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DNA Barcoding of Chiropterans at Minalungao National Park, Nueva Ecija, Philippines

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Chiroptera, or the order of bats, are widespread and considered one of the most diverse group of mammals consisting of about 1200 species. The Philippines, as one of the key biodiversity spots, hosts a great concentration of chiropterans of which 26 are indigenous. Despite this diversity, this group of mammals are the least known fauna in the country. One area that holds a number of bats is the Minalungao National Park located in Central Luzon, Philippines. Minalungao National Park is a conserved Key Biodiversity Area (KBA) that is characterized with dense forests and has not yet been the focus of scientific studies, particular-ly in systematics and biodiversity conservation. It is a biologically significant area where several species are not yet identified and recorded. This study reports the identities of the bats present in the caves of Minalungao National Park. The bats were identified molecularly through the sequences of the mitochondrial cyto-chrome oxidase 1 (mtCOI). Three (3) species were identified, namely: *Hipposideros rotalis, Taphozous melanopogon* and *Cynopterus sphinx*, an unreported species in the Philippines.

Keywords: Chiroptera, Key Biodiversity Area, Minalungao National Park, Mitochondrial cyto-chrome oxidase 1, Molecular identification

Introduction

Minalungao National Park is a protected area and one of the few remaining natural environments in the region north of Manila. Spanning over 2,000 hectares, it lures travellers with the picturesque sight of the emerald colored Penaranda River that runs across most of the park and the towering limestone formations that border it (Bernardino, 2016). The park derived its

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name from “Mina” and “Lungao”, meaning gold mine in caves. It is characterized with dense forests and rock formations, cool springs and flow-ing rivers.

Despite urbanization and persistent threat to the integrity of the forest, Luzon has the most number of site-endemic species of mammals in the world, including bats and rodents inhabiting mountaintops. Luzon Is-land now has the highest concentration of unique species of bats in the world (Balete *et al*., 2016). There are 57 species of bats, most of them live in the hot and humid lowlands. These include the golden-crowned flying fox, which is one of the heaviest bats in the world with weights that reaches up to 2.5 pounds and the smaller flat-headed bat (Balete *et al*., 2016).

With at least 57 known species, chiroptera is the most diverse order of mammals in Luzon. The diversity of bats range terms of size, feeding habit, behavior and appearance. Some bats consume fruits, nectar and blossoms, while others feed on large, hard-shelled beetles, small moths and spiders. A number of species roost in caves, while others in hollow trees, in thick vegetation or in open sunlight on the top of large trees.

The mitochondrial DNA Cytochrome oxidase I (mtCOI) is a gene that is often used as a DNA barcode to identify animal species. CO1 gene sequence is suitable for barcoding because its mutation rate is often fast enough to distinguish closely related species and also because its sequence is con-served among conspecifics (Aoyama *et al*., 1996).

Thus, this study was conducted to identify the Chiroptera collected at Minalungao National Park using the mtDNA COI.

Objectives: 1) obtained amplicons from the PCR products of the COI gene of Chiroptera collected; 2) sequenced the mitochondrial DNA Cytochrome oxidase I (COI) of the collected Chiroptera; and 3) identified the different species of Chiroptera using the sequenced mtCOI.

Materials and methods

Polymerase Chain Reaction

The target region of the mtCOI was amplified using the universal primer pair COI-F GGT-CAA-CAA-ATC-ATA-AAG-ATA-TTG-G and COI-R TAA-ACT-TCA-GGG-TGA-CCA-AAA-AAT-CA. The polymerase chain reaction (PCR) mix had a total of 10µL per reaction composed of 4.7µL double distilled water, 2.0µL buffer, 0.8µL MgCl2, 0.5µL dNTP, 0.2µL roimers, 0.1µL Taq polymerase, and 1.5µL DNA, and ran in a thermal cycler (Applied Biosystems). The PCR program consisted of initial denaturation of 94℃ for 2 minutes, followed by 35 cycles of denaturation at 94℃ for 30 seconds,

annealing at 46 ℃ for 40 seconds, elongation at 72 ℃ for 50 seconds, and final extension for 10 minutes at 72 ℃.

DNA Sequencing and Sequence Validation

The PCR products were sent to 1stBase (Malaysia) for sequencing. Sequences of the amplified products were assembled using the Chromaslite and were subjected to Basic Local Alignment Search Tool (BLAST, NCBI) for identification.

Results

Molecular Identification

The species identified using the mitochondrial *cytochrome oxidase I* (mtCOI) after PCR amplification were *Taphozous melanopogon* (Ngusong kabayo), *Hipposideros rotalis* (Kulilit), and *Cynopterus sphinx* (Bangaag).

Discussion

The species that were identified are *Taphozous melanopogon, Hipposideros rotalis, and Cynopterus sphinx. Taphozous melanopogon* is commonly known as black bearded tomb bats ranges from grayish to multi-brown or red variations. The hair are white tipped and more red or brown towards the base. Black bearded tomb bats favors environment with densely sheltered areas. Commonly found in rainforest, woodlands, tombs, old buildings, roosts caves, cliffs, and arid country plains (Bates and Harrison 1997). These bats has also been recorded in the islands of Biliran, Cebu, Gigante, Leyte, Luzon (Ilocos Norte, Nueva Viscaya, Pangasinan, Rizal provinces), Maripipi, Mindanao (Davao del Sur), Negros, Palawan, Sibuyan, Tincasan, Lubang, Luzon (Abra Province) and Mindoro (Heaney *et al*., 1998). It is found from sea level to 150 masl in the Philippines (Heaney *et al*., 1998).

Another is *Hipposideros rotalis*, which is characterized by a broad noseleaf, covering muzzle, brown upper parts with white bases in individual hairs, brown middles and pale tips and the underparts are paler. This species of bats can commonly be found in Northeastern Luzon, Philippines (Mudar and Allen, 1986). H. rotalis has been recorded to inhabit limestone caves in distributed evergreen forest, and mixed dry dipterocarp and evergreen forest (Francis and Barrett, 2008).

The third species identified was *Cynopterus sphinx*. This species is known to possess greater short-nosed leaf with a relatively long snout. The upper parts are usually bright orange in color with paler underparts. This

species is very common in tropical forests and areas where the fruit crops are cultivated. In addition, they can also be found in grasslands and mangrove forests (Shefferly, 1999). No reports of this species was made in the Philippines. This species usually occurs in Bangladesh, Bhutan, Cambodia, China, Hong Kong, India, Indonesia, Lao People's Democratic Republic, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand, and Vietnam (Bates *et al*., 2008).

Using the mtDNA CO1 marker, three species of bats were identified in Minalungao National Park, Nueva Ecija, Philippines, one of which is unrecorded in the country: Taphozous melanopogon, Hipposideros rotalis, and Cynopteros sphinx.

Only three species were identified using molecular markers out of the 8 morphologically identified bats (Pader and Cruz, unpublished) inhabiting the caves of this national park. It is hoped that the other species will be molecularly identified.

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