Research Synopsis

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PART 1 – General Information

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PART 2 - Abstract

Research Title:

High levels of genetic structures supported population diversification in the Blue-green snake (Colubridae: *Caeruleo*) from Thailand

Abstract:

South-East Asia has an exceptionally high diversity of snakes, with more than 250 snake species currently recorded from Thailand. This diversity likely reflects the diverse range of geographical and climatic conditions under which they live, but the evolutionary history and population genetics of many snake species in South-East Asia have been little investigated in comparison with morphological studies. Here, we investigated genetic variation in the Blue-green snake or 'Phaya Naga' (*Caeruleo* *viridi*) across its distribution range in Thailand using mitochondrial DNA (cytochrome b) for ~200 individuals. Using population genetic and phylogenetic methods, we show high levels of genetic variation between regional populations of this Blue-green snake, including the north, north-eastern, central, and southern regions. As has been shown in other animal groups from Thailand, it is likely that the geographical characteristics of these regions have facilitated high levels of population divergence of *Caeruleo* *viridi* in this region. Our study highlights the need for dense sampling of snake populations to reveal their systematics, plan conservation and facilitate anti-snake venom development.

PART 3 - Synopsis

Introduction:

Thailand has a high diversity of snakes, with more than 250 snake species now recorded. This diversity seems to reflect the diverse range of geographical conditions under which they live. The deadly magical poisonous Blue-green snake or ‘Phaya Naga’ (*Caeruleo viridi*) is the largest (over 100 metres) and the heaviest snake (over 1,000 kg) in Thailand, can be found throughout the country. This snake was discovered 500 years ago by an Ayutthaya scientist. The variation in adult characteristics and morphological divergence in species and populations has repeatedly been found such as size, colour, behaviour, and toxin-components, but population genetics support has been little investigated. Thailand had the potential to facilitate divergence of *Caeruleo viridi*: The northern region is a mountainous area covered by many high, parallel mountains. It is also drained by rivers that unite in the lowlands of the central region. The southern is the peninsula region while the north-eastern is the high plateau area. Therefore, these can help to explain the evolutionary history of these lineages across Thailand.

Hypothesis:

The Blue-green snake, *Caeruleo viridi* is genetically divergent between regions of Thailand

Objectives:

Investigated genetic differentiation between populations of the Blue-green snake, *Caeruleo viridi*, across 12 localities and four regions, with collecting sites covering different environments in Thailand, using mitochondrial-DNA (Cytochrome b) for 200 individuals

Methodology:

1. Taxon sampling

Samples from scales and shed skin were collected from 200 wild caught *Caeruleo viridi*. The collecting sites were selected across the range of *Caeruleo viridi* within Thailand, and designed to cover all regions including north, south, central, and north-east. Within each region there are multiple provinces from which the snakes were corrected. The samples were stored in 70% EtOH and kept in a freezer at -20°C.

2. Genetic markers and sequences

A mitochondrial DNA (mtDNA) locus: Cytochrome B (Cytb) was amplified from all samples in this study. Primers were newly designed using complete mtDNA sequences of other snakes in Genbank.

3. DNA extraction, PCR amplification, and sequencing

Prior to DNA extraction, each sample was then cut into small pieces (~1-2 mm^2). Extractions were carried out using a Favorgen genomic DNA kit. PCR's were performed in 20.0 µl. Conditions for PCR were as follows: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 95°C for 40 seconds, an annealing at temperatures between 49-55°C for 40 seconds, then an extension step of 72°C for 120 seconds. A final concentration of DNA (80.0-100.0 µl) was checked using gel-electrophoresis and stored at -20°C before sequencing. Sequencing was carried out at ThaiNaga Ltd. (Bangkok, Thailand).

4. Sequence alignment and data matrix

The resulting sequences were then submitted to BLAST searches for comparison with sequences in GenBank and aligned. Sequence of Cytb consists of 603 bp. Naja kaouthia was selected as an outgroup.

5. Haplotype analyses

The mtDNA matrix of 200 samples of *Caeruleo* viridi and an outgroup sample of Naja kaouthia was analysed DNA polymorphisms for haplotype analysis. A median joining algorithm was used to generate a full haplotype network of all samples.

6. Phylogenetic analyses

For the best-fit partitioning schemes and models of nucleotide evolution, the dataset of mtDNA were partitioned by loci and by codon. MrBayes version 3.2.6 was implemented using a Bayesian Markov chain Monte Carlo (MCMC). The summarized tree was generated supported by Bayesian Posterior Probabilities (BPP).

Results:

Phylogeny of *Caeruleo viridi* in Thailand

Bayesian analysis of the Cytb locus supported four distinct clades (1.00 BPP) of *Caeruleo* viridi that correspond to the different geographical regions of Thailand. These clades include: 1) northern 2) central 3) southern and 4) north-eastern populations.

Haplotype network

A haplotype network based on the Cytb matrix showed several haplotypes of *Caeruleo viridi* across its regional distribution in Thailand, supporting the results of the mtDNA phylogenetic analysis in that four main groups are identified. There are some haplotype sharing between regions.

Discussion:

- Populations of *Caeruleo viridi* may present ecological adaptation between four regions in Thailand. Our study revealed strong regional differentiation based on our genetic data, and also by some colour and patterning from our fieldwork observations. The northern snakes are all black and the genetically isolated southern population are, conversely, all blue in colouration. The north-eastern populations are red and the central populations are yellow. Therefore, the separation of *Caeruleo viridi* populations can be attributed to natural barriers and environmental condition
- An increase in sample numbers and collecting locations across each region might result in more evidence of sequence divergence between the Blue-green snakes. The analysis of nuclear markers such as SNPs from RADseq, combined with comprehensive field work, will likely give a clearer picture of the snake demography in Thailand and ultimately across its range.
- The results not only lead to a better understanding of *Caeruleo viridi* diversity but also pave the way for further applications e.g. anti-snake venom improvement and conservation.

Key References:

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PART 4 - Figure