si Thammarat (NK), Songkla (SK), Narathiwat (NW), Krabi (KB), Trang (TR), Satun (ST).

Table 3.1 Location information on 10 sampled colonies, the number of individuals sampled and the haplotype codes used.

Location name and abbreviation of colony	Geographic coordinates		N
Trat (TT)	N 12º14´30.8´´	E 102º32´33.0´´	5
Samut Sakhon (SS)	N 13º31´42.8´´	E 100º16´02.6´´	26
Chumphon (CP)	CP1: N 10º29´38.9´´	E 099º10´22.5´´	12
	CP2: N 10º26´23.9´´	E 099º15´08.7´´	
	CP3: N 10º26´26.6´´	E 099º15´08.9´´	
Surat Thani (SRT)	N 08º38´03.3´´	E 099º22´21.9´´	43
Nakohon Si Thammarat (NK)	N 08º02´22.9´´	E 100º19´03.4´´	34
Songkla (SK)	SK1: N 07º01´45.1´´	E 100º28´13.5´´	5
	SK2: N 07º11´31.8´´	E 100º35´35.2´´	
Narathiwat (NW)	N 06º25´57.1´´	E 101º49´39.1´´	19
Krabi (KB)	N 08º05´07.7´´	E 098º54´33.9´´	3
Trang (TR)	N 07º24´30.7´´	E 099º30´46.9´´	10
Satun (ST)	N 06º36´27.3´´	E 100º03´41.5´´	3

3.3 Laboratory procedure

3.3.1 DNA extraction

Three extraction methods; Puregene DNA isolation, phenol-chloroform method, and DNeasy® Tissue extraction were used to extract *A. fuciphagus* DNA.

3.3.1.1 Puregene DNA isolation (Gentra Systems, Minneapolis, Minnesota)

Tissue samples were chopped with razor blade into pieces and were added into 1.5 mL microcentrifuge tube containing 300 μL of cell lysis buffer. 7-10 μL of 10 mg/mL proteinase K was added and the mixture was incubated overnight at 55°C on a rocking platform. 100 μL of protein precipitation solution was added and vortexed. The mixture was centrifuged for 3 min at 13000 rpm. Supernatant was removed carefully into a new 1.5 mL microcentrifuge tube. 300 μL of 100% Isopropanol was added and the solution was mixed by inverting until DNA become visible. If DNA was not visible, the solution was kept overnight at -20°C for more precipitation. The solution was centrifuged for 1-3 min at 13000 rpm. Supernatant was poured off and drained the tube on paper towel. 600 μL of 70% Ethanol was added and mixed by inverting gently then poured off and air dry for 30-60 min. 25-75 μL of distilled H_2O was added and incubated 45-60°C or at room temperature to insure that DNA was dissolved into solution. Extracted DNA has been kept for analyses at 4°C or -20°C for long term storage.

3.3.1.2 Phenol-chloroform method (Hillis, Moritz, and Mable, 1996)

Small pieces of tissue samples were tranferred into 1.5 mL microcentrifuge tube containing the mixture of 500 μL of STE buffer (100 mM NaCl, 50 mM Tris-HCl pH 8.0, 1 mM EDTA), 25 μL of 20% SDS and 25 μL of 10 mg/ml proteinase K.

The mixture was mixed well and incubated at 55°C for 4 hours or overnight at 37°C in a shaking water bath. An equal volume of the solution PCI (phenol:chloroform:isoamyl; 25:24:1) was added and mixed gently. The mixture was centrifuged for 10 min at 8000 rpm. The upper aqueous layer was removed and re-exacted with PCI. An equal volume of the solution CI (chloroform:isoamyl alcohol; 24:1) was added and the mixture was centrifuged for 10 min at 8,000 rpm. To precipitate DNA, 1:10 volume of 3 M NaAc and 2.5 times the sample volume of cold absolute EtOH were added. To increase precipitation, DNA was kept overnight at -20°C. The precipitated DNA was centrifuged at 12,000 rpm for 10 min, and then poured off the solution. The pellet was washed with 70% EtOH and air dried. The pellet was re-suspended with TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) or distilled H_2O and incubated at 45-60 °C or at room temperature. Extracted DNA has been stored at 4°C for further analyses.

3.3.1.3 DNeasy® Tissue kit (QIAGEN, Valencia, California)

DNA isolation from feather samples was performed using DNeasy® Tissue kit. The manufacturer's protocol for animal tissues was slightly modified. Buffer ATL, AL, AW1, AW2 and AE were provided by the kit. An aqueous 1 M dithiothreitol (DTT) solution was prepared just before use. Feathers were cut into small pieces and transferred into a 1.5 mL microcentrifuge tube containing 300 μL of Buffer ATL and added 20 μL of proteinase K. To aid the breakage down of the feather shaft, 20 μL of 1 M DTT was added to the lysis buffer prior to overnight incubation at 55°C on a rocking platform. Completely lysed sample was vortexed for 15 s and 200 μL of Buffer AL was added to the sample and mixed thoroughly by vortexing. 200 μL of

100% EtOH was added and mixed again. The mixture was transferred into the DNeasy Mini spin column and centrifuged for 1 min at 8,000 rpm. The flow-through solution was discarded and placed the DNeasy Mini spin column in a new 2 mL collection tube. The sample was washed for 2 times with Buffer AW1 and AW2. To elute DNA, 200 μL of Buffer AE was added directly onto the membrane and incubated for 1 min. The column was centrifuged for 1 min at 8000 rpm. Extracted DNA has been kept at 4 °C for analyses.

3.3.2 Mitochondrial DNA

PCR amplification and sequencing

Fragments of mitochondrial DNA genes, cyt-*b* and ND2, were amplified by PCR using newly designed primers: cyt-*b*258 and cyt-*b*820 for cyt-*b* and primers L222 and R702 for ND2 (Table 3.2). The design of these primers was based on *A. fuciphagus* sequences from Thomassen *et al*. (2003) for cyt-*b* (GenBank accession numbers AY135627-AY135632) and Price *et al*. (2004) for ND2 (GenBank accession numbers AY294489-AY294491). PCR amplification was carried out in a 25 μL reaction containing 1x PCR buffer (10 mM Tris-HCl and 50 mM KCl, pH 8.3), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 10x BSA, 0.5 U Taq DNA polymerase, 0.2 μM primers and 1-2 μL DNA template. Thermal profiling consisted of an initial denaturation for 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 1 min at 72°C, with a final 10 min extension at 72°C. PCR products were purified using Qiagen PCR purification kit (QIAGEN) or MultiScreen-PCR Filter Plates following the manufacturer's protocol (Millipore, Billerica, Massachusetts). To purify PCR product using MultiScreen-PCR Filter Plates, 80 μL distilled H_2O was added in each PCR

product and transferred into a Millipore plate. The Millipore plate was put on the vacuum for 7 min and re-suspended with 60 μL distilled H_2O. The Millipore plate was put on a shaker for 15 min and transferred the solution into a 1.5 microcentrifuge tube for cycle-sequencing. The purified product was cycle-sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California). Cycle-sequenced reactions were precipitated with EtOH, 3 M NaOAc and 125 mM EDTA and sequencing products were run on an 3730 Analyzer (Applied Biosystems). DNA sequences have been deposited in GenBank under accession numbers EU072051-EU072080 and EU085044-EU085333.

Table 3.2 Primers used in this study for PCR and sequencing of cytochrome-*b* and ND2 genes.

Gene	Primer name		Primer sequence (5'-3')	Tm (°C)
Cyt-*b*	cyt-*b*258	forward	CGGAGCCTCATTCTTCTTCA	55
	cyt-*b*820	reverse	ATCATTCCGGCTTGATATGG	55
ND2	L222	forward	TCAAGCAGCTGCCTCCAC	55
	R702	reverse	TGTTGTGTTCAGGGTGAGGA	55

3.3.3 Microsatellites

Microsatellite development and genotyping

Species specific microsatellite primers were developed using an enrichment protocol (Glenn and Schable, 2005) by Feldheim and primer pairs for PCR amplification were tested and selected by Aowphol at the Pritzker Laboratory for Molecular Systematics and Evolution, the Field Museum of Natural History, Chicago. The enrichment protocol used biotin-labeled microsatellite probes and streptavidin-coated magnetic beads which have been described in detail elsewhere (Feldheim *et al*., 2006; Pauls *et al*., 2007). Genomic DNA fragments were enriched using five biotin-labeled tetranucleotide probes (AAAT; AACT; AAGT; ACAT; AGAT). 144 potential loci were sequenced. The primers flanking core microsatellite repeats were developed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Eleven primer pairs (Table 3.3) were used to genotype all sampled individuals.

PCR amplifications for microsatellite loci were carried out in 10 μL reactions containing 1x PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM $MgCl_2$, 10x BSA, 0.12 mM of each dNTP, 0.16 μM of the fluorescently labeled M13 probe, 0.16 μM of the species-specific reverse primer, 0.4 μM of the species-specific forward primer with a 5'-M13 tail (following Schuelke, 2000), 1U Taq DNA polymerase, 1-3 μL DNA template and sterile water. Cycling conditions consisted of initial denaturation 4 min at 94°C, 30 cycles of 30s at 94°C, 30 s at 57-60°C (Table 3.3), 45 s at 72°C, 8 cycles of 30 s at 94°C, 30 s at 53°C, 45 s at 72°C and a final extension 10 min at 72°C. Samples were run on ABI 3730 with 9.0 μL, HiDi formamide and 0.25 μL LIZ-500 ladder (Applied Biosystems) and 0.5 μL of the PCR

Table 3.3 Characterization of eight microsatellite loci observed in *A. fuciphagus* samples. M, fluorescently labeled M13 tail.

Locus	Primer sequence (5'-3')	Repeat motif	Tm (°C)
Aef4	F: M-AGA ACA TTT CCC CAA CCA CTT R: AAT GTT GGC AAT GTG CCT TA	$(TATC)_{14}$	60
Aef24	F: M-TGG AAG TCT TGT ATG ATG GAC AG R: CCA TAG TTG CAG GGA TAG TCT G	$(TAGA)_{15}$	57
Aef27	F: M-CCA TAA CCT AAA TCC CCC TAC C R: CAG CTG GTG TGC TGA GAA AA	$(GATA)_{16}$	60
Aef28	F: M-AAG CAT TGT TCT TGT TGA TAT TTC C R: GTG TCT TTT TGG CTA CCC CTT AGC TCT T	$(TAGA)_{12}(CAGA)_4$	57
Aef35	F: M-TGT CTG GAT TTA AGT TTC AGT GC R: ACC CAG CAT CCC AGT AAA TG	$(TAGA)_{16}$	57
Aef91	F: M-GGA TCA GCA ATT AAA CCA GCA R: GTG TCT TTA GAA TGC CCA GGC TTA AAA	$(TATC)_{10}TGTCTATCTATT(TATC)_{10}$	57
Aef104	F: M-GGA GAA TCT GGG AGA GCT GA R: GTG TCT TTC TGG TTC CAT CTT TAT GCA G	$(TATC)_{11}(TGCC)_{10}$	57
Aef109	F: M-TGC CTC TAT ATG CAC ACA TGC R: TTT TTA CCA TTT CAT TGC CTT TT	$(TAGA)12$	57
Aef112	F: M-TTT TTG CCC TCA CAG TCT CC R: CAG ACC TCC TTG ATG TCC TGA	$(TATC)13$	57
Aef115	F: M-CAC ACA CTA TTT TTG GGC AGA R: AAG GTG CTT GGC ATT AGT GAA	$(TAGA)_{12}$	57
Aef133	F: M-GTC CAG TGC CTA CAA TGC TG R: AAT CCG GAT AAC ATC TCC TCT T	$(TATC)_{17}$	60

product.   Allele sizes were scored using GENEMAPPER 3.7 software (Applied Biosystems).

3.4 Data analysis

3.4.1 Mitochondrial DNA analysis

Sequences were edited and aligned using SEQUENCHER version 4.2 (Genecodes).   Analyses were carried out for a combined data set of the two mitochondrial DNA genes.   The incongruence length difference test suggested that these genes can be combined (Bull *et al*. 1993; Cunningham, 1997).   Genetic diversity was assessed using indices of haplotype diversity (Hd) and nucleotide diversity (π) and haplotypes were identified using DnaSp 4.10.9 (Rozas *et al*., 2003).

Phylogenetic relationships among haplotypes were reconstructed using the parsimony optimality criterion with PAUP *4.0 (Swofford, 2002).   A heuristic search was performed with equal weighting of nucleotide substitutions, stepwise addition with 100 random addition replicates and tree-bisection-reconstruction (TBR) branch swapping.   A strict consensus tree of all equally most parsimonious trees was constructed.   Nodal support was evaluated with 100 bootstrap pseudoreplicates. Additional sequences for phylogeographic analysis were drawn from GenBank (Table 3.4).

A median-joining network was constructed to describe the haplotype relationship using NETWORK version 4.2.0.1 (Fluxus Technology Ltd.) based on the default parameters.   Pairwise Φ_{ST} values between sampling sites were calculated using Jukes-Cantor distances (Jukes and Cantor, 1969) in ARLEQUIN 3.1 (Excoffier *et al*., 2005).   The significance of departures from zero and variance component was tested

using 10,000 permutations. The exact test was used to assess genetic differentiation between pairs of colonies using ARLEQUIN 3.1. To test population expansion, we used Tajima's D (Tajima, 1989), Fu's *Fs* (Fu, 1997) and mismatch distribution conducted in DnaSp 4.10.9. Their significance was tested by 1,000 simulations.

Table 3.4 DNA sequences retrieved from GenBank and used in the phylogeographic analysis.

Species	Locality	GenBank accession numbers		Authors
		Cyt-*b*	ND2	
Aerodramus salangana	Gomantong cave, Sabah, Malaysia	AY294424	AY294486	Price *et al.* (2004)
Aerodramus francicus	Muaritius	AY294434	AY294496	Price *et al.* (2004)
Aerodramus spodiopygius	Western Samoa	AY294437	AY294499	Price *et al.* (2004)
Aerodramus elaphrus	Seychelles	AY294430	AY294492	Price *et al.* (2004)
Aerodramus fuciphagus	Gomantong cave, Sabah, Malaysia	AY294428	AY294490	Price *et al.* (2004)

3.4.2 Microsatellite analysis

Genotyping errors (i.e. stuttering, large allele dropout and null alleles) in the microsatellite data set were identified by the software Micro-Checker version 2.2.3 (van OosterhoutM *et al*., 2003). Mean number of alleles per locus, observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated for microsatellite loci using ARLEQUIN 3.1. Deviations from Hardy-Weinberg Equilibrium (HWE) and test for linkage disequilibrium were conducted using GENEPOP (Raymond and Rousset, 1995) and the sequential Bonferroni correction was applied to probabilities for multiple comparisons (Rice, 1989).

Genetic divergence among colonies was assessed by genic and genotypic differentiation using GENEPOP and by pairwise F_{ST} calculated using FSTAT (Goudet, 2001). Gene flow was estimated using the private allele method (Slatkin, 1985; Barton and Slatkin, 1986) as implemented in GENEPOP. To examine isolation by distance, we tested the correlation between genetic distance (F_{ST}) and geographic distance using a Mantel test performed in GENEPOP. Geographic distance was measured in two ways. Firstly, we used straight line distances between all sampling sites. Secondly, we used coastline distance between colonies along the same coastline and used straight line distances between colonies of two coastlines; the Gulf of Thailand i.e. SRT, NK, SK and the Thai Andaman Sea i.e. KB, TR, ST based on the assumption that swiftlets possibly fly across non-coast area because the distance is not too far and we found a few swiftlet house colonies located between the two coastlines.

To assess the number of populations in our sample, we analyzed our microsatellite data using a Bayesian clustering approach as implemented in STRUCTURE version 2.2 (Pritchard *et al.*, 2000). Analyses were performed under

the admixture model with correlated allele frequencies and sampling location was not used as prior information. To estimate the number of subpopulations (K), ten independent runs of each K for K = 1 to 10 were carried out at 100,000 Markov chain Monte Carlo (MCMC) repetitions following a burn-in of 100,000 repetitions.

CHAPTER IV

RESULTS

4.1 Current distribution of the white-nest swiftlet *Aerodramus fuciphagus* (Thunberg, 1812) on mainland in Thailand

Based on the observations in this study and information from the literature review (Pothieng, 2005), *A. fuciphagus* was found in two habitat types; natural caves and man-made structures. Original habitat of *A. fuciphagus* was natural caves which were located on the islands and coastal areas of the Gulf of Thailand and Thai Andaman Sea. However, only natural caves on Ko Chan, Prachuap Khiri Khan Province could be visited by the observer in this study due to the high valuable of nests and privately owned operation. The white-nest swiftlet *A. fuciphagus* was found sharing some caves with the black-nest swiftlet, *A. maximus* which was observed from nests within the caves. For man-made structures, three kinds of the building for *A. fuciphagus* i.e. old houses, sacred buildings and newly constructed buildings were found and there was only the white-nest swiftlet *A. fuciphgus* occupying in those buildings. The buildings were located near the river mouth and along the coastalines of the Gulf of Thailand and Thai Anadaman Sea. Comparing the number of swiftlet houses for two coastline areas; Thai Andaman Sea and the Gulf of Thailand, the number of swiftlet houses on Thai Andaman Sea coastline is lower than swiftlet houses on the Gulf of Thailand coastline. Within buildings, environmental condition within houses is controlled as cave-like condition. The temperature ranged from 28.0-31.5 ˚C and the relative humidity was more than 70%. Differing from the white-nest swiftlet populations living in sacred buildings in other areas which usually live and

nest in dark area under the roof, *A. fuciphgus* population at Suthiwatwararam (or Chong Lom) Temple, Samut Sakhon Province can live and nest both under the roof and a light area inside the building (Fig 4.1).

Figure 4.1. The white-nest swiftlet *A. fuciphagus* colony in a sacred building at Suthiwatwararam (or Chong Lom) Temple, Samut Sakhon Province (28 January, 2008).

At present, the newly constructed buildings or modified buildings have been widely expanded over the large areas in Thailand, causing the expansion of *A. fuciphagus* distribution in this region. Current distribution of *A. fuciphagus* along the coastlines on the mainland in Thailand was shown in Table 4.1 and plotted on map in Figure 4.2. Even though the locations of swiftlet houses are usually on the river mouth, A few newly constructed buildings were found to be located at inland areas which are far from the coastline more than 60 km. The number of *A. fucihphgus* foraging in some coastal or river mouth areas i.e. Mueang, Chumphon Province, Ban Leam, Petchburi Province, and Pak Phanang, Nakhon Si Thammarat Province was moderate to high, causing a rapid and large expansion of swiftlet building construction in those areas (Fig 4.3-4.6).

Table 4.1. Occurrence of *Aerodramus fuciphagus* and geographic coordinates of the location of swiftlet houses surveyed in this study.

Province	District	Source of information		Abbreviation	Geographic coordinates	
		Literature	**Observation**			
Trat	Mueang	✓		-	-	-
			●	TT1	N 12º14´30.8´´	E 102º32´33.0´´
			●	TT2	N 12º14´39.8´´	E 102º32´08.5´´
			●	TT3	N 12º14´39.9´´	E 102º32´08.8´´
Rayong	Mueang	✓		-	-	-
	Klaeng	✓		-	-	-
Chanthaburi	Mueang	✓		-	-	-
			●	CB	N 12º35´41.1´´	E 102º07´119.3´´
Samut Prakan	Mueang		●	SP	N 13º33´27.7´´	E 100º32´48.0´´
Samut Sakhon	Mueang	✓		-	-	-
			●	SS	N 13º31´42.8´´	E 100º16´02.6´´
Petchburi	Ban Laem	✓		-	-	-
			●	PB1	N 13º12´39.4´´	E 099º59´09.8´´
			●	PB2	N 13º12´38.5´´	E 099º59´10.0´´
Prachuap Khiri Khan	Thap Sakae		●	PKK	N 11º36´59.11´´	E 099º41´23.89´´
	Pran Buri	✓		-	-	-

Table 4.1. Occurrence of *Aerodramus fuciphagus* and geographic coordinates of the location of swiftlet houses surveyed in this study. (continue).

Province	District	Source of information		Abbreviation	Geographic coordinates	
		Literature	**Observation**			
Chumphon	Mueang	✓		-	-	-
			●	CP1	N 10º29´38.9´´	E 099º10´22.5´´
			●	CP2	N 10º26´23.9´´	E 099º15´08.7´´
			●	CP3	N 10º26´26.6´´	E 099º15´08.9´´
	Pathio		●	CP4	N 10º40´45.5´´	E 099º20´02.3´´
Surat Thani	Mueang	✓		-	-	-
	Wiang Sa		●	SRT	N 08º38´03.3´´	E 099º22´21.9´´
Nakohon Si Thammarat	Hua Sai	✓		-	-	-
			●	NK1	N 08º02´22.9´´	E 100º19´03.4´´
	Pak Phanang	✓		-	-	-
			●	NK2	N 08º20´42.2´´	E 100º12´10.9´´
Phatthalung	Khao Chaison	✓		-	-	-
	Pak Phayun	✓		-	-	-
	Tamot	✓		-	-	-

Table 4.1. Occurrence of *Aerodramus fuciphagus* and geographic coordinates of the location of swiftlet houses surveyed in this study. (continue).

Province	District	Source of information		Abbreviation	Geographic coordinates	
		Literature	Observation			
Songkla	Mueang	✓		-	-	-
			●	SK1	SK1: N 07º01´45.1´´	E 100º28´13.5´´
	Hat Yai		●	SK2	SK2: N 07º11´31.8´´	E 100º35´35.2´´
	Chana		●	SK3	SK3: N 06º54´13.4´´	E 100º44´38.3´´
	Krasae Sin	✓		-	-	-
	Ranot	✓		-	-	-
	Sathing Phra	✓		-	-	-
	Singhanakorn	✓		-	-	-
Pattani	Nong Chik		●	PN	N 06º51´42.2´´	E 101º14´16.2
Narathiwat	Mueang	✓		-		
			●	NW1	N 06º25´57.1´´	E 101º49´39.1´´
			●	NW2	N 06º25´57.2´´	E 101º49´43.3´´
	Tak Bai	✓		-	-	-
Ranong	Mueang	✓		-	-	-
			●	RN	N 09º57´16.8´´	E 098º36´48.7´´
	Kra Buri	✓		-	-	-

Table 4.1. Occurrence of *Aerodramus fuciphagus* and geographic coordinates of the location of swiftlet houses surveyed in this study. (continue).

Province	District	Source of information		Abbreviation	Geographic coordinates	
		Literature	Observation			
Phang-Nga	Mueang	✓		-	-	-
	Takua Pa	✓	●	PNG	N 08º49´26.6´´	E 098º18´19.3´´
	Takua Thung	✓		-	-	-
Phuket	Thai Mueang	✓		-	-	-
Krabi	Mueang	✓		-	-	-
			●	KB	N 08º05´07.7´´	E 098º54´33.9´´
Trang	Kantang	✓		-	-	-
			●	TR	N 07º24´30.7´´	E 099º30´46.9´´
Satun	Mueang	✓		-	-	-
			●	ST	N 06º36´27.3´´	E 100º03´41.5´´
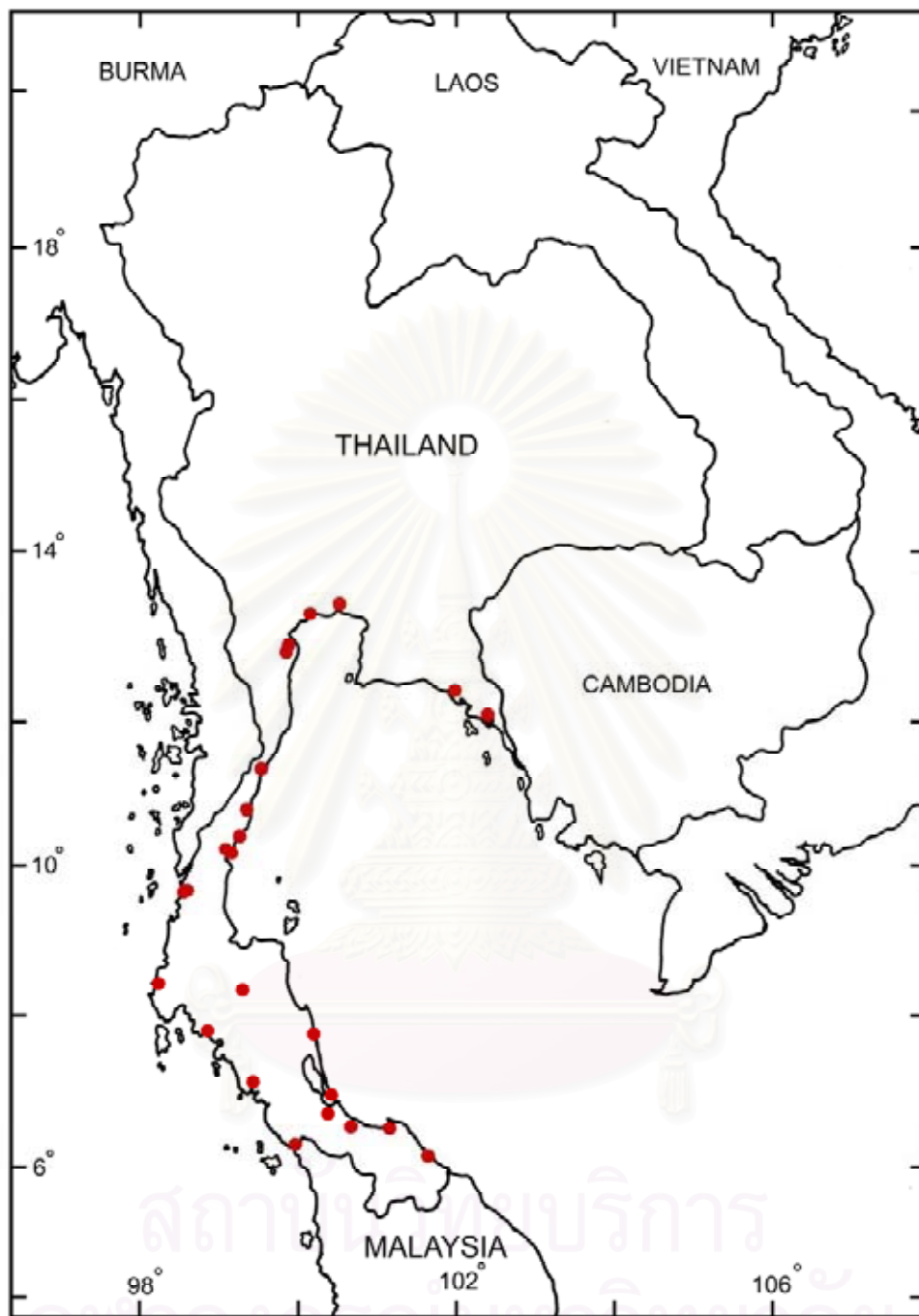
Figure 4.2. Distribution map of the current expansion of the white-nest swiftlet *A.*

fuciphagus colonies in Thailand.

Figure 4.3. Swiftlet houses in the area of Pak Phanang River mouth, Pak Phanang District, Nakhon Si Thammarat Province.

Figure 4.4. Swiftlet houses in Mueang District, Chumphon Province.

Figure 4.5. A newly constructed building for swiftlets located at Ban Leam District, Petchburi Province.

Figure 4.6. An old building modified for *A. fuciphagus* at Pak Phanang District, Nakhon Si Thammarat Province.

4.2 Genetics at population levels

4.2.1 Mitochondrial DNA

4.2.1.1 Genetic diversity

A total of 966 bp from the mitochondrial cytochrome-*b* gene (Cyt-*b*; 523 bp) and the NADH dehydrogenase subunit 2 gene (ND2; 443 bp) revealed 12 polymorphic sites with no alignment gaps. These polymorphic sites defined 15 haplotypes (Table 4.1). The average number of haplotypes per colony was 6.0 with a high number of haplotypes in Samut Sakhon (SS) and Surat Thani (SRT) colonies. Haplotype H05 was the most common and widespread appearing in nine colonies, representing 38.1% of all individual birds. Six unique haplotypes (H03, H08, H10, H12, H14, H15) were found in single individuals or 0.63% of all individuals for each unique haplotype. Haplotype diversity values of swiftlet colonies were moderately high whereas nucleotide diversity values were very low. Overall haplotype diversity was 0.785 ± 0.022 and ranged from 0.667 ± 0.163 in the Trang (TR) colony to 1.000 ± 0.272 in the Krabi (KB) colony. Overall nucleotide diversity was 0.00294 ± 0.00018 and ranged from 0.00069 ± 0.00000 in Satun (ST) to 0.00483 ± 0.00198 in the KB colony. The haplotype distribution of 160 individuals from 10 colonies is summarized in Table 4.3 and haplotype frequencies in each population are shown in Figure 4.7.

Table 4.2. Variable sites of the identified haplotypes from the mtDNA combined dataset and percentage of haplotypes in the white-nest swiftlet colonies in Thailand.

Haplotype code	Variable sites												N	Percentage of haplotypes
	1 0 4	1 1 6	2 9 6	4 1 4	5 7 5	5 7 8	6 4 2	6 4 5	6 9 6	7 5 5	8 2 1	8 8 7		
H01	C	T	A	C	T	T	C	G	A	C	A	A	30	18.75
H02	T	C	A	T	T	C	C	G	A	A	G	A	25	15.63
H03	C	T	A	C	T	T	C	A	A	C	A	A	1	0.63
H04	T	T	A	C	T	T	C	G	A	C	A	A	3	1.88
H05	T	T	A	C	T	T	C	G	G	C	A	A	61	38.13
H06	T	C	C	T	T	C	C	G	A	A	G	A	6	3.75
H07	T	T	A	C	T	T	C	G	A	C	A	G	15	9.38
H08	C	T	A	C	T	T	C	G	A	C	A	G	1	0.63
H09	T	C	A	T	T	T	C	G	A	C	A	A	10	6.25
H10	C	T	A	C	C	T	C	G	A	C	A	A	1	0.63
H11	T	T	A	C	T	C	C	G	A	A	G	A	2	1.25
H12	T	C	C	T	T	T	C	G	A	C	A	A	1	0.63
H13	T	T	A	C	T	T	T	G	A	C	A	A	2	1.25
H14	T	C	A	T	T	C	C	G	A	C	A	A	1	0.63
H15	T	T	A	C	T	T	C	A	A	C	A	A	1	0.63
													160	100.00

Table 4.3. Haplotype distribution, haplotype diversity and nucleotide diversity of the white-nest swiftlet colonies in Thailand.

Location name and abbreviation of colony	N	Number of haplotypes and haplotype codes	Haplotype diversity	Nucleotide diversity
Trat (TT)	5	4; H01,H06, H08, H09	0.900 ± 0161	0.00166 ± 0.00000
Samut Sakhon (SS)	26	9; H01,H02,H05,H06,H07,H09,H10,H13,H14	0.843 ± 0.051	0.00326 ± 0.00000
Chumphon (CP)	12	7; H01,H02,H,05,H06,H07,H09,H15	0.864 ± 0.079	0.00281 ± 0.00082
Surat Thani (SRT)	43	9; H01,H02,H03,H05,H06,H07,H09,H11,H12	0.793 ± 0.040	0.00328 ± 0.00024
Nakohon Si Thammarat (NK)	34	7; H01,H02,H04,H05, H07,H09,H11	0.758 ± 0.043	0.00272 ± 0.00041
Songkla (SK)	5	2; H04,H05	0.400 ± 0.237	0.00083 ± 0.00049
Narathiwat (NW)	19	6; H01,H02,H04,H05,H07,H09	0.813 ± 0.060	0.00315 ± 0.00056
Krabi (KB)	3	3; H01, H05, H07	1.000 ± 0.272	0.00483 ± 0.00198
Trang (TR)	10	5; H01,H02,H05,H07,H13	0.667 ± 0.163	0.00246 ± 0.00000
Satun (ST)	3	2; H05,H06	0.667 ± 0.314	0.00069 ± 0.00000
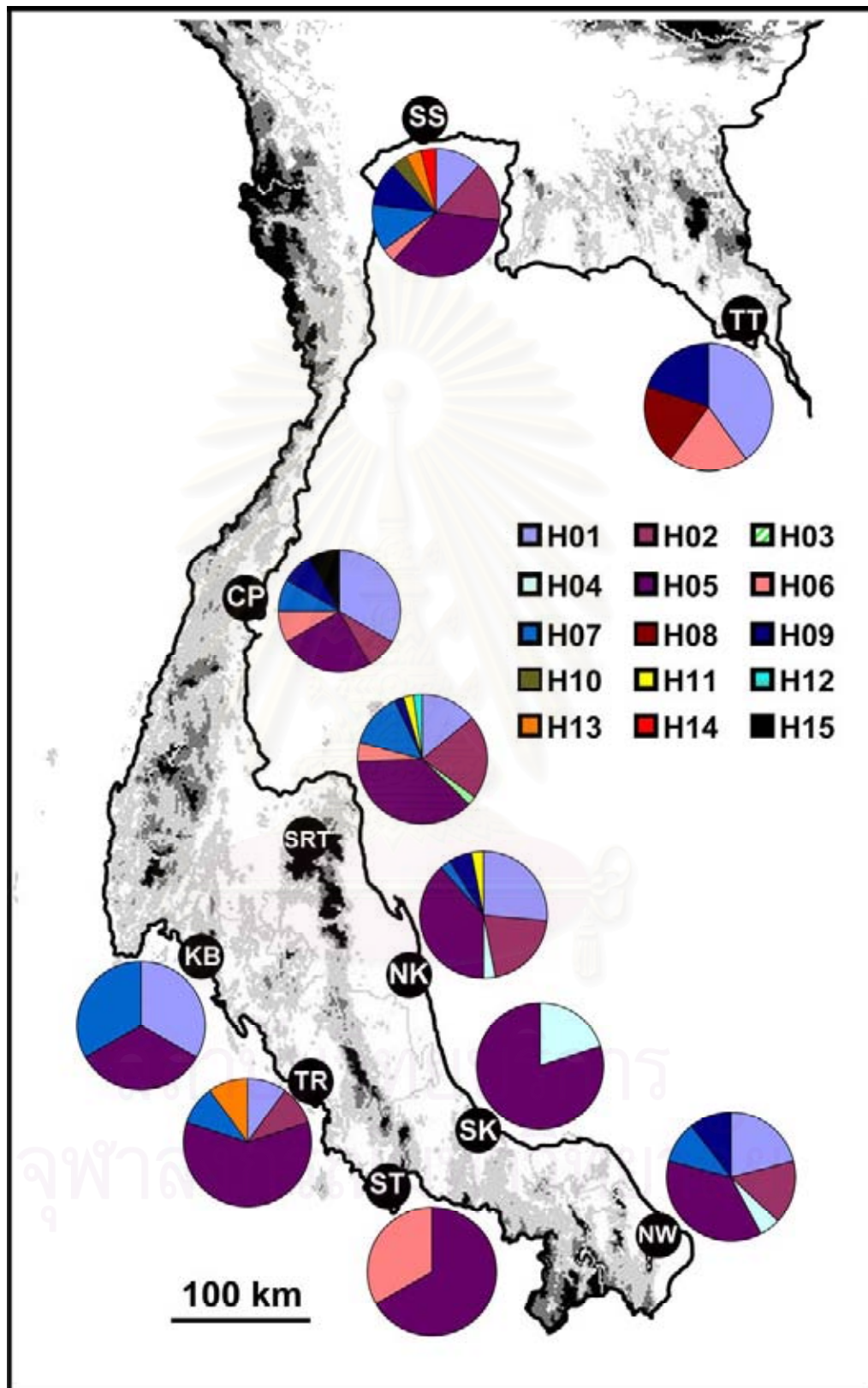
Figure 4.7. Haplotype frequencies of *A. fuciphagus* populations in Thailand.

4.2.1.2 Relationship between haplotypes and geographic structure

Parsimony analysis (MP) of the 15 haplotypes yielded 96 equally most parsimonious trees with a length of 102 steps (consistency index (CI) = 0.745; retention index (RI) = 0.705). The maximum parsimony trees showed two clades of Thai haplotypes, with short branches separating all haplotypes (Fig. 4.8). The Thai haplotypes are placed as the sistergroup of *A. fuciphagus* from Sabah, Malaysia with 96% bootstrap support. Considering geographic distribution of the haplotypes (Table at the right in Fig. 4.9) and haplotype relationship, several haplotypes were spread in both clades and geographic structure within and between clades was not found.

Similarly to the parsimony analysis, the median-joining network (MNJ; Fig. 4.9) did not show geographic structure among white-nest swiftlets from different colonies sharing haplotypes nor did it show clustering of haplotypes from any colonies. The haplotypes of each clade were separated from one another by only 1 or 2 mutation steps. Central colony, SS showed two unique haplotypes, H14 and H10, which could be the haplotypes linking the central and southern colonies. Due to no geographic distribution pattern of Thai haplotypes, the original haplotype which derived into other haplotypes could not be concluded for Thai populations from these analyses.

		1 TT	2 SS	3 CP	4 SRT	5 NK	6 SK	7 NW	8 KB	9 TR	10 ST	Total
H10			1									1
H14			1									1
H03					1							1
H08		1										1
H09		1	3	1	1	2		2				10
H01		2	3	4	6	9		4	1	1		30
H11					1	1						2
H15			1									1
H04					1	1	1					3
H06		1	1	1	2						1	6
H05			9	3	16	13	4	7	1	6	2	61
H13		1							1			2
H07			3	1	6	1		2	1	1		15
H02			4	1	9	7		3		1		25
H12			1									1
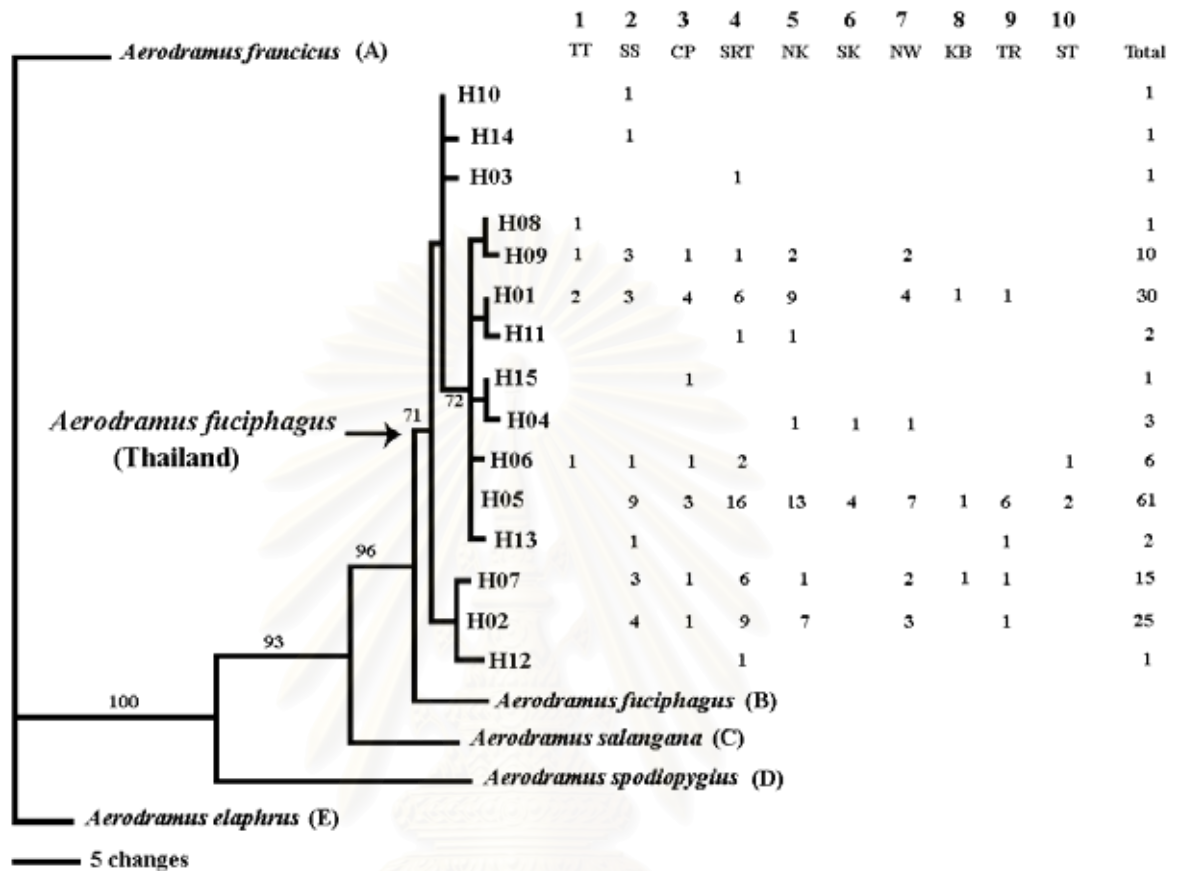
Figure 4.8. Phylogram of one of 96 equally most parsimonious trees showing haplotype relationships among the white-nest swiftlet colonies (CI = 0.745, RI = 0.705). Tree is constructed from a combined mtDNA data set of partial cyt-*b* and ND2 genes. Bootstrap values ≥ 50% (100 replications) are displayed above branches. Outgroups; (A) *Aerodramus francicus*, (B) *Aerodramus fuciphagus* (from Gamantong, Sabah, Malaysia) (C) *Aerodramus salagana* (D) *Aerodramus elaphrus* and (E) *Aerodramus spodiopygius* are from GenBank. The table at the right shows geographic distribution of the haplotypes.
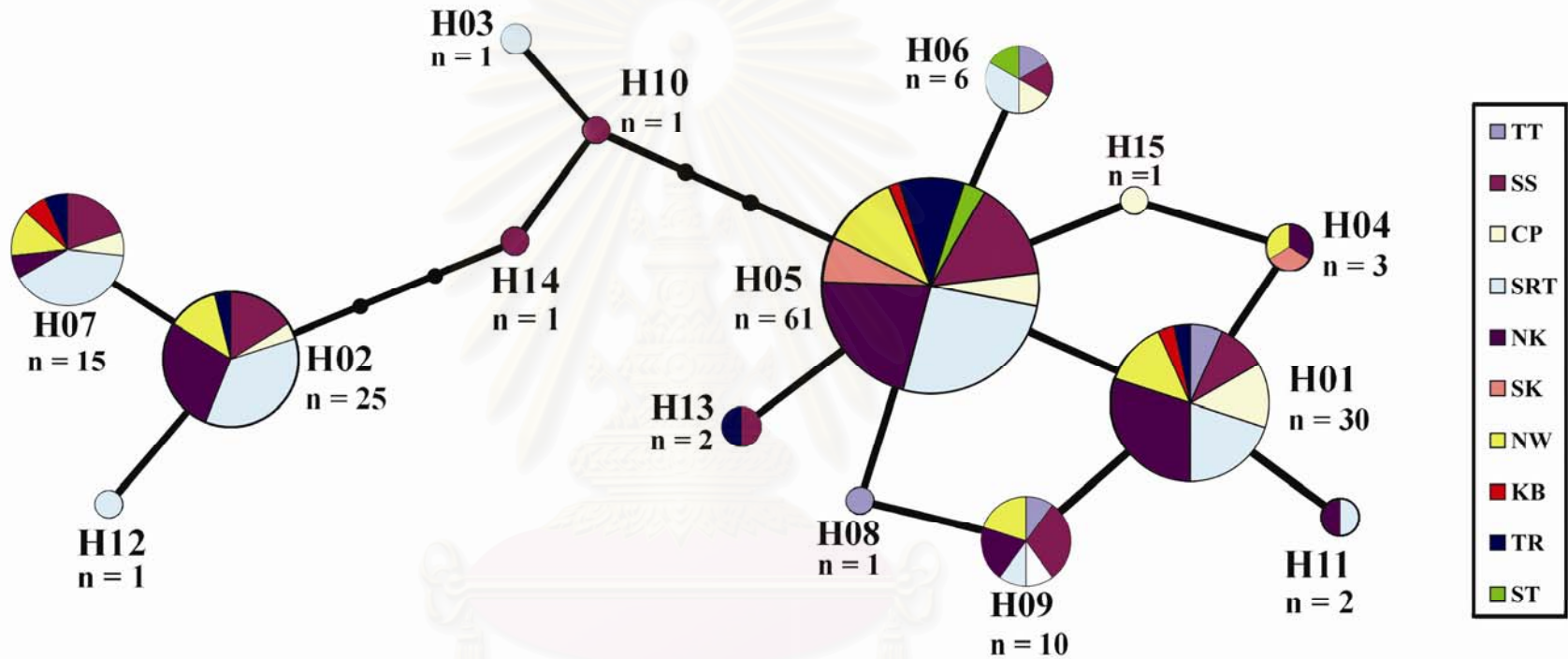
Figure 4.9. Median-joining network showing the relationship among 15 mitochondrial haplotypes of the white-nest swiftlets sampled.

The size of each circle represents the relative frequency of the haplotype in the total sample. Each black dot indicates one mutation event.

4.2.1.3 Genetic differentiation of *A. fuciphagus* populations

Based on mtDNA data set, a global test for genetic differentiation among colonies was not significant (P > 0.05), although some significant pairwise comparisons were found (TT-SRT, TT-NK, TT-SK and TT-TR). Analysis was not performed by grouping colonies due to man-made habitat expansion and high dispersal ability. For pairwise comparisons of genetic differentiation, Φ_{ST} values between colonies were very low and ranged from -0.26186 to 0.63565 (Table 4.4). The largest pairwise difference was found between TT-SK. For several pairwise comparisons, Φ_{ST} values showed negative values, indicating that genetic variability within colonies higher than between colonies. The only significant pairwise Φ_{ST} values were found for pairs of small colonies (TT-NK, TT-SK, TT-TR, TT-ST and SK-CP; P < 0.05); however, the power of these tests is limited by small sample sizes.

Table 4.4. Pairwise Φ_{ST} values among 10 colonies across all loci from the mtDNA data set. The names for the colony abbreviations are given in Table 4.3.

Colony	TT	SS	CP	SRT	NK	SK	NW	KB	TR	ST
TT	*									
SS	0.18027	*								
CP	-0.00875	-0.00225	*							
SRT	0.24303	-0.02465	0.04282	*						
NK	0.29261*	-0.02051	0.06620	-0.02154	*					
SK	0.63565*	0.09789	0.28179*	0.05980	0.03709	*				
NW	0.15622	-0.04576	-0.01957	-0.02891	-0.02873	0.09632	*			
KB	-0.16998	-0.12198	-0.25335	-0.06466	-0.02826	0.22957	-0.14610	*		
TR	0.41966*	0.00199	0.14111	-0.02045	-0.03628	-0.10386	0.00244	0.05510	*	
ST	0.46797*	-0.09601	0.06741	-0.12766	-0.12746	-0.26186	-0.09361	-0.10404	-0.24902	*

* significance at P < 0.05

4.2.1.4 Historical demography

Tests of neutrality; Tajima's D and Fu's F_s, on the total mtDNA data set showed positive values and were not significantly deviated from the null hypothesis of neutrality when all samples were combined (Tajima's D = 0.837, P > 0.1; Fu's F_s = 0.157, P > 0.1). Therefore, it could be suggested that the observed nucleotide polymorphism is selectively neutral.

When mismatch distribution analysis for entire populations was tested, the curve appeared to be bimodal (Fig. 4.10). Both the variance (SSD) and raggedness index (*r*) tests suggested that the curve did not significantly differ from the distribution expected from a model of population expansion (SSD = 0.109, P = 0.181; *r* = 0.31389, P = 0.232).
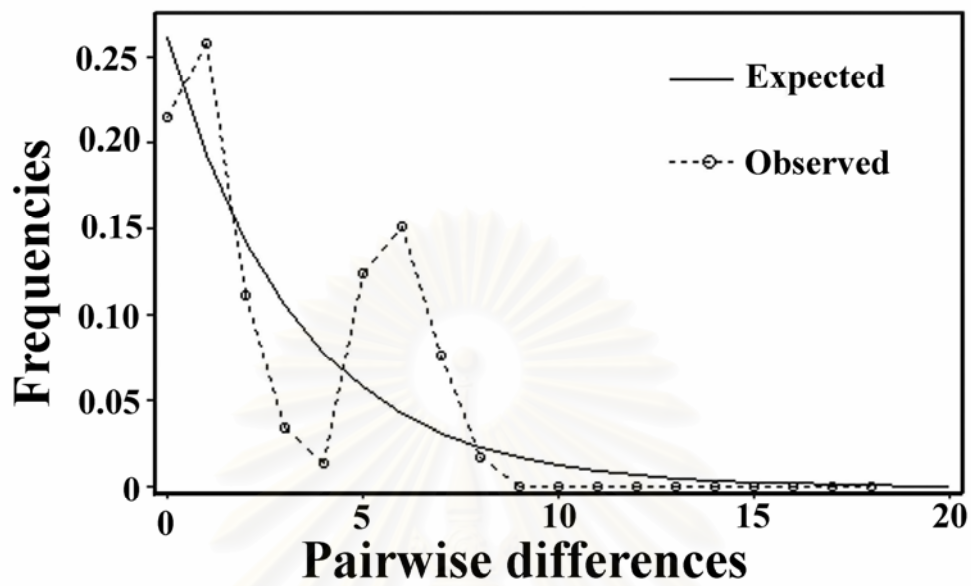
Figure 4.10. Mismatch distributions of the mitochondrial haplotypes of *A. fuciphagus*. The dashed line indicates the observed distribution of pairwise differences and the solid line shows the expected distribution under the sudden expansion model.

4.2.2 Microsatellites

4.2.2.1 Detection of scoring errors of microsatellite allele data and the presence of null alleles

According to the processes of primer design and polymerase chain reaction (PCR), eleven microsatellite loci were successfully amplified in *A. fuciphagus* samples. Analysis of microsatellite data using Micro-Checker program indicated that some loci i.e. Aef28, Aef35, Aef91 prominently exhibited an excess of homozygotes for most allele size classes, indicating by significant values of combined probability for all classes ($p < 0.001$). An excess of homozygotes may be a result of either presence of null alleles in these loci in the sampled individuals or stutter bands in PCR amplification. Moreover, no evidence of large allele dropout in all loci due to genotyping errors of microsatellite data set was found. Therefore, microsatellite data of these three loci were excluded for further analyses. At locus Aef109, an excess of homozygotes was found only in some populations with no significantly combined probability for all allele size classes ($P > 0.05$); thus, microsatellite data of this locus was included for diversity analysis.

4.2.2.2 Genetic diversity of microsatellite loci

All eight loci were polymorphic. The maximum number of alleles per locus was 18 for Aef27 and the minimum number of alleles per locus was 9 for Aef4 and Aef115 (Table 4.5). The mean number of alleles per locus ranged from 3.63 alleles in the ST colony to 9.63 in the SRT colony. Observed heterozygosity (H_o) and expected heterozygosity (H_e) were high for all loci across all 10 colonies. The highest H_o

values were observed at Aef104 (H_o = 0.880) and the highest H_e values were observed

at Aef24 (H_e = 0.929). High heterozygosity values were found for all sampling sites

across all loci. Observed heterozygosity (H_o) and expected heterozygosity (H_e) ranged

from 0.6667 in ST colony to 0.9750 in TT colony and 0.7667 in SK colony to 0.8554

in SS colony, respectively (Table 4.6). Several private alleles were present in each

colony, except SK, KB, TR colonies.

Table 4.5. Size range of alleles, number of alleles, observed (H_o) and expected (H_e)
heterozygoisty at each microsatellite locus.

Locus	Size range (bp)	No. of alleles	H_o	H_e
Aef4	251-283	9	0.834	0.819
Aef24	227-267	10	0.820	0.929
Aef27	215-247	18	0.843	0.856
Aef104	176-216	13	0.880	0.884
Aef109	143-210	16	0.882	0.824
Aef112	224-248	10	0.794	0.781
Aef115	215-243	9	0.806	0.819
Aef133	204-232	10	0.862	0.910

Table 4.6. Estimates of the average number of alleles, observed heterozygosity (Ho), expected heterozygosity (H_e) and number of private alleles in 10 colonies across eight microsatellite loci of *A. fuciphagus*.

Colony	N	Average number of alleles	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Number of private alleles
TT	5	5.25 ± 1.49	0.9750 ± 0.0707	0.8389 ± 0.0514	1
SS	26	9.00 ± 1.14	0.8590 ± 0.0953	0.8554 ± 0.0328	6
CP	12	7.25 ± 1.98	0.8646 ± 0.1173	0.8397 ± 0.0588	2
SRT	43	9.63 ± 2.00	0.8256 ± 0.0963	0.8398 ± 0.0323	6
NK	34	8.88 ± 2.10	0.8534 ± 0.0645	0.8358 ± 0.0359	3
SK	5	4.38 ± 1.51	0.9250 ± 0.2121	0.7667 ± 0.1279	0
NW	19	8.00 ± 1.51	0.8701 ± 0.0738	0.8468 ± 0.0345	2
KB	3	3.88 ± 0.99	0.8958 ± 0.1980	0.8453 ± 0.1321	0
TR	10	6.75 ± 1.58	0.8625 ± 0.1303	0.8290 ± 0.0587	0
ST	3	3.63 ± 0.58	0.6667 ± 0.2520	0.8000 ± 0.0943	1

4.2.2.3 Tests for deviation from Hardy-Weinberg Equilibrium and

linkage disequilibrium

After sequential Bonferroni correction for multiple tests (Rice, 1989), global tests of heterozygote deficiencies across colonies within each locus revealed significant departure from Hardy–Weinberg expectation at locus Aef109 ($P < 0.05$). Heterozygote deficiencies at Aef109 were found in CP, SRT and NK colonies, indicating that a null allele might be present at this locus; therefore, this locus was included in further analyses as suggested by many authors. Exact tests showed no evidence of linkage disequilibrium between pairwise microsatellite loci comparisons across all ten colonies ($P > 0.05$). Genetic independence of these loci was assumed for all subsequent analyses.

4.2.2.4 Genetic differentiation among colonies and gene flow

Pairwise F_{ST} values of microsatellite data set were non-significant between all pairs of colonies (Table 4.7). The distribution of pairwise F_{ST} values was uniformly low, ranging from -0.0128 for the KB-ST comparison to 0.0694 for the SK-ST comparison. Most values were at or around zero and many were negative including pairwise F_{ST} values of large colonies such as SS-SRT, SS-NK and SRT-NW. The negative values indicate more variance within colonies than there was between colonies. As with F_{ST}, pairwise comparisons of allelic and genotypic differentiation were non-significant between all colonies ($P > 0.05$).

Table 4.7. Pairwise F_{ST} values among 10 colonies across all loci from the microsatellite data set. The names for the colony abbreviations are given in Table 4.3.

Colony	TT	SS	CP	SRT	NK	SK	NW	KB	TR	ST
TT	*									
SS	-0.00890	*								
CP	-0.01080	-0.00820	*							
SRT	-0.00040	-0.00160	0.00010	*						
NK	-0.00094	-0.00110	-0.00790	0.00360	*					
SK	0.03750	0.02520	0.04480	0.02940	0.03950	*				
NW	-0.00060	-0.00390	-0.00600	-0.00790	-0.00460	0.04260	*			
KB	0.03150	-0.00760	0.01130	0.00410	-0.00250	0.05230	0.01180	*		
TR	-0.00090	-0.00180	0.00060	0.00480	0.01040	0.02850	0.00470	0.02150	*	
ST	0.05400	0.00260	0.00240	0.00420	0.01330	0.06940	-0.00240	-0.01280	0.04450	*

The individual assignment to population clusters (K) without prior information on the origin of individuals by program STRUCTURE analyzed a number of estimated populations between 1 and 10. The results of the STRUCTURE analysis showed that the most probable number of subpopulations was K = 1 (ln Pr(X\K) = -4277.7) and the higher values of K showed lower log-likelihood (Table 4.8). Membership proportions into each cluster were found evenly and declined regularly when K was higher than 1 (see Appendix VI). This result provided strong support for a single genetic population.

A measure of the level of gene flow among the white-nest swiflet colonies in Thailand was calculated from private allele method and was inferred in term of the effective number of migrants per generation. The result showed high level of gene flow among colonies indicating by the high number of migrants per generation (Nm = 8.0).

Table 4.8. Posterior probabilities and variances for models assuming various

numbers of clusters (K) for the entire data set of microsatellites. Statistics for each K

value represent average values over ten simulations.

K	ln Pr(X\K)	Var [ln Pr(X\K)]	Pr(K\X)
1	-4277.7	36.6	~1
2	-4300.7	105.4	~0
3	-4335.9	197.0	~0
4	-4348.0	244.8	~0
5	-4340.9	258.2	~0
6	-4348.6	286.1	~0
7	-4340.3	261.1	~0
8	-4338.1	254.6	~0
9	-4329.5	233.7	~0
10	-4348.9	277.8	~0

4.2.2.5 Isolation by distance

Isolation by distance was analyzed to test the correlation between F_{ST} values and geographic distance which was measured in two ways, straight line distances and coastline distances. The straight line distances between colonies were shorter than the coastline distances between colonies. The maximum straight line distance between colonies was 802.46 km whereas the maximum coastline distance between colonies was 1,503.33 km. A Mantel test showed no significant correlation between all pairwise values of F_{ST} and geographic distance across observed swiftlet colonies which occur over hundreds of kilometers (Straight line distance: P = 0.938; coastline distance: P = 0.939). Figure 4.11 and figure 4.12 showed isolation by distance plots for all pairwise values of F_{ST} values and the geographic distance with non significant correlation (P > 0.05).
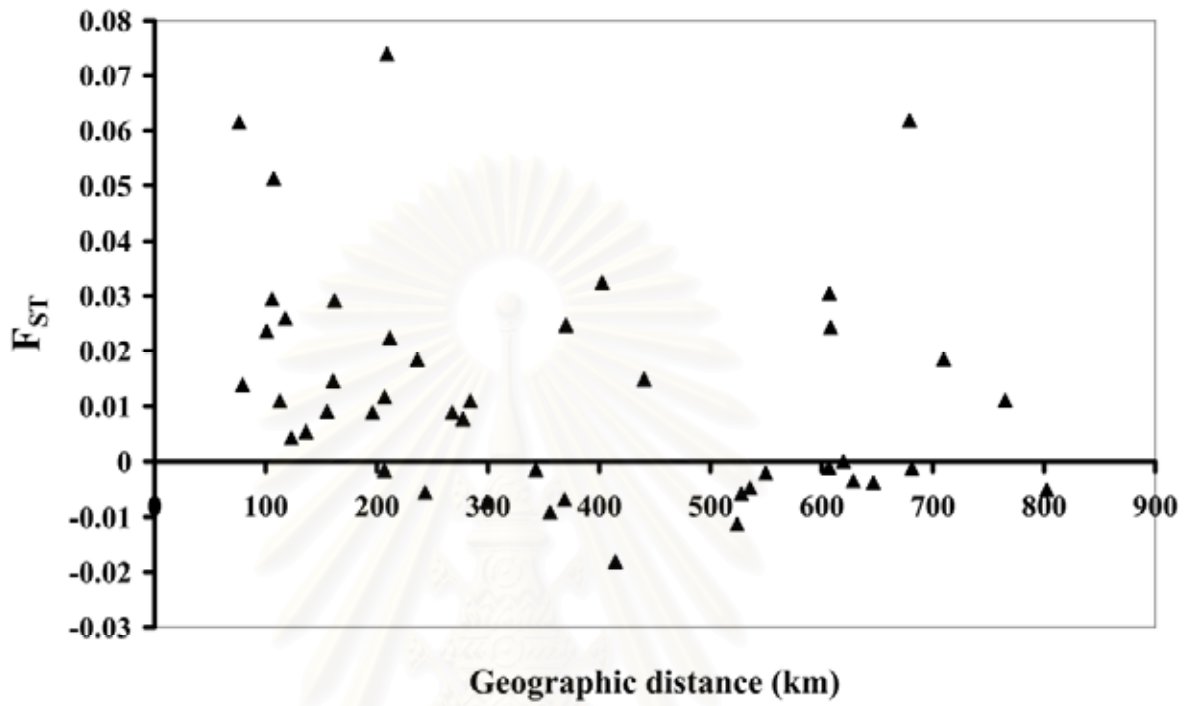
Figure 4.11. Isolation by distance plots for all pairwise values of F_{ST} and straight line distance over all colonies based on microsatellite data.
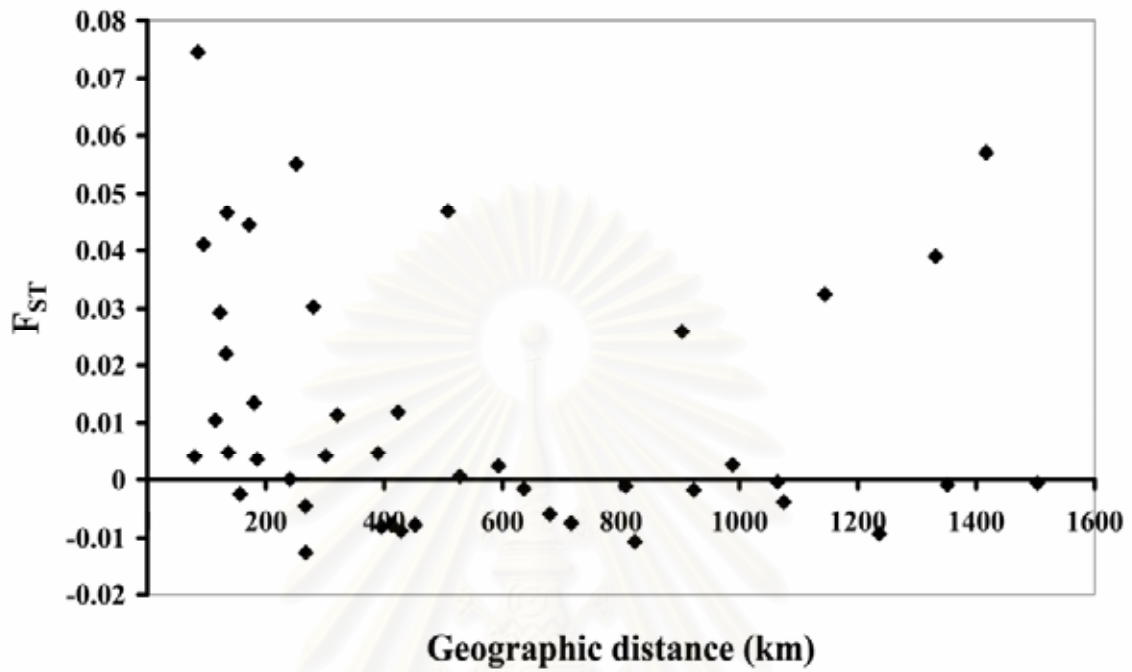
Figure 4.12. Isolation by distance plots for all pairwise values of F_{ST} and coastline distance over all colonies based on microsatellite data.

CHAPTER V

DISCUSSION

5.1 Colony expansion of the white-nest swiftlet, *A. fuciphagus* in Thailand

The distribution of natural colonies of the white-nest swiftlet, *A. fuciphagus* has been reported across its distribution range. However, since this species has adapted to human environments and been encouraged to establish colonies in man-made buildings, the distribution range of *A. fuciphagus* colonies has been increased. The observations in this study found that the number of buildings for swiftlets has been rapidly increased and expanded along the coastlines, ranging from the East through the South of Thailand. This expansion has occurred during the few years of this study and will likely increase enormously over the next several years. The major areas of swiftlet houses are usually located on the mainland opposite to the natural habitats on the islands and at the river mouth areas, one of foraging areas for this insectivorous bird. For example, swiftlet houses were found on the coastlines opposite to the islands of marine national parks such as Mu Ko Chumphon National Park, Chumphon Province, Hat Wanakon National Park, Prachuap Khiri Khan Province. Numerous swiftlet houses were found in the areas of the river mouth such as Pak Phanang District, Nakhon Si Thammarat Province, Mueang District, Chumphon Province, Kantang District, Trang Province. Moreover, the observations found that some swiftlet houses are also established along the foraging route of this swiftlet. Swiftlet houses in some city areas may have been colonized from the swiftlet colonies that were naturally established in abandon houses or sacred

buildings, for example, a sacred building at Suthiwatwararam Temple located at Tha Chin River Mouth.

Even though swiftlet houses have been widespread over large areas of both coastlines of the Gulf of Thailand and the Thai Andaman Sea, information on the number of swiftlet houses has not been studied and to date there is no governmental regulation or law for controlling the construction of the swiftlet buildings by the government. Some swiftlet buildings were constructed without permission from the government and have been causing some pollution such as noise pollution (from swiftlet song) and air pollution (dust from many swiftlets flying around their colonies) that can affect to people who live in those areas. At present, there is no report of epidemics due to the swiftlets, but it is unpredictable in case of some diseases such as bird flu and if it did occur, it would be a big disaster for humans. Therefore, the government should pay more attention to the construction of swiftlet houses, especially in city areas. In addition, an environmental risk assessment should be conducted for short and long term management.

5.2 Genetics of *A. fuciphagus* populations living in man-made buildings in Thailand

5.2.1 Genetic diversity and genetic differentiation of colonies

In this study mtDNA, the nucleotide diversity was relatively low and similar across colonies (π = 0.00294) whereas haplotype diversity approached 1.0 for both all colonies (Hd = 0.785) and for individual colonies. When genetic diversity was compared for unequal sized colonies, haplotype diversity of ST, was extremely high (N = 3; Hd = 1.000 ± 0.272) compared to that of larger colony, SRT (N = 43; Hd = 0.793 ± 0.040) probably because of its small sample size where three samples belong to three different haplotypes.

Microsatellite loci also showed high levels of genetic diversity; however, the number of alleles increases quickly with sample size and levels off at about N = 20, increasing slowly with larger sample sizes. This might indicate the effect of small sample sizes (N < 20). For the tests of Hardy-Weinberg Equilibrium (HWE), seven of eight loci were in HWE. Only locus Aef 109 deviated from HWE; therefore, this locus was excluded for further population analyses because a null allele might be present at this locus and may affect the estimation of genetic differentiation. The exclusion of microsatellite loci where a null allele might be present for the population genetic analysis has been previously reported (Carlsson *et al*., 2004; Dakin and Avise, 2004; Chapuis and Estoup, 2007).

Estimates of genetic differentiation for both mitochondrial DNA and microsatellites did not show genetic structure among recently established swiftlet colonies in buildings in Thailand, even though some significant Φ_{ST} values of mtDNA were found. The latter cases may be indicative of low sample sizes which may have

also caused the observation of negative pairwise Φ_{ST} and F_{ST} values. However, these negative values were also found in pairs of larger colonies, indicating higher genetic variation within those colonies than between colonies.

Because unequal and small sample size may affect the pairwise F_{ST} values, analysis of the microsatellite data using STRUCTURE which uses an individual-based approach to calculate the most probable number of subpopulations from the dataset without prior information on population sampling was conducted and STRUCTURE results provided strong support for a single genetic population ($K = 1$). The absence of genetic structure was not due to the low genetic variation within colonies as indicated by microsatellite data (Table 4.5).

5.2.2 Why genetic homogeneity?

In general, genetic heterogeneity in any species has been typically explained by three factors; 1) geographical barriers to gene flow; 2) behavioral barriers to gene flow, e.g. natal philopatry and 3) historical demographic events, e.g. bottleneck (Avise, 2004). Some highly mobile species e.g. the ground tit *Pseudopoces humilis*, the greater sage-grouse *Centrocercus urophasianus* and the Atlantic bluefin tuna *Thunnus thynnus thynnus* have been found to have genetic structure across geographic areas surveyed (Carlsson *et al*., 2004; Oyler-McCance *et al*., 2005; Yang *et al*., 2006b). In contrast to those species, a lack of genetic differentiation has been found in some bird species e.g. the grey-headed albatross (*Thalassarche chrysostoma*) and the Adélie penguin (*Pygoscelis adeliae*), even though they have high levels of natal philopatry (Burg and Croxall, 2001; Roeder *et al*., 2001). The lack of genetic differentiation in the grey-headed albatross could be explained by the mixing of

juveniles on feeding grounds (Burg and Croxall, 2001), whereas that of Adélie penguin is due to movement of individuals between colonies over very large distances and the large effective population size (Roeder *et al.*, 2001).

High levels of nest-site fidelity and monogamy (Nugroho and Whendrato, 1996; Viruhpintu, 2002) in white-nest swiftlets could lead to genetic differentiation between colonies. However, a lack of genetic structure was found between recently established man-made colonies. One likely explanation for the high level of genetic homogeneity observed could be movements of young birds seeking nest sites at new colonies, leading to the mixing between young breeders from both natural cave colonies and other recent man-made colonies. Natal dispersal from natural caves on islands and established man-made houses on the mainland likely contributes to the genetic homogeneity observed across their expanding range. Natal dispersal of juveniles has been explained by hypotheses such as resource competition (food and space) and intrasexual mate competition and inbreeding avoidance (Greenwood, 1980; Dobson, 1982; Greenwood and Harvey, 1982; Johnson and Gaines, 1990).

A study on nest site selection of the white-nest swiftlet in natural caves suggested that space for nest sites is limited in crowded swiftlet colonies (Viruhpintu *et al.*, 2002). The white-nest swiftlet usually builds its nest on smooth and concave sites with supports such as protruding U-shape rocks. When a nest site is occupied by a breeding pair, that site is unavailable to other pairs until one of the first pair dies. Thus, most new breeders must choose to occupy lower quality nest sites causing mortality to their offspring or seek space in other locations. Therefore, competition for nest sites within crowded colonies may encourage natal dispersal but there is no confirmation of this from bird banding studies of this species. However, it is

noteworthy that Nguyen Quang *et al*. (2002) mentioned that white-nest swiftlets forage in areas which are more than 80 km from their breeding sites so the birds clearly frequent areas where other colonies occur and where new houses are being constructed. Moreover, the common practice of using swiftlet song playbacks over many months to draw breeding pairs of birds to the new colonies results in numerous pairs establishing the new colony. This practice greatly reduces the possibility of founder effects that might have contributed to differences between new colonies.

5.2.3 History of colony growth

The history of swiftlet colonies was investigated by tests of the departure from neutrality test and mismatch distribution tests. Neutrality tests, Tajima's D and Fu's Fs values indicated a history of expansion. Positive values are observed in stationary populations, in which a substantial number of mutations are shared by different lineages (Rogers and Harpending, 1992). In contrast, negative values are indicative of the possible departure from neutral expectations and most mutations tend to be unique to a single lineage. A lack of genetic differentiation was found in other species such as the white-rump snowfinch (*Onychostruthus taczanowskii*, Yang *et al*., 2006a), the black-throated blue warbler (*Dendroica caerulescens*, Davis *et al*., 2006) and the hooded seal (*Cystophora cristata*, Coltman *et al.,* 2007). These species have high haplotype diversity and low nucleotide diversity. Their mismatch distributions showed the unimodal pattern and tests of the departure from the neutrality tests, Tajima's D or Fu's Fs showed significant negative values. These results indicated that the populations of those three species had experienced a bottleneck recently and have not yet reach equilibrium. In contrast to those species, neutrality tests, Tajima's

D and Fu's Fs values of all white-nest swiftlets sampled in Thailand were positive, indicating that the swiftlet population could be in equilibrium or a stationary population. The mismatch distribution for all samples reveals a bimodal shape, but in this case there is no evidence that groups of haplotypes evolved in different geographic regions.

5.3 Taxonomic status of *Aerodramus fuciphagus* (Thunberg, 1812) in Thailand

The taxonomic status of the subspecies of *A. fuciphagus* has been a contentious issue among ornithologists. Some authors reported the geographic distribution of eight *A. fuciphagus* subspecies; however, only small morphological differences in these subspecies have been used to distinguish among them (Medway, 1966; Chantler and Driessens, 2000; Nguyên Quang *et al.*, 2002) and there is no other firm evidence for the subspecies boundaries. Two proposed subspecies; *Aerodramus fuciphagus germani* and *A. f. amechanus* occur in Thailand and the boundary between these two subspecies might be in the extreme South of Thailand (Lekagul and Round, 1991).

The results of this present study based on two molecular markers, mtDNA genes and microsatellite loci did not support the occurrence of two proposed subspecies, *A. f. germani* and *A. f. amechanus* in Thailand. Lack of support for the subspecies designations is emphasized by several population genetic analyses. Median Joining Network did not show a geographic pattern based on mtDNA haplotypes. Although, several haplotypes; H03, H08, H10, H12, H14 and H15 were restricted to the colonies, a geographic pattern was not observed. Furthermore, a few common haplotypes; H05, H01 and H02 were also shared in many *A. fuciphagus*

colonies (Fig. 4.9). Similarly, analyses of microsatellite data set i.e. isolation by distance (IBD), revealed no association between levels of genetic differentiation and geographic distances (Fig. 4.11 and 4.12) and STRUCTURE analysis also indicated that *A. fuciphagus* in Thailand is a single genetic population. Low genetic differentiation of *A. fuciphagus* colonies were found for both mtDNA (Φ_{ST}) and microsatellites (F_{ST}) and no population subdivision was detected across distribution range in Thailand. Thus, *A. fuciphagus* populations in Thailand should not be treated as different subspecies.

Lack of genetic support for subspecies designations was found in some avian taxa e.g. the least tern, *Sterna antiliarm* indicated by the star-like phylogeny of haplotypes that shows no correlation with geography based on mtDNA. Moreover, AMOVA analyses of mtDNA and microsatellites did not detect genetic structure among *S. antiliarm* populations (Draheim, 2006). Therefore, this finding suggested that there was no support for the three traditional subspecific designations in this bird. Contrary to the above report and this present study, validity of subspecies designations was observed in some birds e.g. gulls in *Larus cachinnanas-fuscus* group (Liebers *et al*., 2001) and the rock partridge, *Alectoris graeca* (Randi *et al*., 2003).

The gulls in *Larus cachinnanas-fuscus* group were investigated for genetic differentiation and phylogeography including current taxonomic boundaries (Liebers *et al*., 2001). The results showed two major clades; Atlantic-Mediterranean clade and NW Palearctic-Central Asian clade. At subspecies level, high molecular variance of over 80% was found among six (groups of) taxa based on the hypervariable control region (HVR-I). This finding indicated that the molecular genetic structure of the gulls in *Larus cachinnanas-fuscus* group was concordant with the current taxonomy.

Population structure of the rock partridge, *Alectoris graeca* sampled from throughout the species' distribution range with the exception of the central Balkans region was observed based on mtDNA control region haplotypes and microsatellites (Randi *et al*., 2003). The rock partridge was split into two phylogroups; partridges from Sicily and all the other sampled populations. The population subdivision in this bird could be a result of the bottleneck effect without evidence of recent population expansion. The results suggested that *P. violaceus whiteakeri* subspecies in Sicily should be recognized as a distinct evolutionary unit.

5.4 Ecological and environmental implications

The high levels of genetic variability observed at microsatellite loci within and between colonies of white-nest swiftlets in man-made buildings suggests a genetically viable future for this species. In this study all colonies from swiftlet houses that were represented by three or more individuals showed a high proportion of the total variation observed across all colonies and thus it appears that individual colonies hold very little unique genetic material. Thus, if a few natural colonies shrink or are lost the species as a whole is unlikely to become threatened due to a loss of genetic variation.

Lim and Cranbrook (2002) mentioned that there are two schools of thought on conservation of swiftlet species. One promotes the advantages of swiflet farming, while the other strongly opposes it. Because swiftlets roost and nest in caves, the opposing position has proposed that the introduction of swiftlet farming would eliminate a strong attention to protect natural caves. This could be a risk for other species in a cave ecosystem, especially in limestone habitats of Southeast Asia, which

are well known for harboring many plants and animals such as the naked bat, *Cheiromeles torquatus*, (Clements *et al.*, 2006). The other opinion on swiflet farming has reasoned that such undertakings offer stronger support to protect the wild populations as primary stocks, as well as maintaining much larger numbers of birds that would reduce the risk of inbreeding. Furthermore, the increasing of swiftlet colonies in man-made buildings may provide other possible positive impacts e.g. 1) increasing the overall nest yield, 2) controlling insect pests and 3) reducing over-harvesting risk to the natural cave populations.

However, a close watch over the species is needed and special management attention should be given to the original natural cave colonies of the white-nest swiftlet due to reports of the decreasing numbers of swiftlets in some areas (Lau and Melville, 1994; Chantler and Driessens, 2000; Sankaran, 2001; Lim and Cranbrook, 2002). Unforeseen economic factors could lead to a rapid collapse of the colonies in man-made buildings and leave the species dependent on its original cave-dwelling colonies. Thus, the natural cave dwelling colonies should not be allowed to be over-harvested in order to avoid the possibility of genetic bottle-neck effects due to small population sizes. Due to the lack of governmental regulation or laws to control the bird house construction, the expansion of swiftlet buildings in Thailand is likely to increase enormously in the near future and this could cause some negative impacts to humans and other sympatric species. The white-nest swiftlets living in city areas can cause some pollution e.g. noise pollution from swiftlet song, air pollution from dust and dried feces, and diseases from bird ectoparasites. Genetic homogeneity of this bird in Thailand may also result in susceptibility to disease and might have potential interaction with the bird flu issues. Moreover, the ecological consequences for other

aerial insectivorous species should be taken into consideration if the number of swiftlets is increased through human activities, and studies on these aspects are, therefore, urgently needed.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Current distribution of the white-nest swiftlet, *A. fuciphagus* has been extensively increased from original caves on the islands to man-made buildings on the mainland and also expanded from those buildings to newly constructed swiftlet buildings. Artificial habitats i.e. abandon or old buildings and sacred buildings have been originally found in some areas in Thailand for decades, for example, old houses at Pak Phanang District, Nakhon Si Thammarat Province in the South, a sacred building at Suthiwatwararam Temple, Samut Sakhon Province in Central Thailand and an old factory at Mueang District, Trat Province in Eastern Thailand. Until the past few decades, the ideas of the construction of swiftlet building originated from Indonesia have been introduced to Thailand and the swiftlet buildings have been largely expanded along the coastlines; Gulf of Thailand and Thai Andaman Sea. Without controlling of the swiftlet building construction by the government, the swiftlet buildings have been enormously increased and many of them are located in some city areas. Although there has been no report or evidence of negative impact from swiftlet farming to human, the rapid increasing of swiftlet buildings should be concerned by the government and local administrator. The short and long term management of ecological impact and health risk assessment should be conducted.

2. Historically, the natural colonies were relatively sparse and limited by the availability of cave sites. The construction of buildings for swiftlet farming has increased the availability of nest sites. In Thailand, the white-nest swiftlet, *A.*

fuciphgus colonies in man-made buildings have expanded over large areas within the past decades and this could affect genetic variation within the species. In this study, genetic relationship between *A. fuciphagus* recently established colonies in Thailand was investigated using two molecular markers; maternal (mtDNA) and paternal inheritance (nuclear DNA microsatellites). Genetic diversity for mtDNA data showed low nucleotide diversity, but high haplotype diversity whereas genetic diversity of microsatellite data showed high heterozygosity for all colonies. Estimates of genetic differentiation for mitochondrial DNA (Φ_{ST}) and microsatellites (F_{ST}) did not detect population subdivision within *A. fuciphagus* recently established colonies in Thailand. The absence of genetic structure in *A. fuciphagus* colonies in Thailand could be a result of high gene flow among colonies, causing genetic homogeneity for *A. fuciphagus* colonies. Therefore, *A. fuciphagus* living in recently established man-made colonies in Thailand should be considered members of a single panmictic population. Moreover, based on above evidence of genetic homogeneity, *A. fuciphagus* populations in Thailand should not be designated as different subspecies.

3. Although the opinion of swiftlet farming has been supported for maintaining genetic variability of *A. fuciphagus* in natural colonies and would reduce the risk of inbreeding for a species, natural colonies of the swiftlets should be paid more attention for protecting from over harvesting since there have been some reports on decreasing of numbers of the swiftlets in many natural colonies. In addition, an explosive increasing number of swiftlets could be also affected to other aerial insectivorous birds in the ecosystem.

6.2 Recommendations

     1. To confirm the information on genetic relationship of *A. fuciphagus* colonies and origin of *A. fuciphagus* colonies in man-made buildings, the samples from natural colonies should be included and the results will be determined whether this panmixia is stable or a temporary result of the recent explosive expansion of the number of colonies and comparisons to natural colonies may provide an understanding of mechanisms producing the lack of genetic structure in swiftlet house colonies. Moreover, the comparison can provide information on the impact of human activities and biodiversity.

     2. Negative impacts to human e.g. allergy from dust producing by swiftlets living in city areas, diseases from secretion and excretion of swiftlets and possible disease-carrier to human could be caused by an enormous increasing number of swiftlet populations. Therefore, further studies on these concerns should be conducted as soon as possible, including the impact to other aerial insectivorous birds that have trophic niche overlapping with swiftlets.

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APPENDICES

APPENDIX I

Catalogue numbers of *Aerodramus fuciphagus* specimens deposited at the Natural History Museum of Chulalongkorn University.

Catalogue number	Field number	Locality
CUMZ.AV.079.2006.1	AA01	Trang
CUMZ.AV.079.2006.2	AA02	Samut Sakhon
CUMZ.AV.079.2006.3	AA03	Samut Sakhon
CUMZ.AV.079.2006.4	AA04	Samut Sakhon
CUMZ.AV.079.2006.5	AA06	Chumphon1
CUMZ.AV.079.2006.6	AA07	Chumphon1
CUMZ.AV.079.2006.7	AA08	Chumphon1
CUMZ.AV.079.2006.8	AA10	Nakhon Si Thammarat
CUMZ.AV.079.2006.9	AA11	Nakhon Si Thammarat
CUMZ.AV.079.2006.10	AA14	Trang
CUMZ.AV.079.2006.11	AA21	Surat Thani
CUMZ.AV.079.2006.12	AA23	Surat Thani
CUMZ.AV.079.2006.13	AA24	Surat Thani
CUMZ.AV.079.2006.14	AA31	Narathiwat
CUMZ.AV.079.2006.15	AA32	Surat Thani
CUMZ.AV.079.2006.16	AA33	Surat Thani
CUMZ.AV.079.2006.17	AA34	Surat Thani
CUMZ.AV.079.2006.18	AA35	Surat Thani
CUMZ.AV.079.2006.19	AA36	Surat Thani
CUMZ.AV.079.2006.20	AA37	Surat Thani
CUMZ.AV.079.2006.21	AA38	Surat Thani
CUMZ.AV.079.2006.22	AA40	Samut Sakhon
CUMZ.AV.079.2006.23	AA41	Samut Sakhon
CUMZ.AV.079.2006.24	AA42	Samut Sakhon
CUMZ.AV.079.2006.25	AA43	Samut Sakhon
CUMZ.AV.079.2006.26	AA44	Samut Sakhon
CUMZ.AV.079.2006.27	AA45	Samut Sakhon
CUMZ.AV.079.2006.28	AA46	Samut Sakhon
CUMZ.AV.079.2006.29	AA50	Surat Thani

Catalogue number	Field number	Locality
CUMZ.AV.079.2006.30	AA51	Surat Thani
CUMZ.AV.079.2006.31	AA52	Surat Thani
CUMZ.AV.079.2006.32	AA53	Surat Thani
CUMZ.AV.079.2006.33	AA54	Chumphon1
CUMZ.AV.079.2006.34	AA55	Chumphon1
CUMZ.AV.079.2006.35	AA56	Chumphon1
CUMZ.AV.079.2006.36	AA57	Samut Sakhon
CUMZ.AV.079.2006.37	AA61	Samut Sakhon
CUMZ.AV.079.2006.38	AA62	Samut Sakhon
CUMZ.AV.079.2006.39	AA63	Samut Sakhon
CUMZ.AV.079.2006.40	AA66	Samut Sakhon
CUMZ.AV.079.2006.41	AA68	Samut Sakhon
CUMZ.AV.079.2006.42	AA71	Trang
CUMZ.AV.079.2006.43	AA72	Trang
CUMZ.AV.079.2006.44	AA73	Trang
CUMZ.AV.079.2006.45	AA74	Trang
CUMZ.AV.079.2006.46	AA77	Narathiwat
CUMZ.AV.079.2006.47	AA78	Narathiwat
CUMZ.AV.079.2006.48	AA80	Narathiwat
CUMZ.AV.079.2006.49	AA81	Narathiwat
CUMZ.AV.079.2006.50	AA82	Nakhon Si Thammarat
CUMZ.AV.079.2006.51	AA83	Nakhon Si Thammarat
CUMZ.AV.079.2006.52	AA84	Nakhon Si Thammarat
CUMZ.AV.079.2006.53	AA88	Trang
CUMZ.AV.079.2006.54	AA89	Trang
CUMZ.AV.079.2006.55	AA90	Trang
CUMZ.AV.079.2006.56	AA91	Trang
CUMZ.AV.079.2006.57	AA92	Trang
CUMZ.AV.079.2006.58	AA93	Chumphon2
CUMZ.AV.079.2006.59	AA94	Chumphon2
CUMZ.AV.079.2006.60	AA95	Chumphon2
CUMZ.AV.079.2006.61	AA96	Chumphon3
CUMZ.AV.079.2006.62	AA98	Chumphon1
CUMZ.AV.079.2006.63	AA99	Chumphon1
CUMZ.AV.079.2006.64	AA101	Songkla1
CUMZ.AV.079.2006.65	AA102	Songkla1

Catalogue number	Field number	Locality
CUMZ.AV.079.2006.66	AA103	Songkla1
CUMZ.AV.079.2006.67	AA104	Satun
CUMZ.AV.079.2006.68	AA105	Satun
CUMZ.AV.079.2006.69	AA106	Satun
CUMZ.AV.079.2006.70	AA107	Krabi
CUMZ.AV.079.2006.71	AA108	Krabi
CUMZ.AV.079.2006.72	AA109	Krabi
CUMZ.AV.079.2006.73	AA110	Songkla2
CUMZ.AV.079.2006.74	AA111	Songkla2
CUMZ.AV.079.2006.75	AA115	Trang
CUMZ.AV.079.2006.76	AA117	Trang
CUMZ.AV.079.2006.77	AA118	Trang
CUMZ.AV.079.2006.78	AA119	Trang

APPENDIX II

GenBank Accession numbers of mtDNA sequences; cytochrome-*b* and ND2 genes.

Sequence name	Accession number	Sequence name	Accession number	Haplotype
AF1.cyt	EU072052	AF1.nd2	EU072067	H01
AF2.cyt	EU072053	AF2.nd2	EU072068	H10
AF3.cyt	EU085082	AF3.nd2	EU085227	H02
AF4.cyt	EU085101	AF4.nd2	EU085246	H05
AF6.cyt	EU072054	AF6.nd2	EU072069	H06
AF7.cyt	EU085152	AF7.nd2	EU085297	H07
AF8.cyt	EU085168	AF8.nd2	EU085313	H01
AF10.cyt	EU085044	AF10.nd2	EU085189	H02
AF11.cyt	EU085053	AF14.nd2	EU085207	H05
AF14.cyt	EU085062	AF21.nd2	EU085215	H05
AF21.cyt	EU085070	AF23.nd2	EU085218	H05
AF23.cyt	EU085073	AF24.nd2	EU085220	H01
AF24.cyt	EU085075	AF31.nd2	EU085229	H01
AF31.cyt	EU085084	AF32.nd2	EU085231	H05
AF32.cyt	EU085086	AF33.nd2	EU085233	H05
AF33.cyt	EU085088	AF34.nd2	EU072071	H01
AF34.cyt	EU072056	AF35.nd2	EU085236	H12
AF35.cyt	EU085091	AF36.nd2	EU085238	H06
AF36.cyt	EU085093	AF37.nd2	EU085240	H02
AF37.cyt	EU085095	AF38.nd2	EU085242	H06
AF38.cyt	EU085097	AF40.nd2	EU085247	H01
AF40.cyt	EU085102	AF41.nd2	EU085249	H09
AF41.cyt	EU085104	AF42.nd2	EU085251	H05
AF42.cyt	EU085106	AF43.nd2	EU085253	H05
AF43.cyt	EU085108	AF44.nd2	EU085255	H05
AF44.cyt	EU085110	AF45.nd2	EU085257	H05
AF45.cyt	EU085112	AF46.nd2	EU085259	H06
AF46.cyt	EU085114	AF50.nd2	EU085265	H01
AF50.cyt	EU085120	AF51.nd2	EU085267	H11
AF51.cyt	EU085122	AF52.nd2	EU072072	H05
AF52.cyt	EU072057	AF53.nd2	EU085270	H03
AF53.cyt	EU085125	AF54.nd2	EU085272	H05
AF54.cyt	EU085127	AF55.nd2	EU085274	H01
AF55.cyt	EU085129	AF56.nd2	EU085276	H01
AF56.cyt	EU085131	AF57.nd2	EU085277	H05

Sequence name	Accession number	Sequence name	Accession number	Haplotype
AF57.cyt	EU085132	AF61.nd2	EU072073	H13
AF61.cyt	EU072058	AF62.nd2	EU085284	H14
AF62.cyt	EU085139	AF63.nd2	EU085286	H05
AF63.cyt	EU085141	AF66.nd2	EU085290	H01
AF66.cyt	EU085145	AF68.nd2	EU085293	H05
AF68.cyt	EU085148	AF71.nd2	EU085299	H07
AF71.cyt	EU085154	AF72.nd2	EU085301	H05
AF72.cyt	EU085156	AF73.nd2	EU072074	H05
AF73.cyt	EU072059	AF74.nd2	EU072075	H02
AF74.cyt	EU072060	AF77.nd2	EU085307	H13
AF77.cyt	EU085162	AF78.nd2	EU085309	H04
AF78.cyt	EU085164	AF80.nd2	EU085314	H05
AF80.cyt	EU085169	AF81.nd2	EU085316	H07
AF81.cyt	EU085171	AF82.nd2	EU085318	H05
AF82.cyt	EU085173	AF83.nd2	EU085320	H01
AF83.cyt	EU085175	AF84.nd2	EU085322	H05
AF84.cyt	EU085177	AF88.nd2	EU085324	H05
AF88.cyt	EU085179	AF89.nd2	EU085325	H01
AF89.cyt	EU085180	AF90.nd2	EU085327	H05
AF90.cyt	EU085182	AF91.nd2	EU085328	H05
AF91.cyt	EU085183	AF93.nd2	EU085329	H05
AF92.cyt	EU072061	AF94.nd2	EU085330	H07
AF93.cyt	EU085184	AF96.nd2	EU085331	H05
AF94.cyt	EU085185	AF98.nd2	EU085332	H05
AF95.cyt	EU072062	AF92.nd2	EU072076	H09
AF96.cyt	EU085186	AF95.nd2	EU072077	H02
AF98.cyt	EU085187	AF99.nd2	EU072078	H01
AF99.cyt	EU072063	AF101.nd2	EU072079	H15
AF101.cyt	EU072064	AF102.nd2	EU085190	H04
AF102.cyt	EU085045	AF104.nd2	EU072080	H05
AF103.cyt	EU085046	AF103.nd2	EU085191	H05
AF104.cyt	EU072065	AF105.nd2	EU085192	H05
AF105.cyt	EU085047	AF106.nd2	EU085193	H05
AF106.cyt	EU085048	AF107.nd2	EU085194	H06
AF107.cyt	EU085049	AF108.nd2	EU085195	H01
AF108.cyt	EU085050	AF109.nd2	EU085196	H05
AF109.cyt	EU085051	AF11.nd2	EU085198	H07
AF110.cyt	EU085054	AF110.nd2	EU085199	H05
AF111.cyt	EU085055	AF111.nd2	EU085200	H05
AF115.cyt	EU085056	AF115.nd2	EU085201	H01
AF117.cyt	EU072051	AF117.nd2	EU072066	H08
AF118.cyt	EU085057	AF118.nd2	EU085202	H06
AF119.cyt	EU085058	AF119.nd2	EU085203	H09
AF1f.cyt	EU085068	AF1f.nd2	EU085213	H07

Sequence name	Accession number	Sequence name	Accession number	Haplotype
AF2f.cyt	EU085081	AF2f.nd2	EU085226	H02
AF3f.cyt	EU085100	AF3f.nd2	EU085245	H05
AF4f.cyt	EU085119	AF4f.nd2	EU085264	H09
AF5f.cyt	EU085136	AF5f.nd2	EU085281	H09
AF6f.cyt	EU085151	AF6f.nd2	EU085296	H07
AF7f.cyt	EU085167	AF7f.nd2	EU085312	H05
AF8f.cyt	EU085181	AF8f.nd2	EU085326	H02
AF9f.cyt	EU085188	AF9f.nd2	EU085333	H01
AF10f.cyt	EU085052	AF10f.nd2	EU085197	H02
AF11f.cyt	EU085059	AF11f.nd2	EU085204	H05
AF12f.cyt	EU085060	AF12f.nd2	EU085205	H01
AF13f.cyt	EU085061	AF13f.nd2	EU085206	H01
AF14f.cyt	EU085063	AF14f.nd2	EU085208	H05
AF15f.cyt	EU085064	AF15f.nd2	EU085209	H04
AF16f.cyt	EU085065	AF16f.nd2	EU085210	H05
AF17f.cyt	EU085066	AF17f.nd2	EU085211	H01
AF18f.cyt	EU085067	AF18f.nd2	EU085212	H01
AF20f.cyt	EU085069	AF20f.nd2	EU085214	H01
AF21f.cyt	EU085071	AF21f.nd2	EU085216	H02
AF22f.cyt	EU085072	AF22f.nd2	EU085217	H09
AF23f.cyt	EU085074	AF23f.nd2	EU085219	H01
AF24f.cyt	EU085076	AF24f.nd2	EU085221	H02
AF25f.cyt	EU085077	AF25f.nd2	EU085222	H02
AF26f.cyt	EU085078	AF26f.nd2	EU085223	H01
AF27f.cyt	EU072055	AF27f.nd2	EU072070	H11
AF28f.cyt	EU085079	AF28f.nd2	EU085224	H05
AF29f.cyt	EU085080	AF29f.nd2	EU085225	H07
AF30f.cyt	EU085083	AF30f.nd2	EU085228	H05
AF31f.cyt	EU085085	AF31f.nd2	EU085230	H05
AF32f.cyt	EU085087	AF32f.nd2	EU085232	H09
AF33f.cyt	EU085089	AF33f.nd2	EU085234	H01
AF34f.cyt	EU085090	AF34f.nd2	EU085235	H02
AF35f.cyt	EU085092	AF35f.nd2	EU085237	H02
AF36f.cyt	EU085094	AF36f.nd2	EU085239	H05
AF37f.cyt	EU085096	AF37f.nd2	EU085241	H02
AF38f.cyt	EU085098	AF38f.nd2	EU085243	H05
AF39f.cyt	EU085099	AF39f.nd2	EU085244	H04
AF40f.cyt	EU085103	AF40f.nd2	EU085248	H05
AF41f.cyt	EU085105	AF41f.nd2	EU085250	H07
AF42f.cyt	EU085107	AF42f.nd2	EU085252	H05
AF43f.cyt	EU085109	AF43f.nd2	EU085254	H05
AF44f.cyt	EU085111	AF44f.nd2	EU085256	H07
AF45f.cyt	EU085113	AF45f.nd2	EU085258	H02
AF46f.cyt	EU085115	AF46f.nd2	EU085260	H07

Sequence name	Accession number	Sequence name	Accession number	Haplotype
AF47f.cyt	EU085116	AF47f.nd2	EU085261	H01
AF48f.cyt	EU085117	AF48f.nd2	EU085262	H02
AF49f.cyt	EU085118	AF49f.nd2	EU085263	H07
AF50f.cyt	EU085121	AF50f.nd2	EU085266	H02
AF51f.cyt	EU085123	AF51f.nd2	EU085268	H05
AF52f.cyt	EU085124	AF52f.nd2	EU085269	H01
AF53f.cyt	EU085126	AF53f.nd2	EU085271	H05
AF54f.cyt	EU085128	AF54f.nd2	EU085273	H05
AF55f.cyt	EU085130	AF55f.nd2	EU085275	H02
AF57f.cyt	EU085133	AF57f.nd2	EU085278	H09
AF58f.cyt	EU085134	AF58f.nd2	EU085279	H02
AF59f.cyt	EU085135	AF59f.nd2	EU085280	H07
AF60f.cyt	EU085137	AF60f.nd2	EU085282	H07
AF61f.cyt	EU085138	AF61f.nd2	EU085283	H02
AF62f.cyt	EU085140	AF62f.nd2	EU085285	H05
AF63f.cyt	EU085142	AF63f.nd2	EU085287	H05
AF64f.cyt	EU085143	AF64f.nd2	EU085288	H05
AF65f.cyt	EU085144	AF65f.nd2	EU085289	H02
AF66f.cyt	EU085146	AF66f.nd2	EU085291	H02
AF67f.cyt	EU085147	AF67f.nd2	EU085292	H05
AF68f.cyt	EU085149	AF68f.nd2	EU085294	H05
AF69f.cyt	EU085150	AF69f.nd2	EU085295	H05
AF70f.cyt	EU085153	AF70f.nd2	EU085298	H05
AF71f.cyt	EU085155	AF71f.nd2	EU085300	H01
AF72f.cyt	EU085157	AF72f.nd2	EU085302	H01
AF73f.cyt	EU085158	AF73f.nd2	EU085303	H09
AF74f.cyt	EU085159	AF74f.nd2	EU085304	H09
AF75f.cyt	EU085160	AF75f.nd2	EU085305	H05
AF76f.cyt	EU085161	AF76f.nd2	EU085306	H01
AF77f.cyt	EU085163	AF77f.nd2	EU085308	H05
AF78f.cyt	EU085165	AF78f.nd2	EU085310	H02
AF79f.cyt	EU085166	AF79f.nd2	EU085311	H05
AF80f.cyt	EU085170	AF80f.nd2	EU085315	H02
AF81f.cyt	EU085172	AF81f.nd2	EU085317	H02
AF82f.cyt	EU085174	AF82f.nd2	EU085319	H07
AF83f.cyt	EU085176	AF83f.nd2	EU085321	H05
AF84f.cyt	EU085178	AF84f.nd	EU085323	H01

APPENDIX III

PUBLICATION

Aowphol, A., Voris, H.K., Feldheim, K.A., Harnyuttanakorn, P. and Thirakhupt, K.
2008. Genetic Homogeneity Among Colonies of the White-Nest Swiftlet
(*Aerodramus fuciphagus*) in Thailand. <u>Zoological Science</u> 25: 372-380.
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BIOGRAPHY

Miss Anchalee Aowphol was born on September 4, 1976 in Saraburi Province. After graduating from Saraburi Witthayakom School, Anchalee enrolled in the Department of Biology, Faculty of Science, Chulalongkorn University, majoring in Biology and received her Bachelor's degree in May 1999. She enrolled in the same Department, majoring in Zoology in June 1999 and conducted a research on ecology of a gecko under supervised of Associate Professor Dr. Kumthorn Thairakhupt. She finished her Master degree in May 2003. She continued her PhD study in Biological Sciences Program, Faculty of Science, Chulalongkorn University in June 2003. She was awarded the scholarship from the Development and Promotion of Science Talents program (DPST) for her study at Chulalongkorn University.