POPULATION GENETICS OF WHITE-NEST SWIFTLET
AERODRAMUS FUCIPHAGUS IN VIETNAM

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ABSTRACT

To address bird nest farming development in Vietnam today, a study on the population genetics of white-nest swiftlet subspecies in Vietnam was conducted under the scientific research project “Science-based research and propose solutions for sustainable development in the bird nest farming in Vietnam”, which was carried out by Khanh Hoa Salanganes Nest Company in coordination with the Institute of Biotechnology and Environment, Nha Trang University. White-nest swiftlets (Aerodramus fuciphagus) are birds of high economic value from their nests. Island and house swiftlets were collected from 5 geographical areas: Khanh Hoa, Trang Bom - Dong Nai, Kien Giang, Binh Phuoc, and Con Dao between 01/2012 - 6/2014. Phylogeographic trees were constructed based on four algorithms, Maximum Parsimony, Maximum Likelihood, Bayesian Inference and Neighbour Joining using cytochrome b (mitochondrial DNA), and GAPDH (genomic DNA) from the subspecies Aerodramus fuciphagus in combination with sequences from GenBank. Haplotype network was built based on Network use Netwoek Draw. The results showed that Vietnamese white- nest swiftlet was classified into two major groups. The samples from Trang Bom - Dong Nai, Binh Phuoc, and Kien Giang have close relationship with the subspecies A. fuciphagus amechanus and A. fuciphagus vestitus. Island swiftlets from Khanh Hoa and Con Dao show clear separation from the subspecies in Trang Bom - Dong Nai, Kien Giang, Binh Phuoc and subspecies data from GenBank. A common haplotype was shared by the house-swiftlet populations from Khanh Hoa, Trang Bom - Dong Nai, Binh Phuoc, Kien Giang and subspecies vestitus, germanium and amechanus. Analyses based on gene Cyt-b and GAPDH show that population in Trang Bom - Dong Nai is A. fuciphagus vestitus, the Kien Giang population is probably A. fuciphagus amechanus, and, the population from Khanh Hoa and Con Dao is likely endemic subspecies in Vietnam.

Keywords: swiftlet, Aerodramus fuciphagus, subspecies, population genetics, Vietnam

I. INTRODUCTION

White-nest swiftlets, Aerodramus fuciphagus, (Apodidae: Collocaliini) are small birds, insectivorous, distributed across the area from Australia, the Indian Ocean to the South Pacific. They usually nest in caves, and can locate by echo (Koon and Cranbrook in 2002, Nguyen et al, 2002).

Presently, many studies have focused on the phylogenetic relationship of swiftlet (Le et al, 1996, Thomassen et al, 2003, 2005, Thomassen and Povel, 2006 Parkert et al, 2012), the behavior of living swiftlet (Fullard et al, 2010), and the evolution of echolocation (Price et al, 2004, 2005). Studies on swiftlet in Vietnam largely have been done at the level of the survey, collect samples and classification, morphological description, the economic value of subspecies of white-nest swiftlet, (Aerodramus fuciphagus), artificial breeding and swiftlet house growing and migrating (Nguyen et al, 2002 Nguyen Khoa Dieu Thu et al, 2009, Le Huu Hoang et al, 2010). Currently, the study on genetic diversity and population structure of

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swiftlet in Vietnam and around the world is still limited (Lee et al, 1996).

The analysis of phylogenetic relationship of swiftlet on the morphological characteristics (shape, color, behavior, ability to echolocation ...) sometimes give inaccurate results, especially for species with a close relationship because they have many similar morphological characteristics (Price et al, 2005). Therefore, the use of molecular markers to identify species and determine precisely the phylogenetic relationships and genetic diversity is essential.

II. MATERIALS AND METHODS

1. Sampling

![map of sampling sites](image)

**Fig. 1. Sampling sites of *Aerodramus fucifagus* in Vietnam, Map shows subspecies of *A. fucifagus* distributed in South East countries. Red circles display subspecies in Vietnam *Aerodramus fuciphagus*.**

Island swiftlets were collected in Nha Trang - Khanh Hoa (Hon Noi A2 - 10 specimens, Hon Mun A6 - 10 specimens), and Con Dao (15 specimens). House swiftlets were collected in Nha Trang - Khanh Hoa (155 Thong Nhat Street - 16 specimens), Trang Bom - Dong Nai (18 specimens), Kien Giang (21 specimens) and Binh Phuoc (19 specimens) ([Figure 1](image)). Samples (tissue or feather) were collected between 2013 and 2014. 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 island workers, stored in 95% ethanol. Feather was collected from each individual, kept separately.

The construction of the phylogenetic tree can be based on sequence analysis of a gene or a gene family. However, data analysis also can expand more, such as combination of genes or different parts of DNA (Lee et al, 1996).

This study aimed to construct phylogenetic trees to subspecies of swiftlet, thereby detecting and determining the phylogenetic relationships of swiftlet populations in geographical areas in Vietnam, provide the data for the conservation, sustainable exploitation of the bird nest resources.
2. DNA extraction, PCR amplification and sequencing

Total genomic DNA from tissues and feather was extracted by using Megazorb® DNA Mini-Prep Kit (Promega)) following the manufacturer’s protocol.

Fragments of genomic GAPDH and mitochondrial cyt-b genes were amplified by PCR using primers G3P13b: 5’TCC ACC TTT GAT GCG GGT GCT GG3’, and G3P14b: 5’AAG TCC ACA ACA CGG TTG CTG TA3’ 3’ (Geland & Cs, 2009) for GAPDH, and W1 5’-GGG GGA TTC TCA GTA GAC AA-3’ (Thomassen và cs, 2003) and RV2 5’-TCT TTG GTT TAC AAA CCA AT-3’ (Desjardins và Morais, 1990) for Cyt-b.

PCR amplification was carried out in 25 μL reactions containing 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 0.2 mM each dNTP, 0.5 U Taq DNA polymerase, 0.2 μM primers and 1–2 μL DNA template. Cycling conditions 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 a Qiagen PCR Purification Kit (Qiagen). The purified product was cycle sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

3. Data analysis

3.1. Haplotype diversity

Sequences were edited and aligned using Bioedit version 7.0. 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).

3.2. Haplotype network

A median-joining network was constructed to describe the haplotype relationship using NETWORK version 4.2.0.1 (Fluxus Technology Ltd.) with the default parameters.

III. RESULT AND DISCUSSION

1. Haplotype diversity

Among 104 GAPDH sequences obtained in Khanh Hoa, Con Dao, Trang Bom - Dong Nai, Binh Phuoc, 4 haplotypes were observed (H = 4) with haplotype diversity Hd = 0.693 ± 0.022, nucleotide diversity π = 0.00285, polymorphic sites S = 2. In particular, A. fucifagus in Khanh Hoa has 3 haplotypes, Con Dao 2 haplotypes, Trang Bom - Dong Nai 2 haplotypes, Kien Giang 2 haplotypes, and Binh Phuoc 1 haplotype, respectively.

Among 85 Cyt-b sequences obtained in Khanh Hoa, Con Dao, Trang Bom - Dong Nai, Kien Giang, 11 haplotypes were observed with Hd = 0.780 ± 0.036, π = 0.00878, s = 17. A. fucifagus in Khanh Hoa has 6 haplotypes, Con Dao 2 haplotypes, Trang Bom - Dong Nai 5 haplotypes, and Kien Giang 2 haplotypes, respectively.

Common haplotype (ancestor haplotype) was shared by populations in Trang Bom - Dong Nai, and Kien Giang. Island A. fucifagus in Khanh Hoa and Con Dao do not share common haplotype with other remaining populations.
2. Haplotype network

The haplotype network of *Aerodramus ficifagus* in Vietnam based on GAPDH (A) and Cytochrome b genes was shown in Figure 2. Based on GAPDH gene (A), four groups (4 haplotypes) were observed. The first two groups included *A. ficifagus* in Khanh Hoa (both house and island swiftlets), and Con Dao. The third group had common haplotype with house *A. ficifagus* in Khanh Hoa, Trang Bom – Dong Nai, Binh Phuoc and Kien Giang. The last group included swiftlet in Kien Giang, and Trang Bom - Dong Nai. Haplotype network from Cyt-b displayed almost similar pattern. Of which, first group included *A. ficifagus* in Khanh Hoa (house and island) and Con Dao. Among that, two subgroups have been observed, one contained only swiftlet in Con Dao, and the other included house and island swiftlet in Khanh Hoa. The second group shared common haplotype with populations in Khanh Hoa (house swiftlet), Trang Bom – Dong Nai, and Kien Giang. In addition, subgroups of swiftlet in Kien Giang, Trang Bom - Dong Nai were also presented.

According to Nguyen et al (2002), in Vietnam exists subspecies *A. ficifagus germani*. This subspecies is also found in Thailand and Burma border, and in the Philippines, meanwhile, subspecies *A. ficifagus amechanus* distributes in Malaysia, and *A. ficifagus vestitus* in Indonesia, respectively (Fig. 1).

According to Le et al. (2014), house *A. ficifagus* collected in Trang Bom - Dong Nai has a close relationship with *A. ficiphagus vestitus* subspecies native to Indonesia, house *A. ficifagus* in Kien Giang shares genotypes with subspecies *A. ficiphagus amechanus* in Malaysia. House *A. ficifagus* in Khanh Hoa shares haplotypes with all 3 subspecies, however, islands *A. ficifagus* in Khanh hoa and Con Dao formed a separated clade.

In terms of geography, house swiftlets in Trang Bom – Dong Nai and Kien Giang may originate from the subspecies *A. ficifagus vestitus* and amechamus from Indonesia and Malaysia, respectively (Figure 1). Due to the behavior of the swiftlet, the bird often fly long way for feeding. They can find suitable habitat (the swiftlet house) with abundant food resources in the South Central and South East, Vietnam (Kien Giang and Trang Bom - Dong Nai).
Khanh Hoa and Con Dao island *A. fucifagus* populations did not share common haplotypes with the other subspecies. Additionally, genetic data from genomic and mitochondrial genes also showed a clear separation (Fig 2 A, B), there may be a subspecies of *A. ficiphagus* endemic to Vietnam. Meanwhile, Khanh Hoa house *A. fucifagus* shared a common haplotype with both house swiftlet (Kien Giang, Trang Bom, Binh Phuoc), and island swiftlet (Khanh Hoa and Con Dao). This population showed a complex mix of house and island *A. fucifagus* in Vietnam. Therefore, the need for comprehensive research on populations of *A. fucifagus* subspecies in Vietnam to provide scientific base for issuing appropriate conservation policies on the endemic genetic resources of Vietnam.

IV. CONCLUSION

By using molecular markers (GAPDH and Cty-b), we have built haplotype network of *A. fucifagus* subspecies of swiftlets in Vietnam. Results showed that swiftlets from Trang Bom - Dong Nai and Kien Giang have close relationships with *A. fucifagus vestitus* and *A. fucifagus amechanus* subspecies. Swiftlet populations in Khanh Hoa and Con Dao were likely endemic subspecies of Vietnam. However, further researches need to be performed on morphological characteristics, ecology and extended sampling area (especially Con Dao) to be able to draw conclusions about the exact subspecies. Khanh Hoa and Con Dao swiftlet population should be included in the national genetic resource conservation program.

REFERENCES


