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Molecular data confirm the presence of *Nycticebus bengalensis* on Langkawi Island, Malaysia

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Abstract. Md-Zain BM, Mohhoyua KS, Aifat NR, Ngadi E, Ayob N, Rovie-Ryan JJ, Ampeng A, Mohd-Ridwan AR, Blair ME, Abdul-Latiff MAB. 2019. Molecular data confirm the presence of *Nycticebus bengalensis* on Langkawi Island, Malaysia. *Biodiversitas* 20: 1115-1120. Recent taxonomic reviews have stated the possibility of Bengal Slow Loris (*Nycticebus bengalensis*) presence in the Northern part of the Malay Peninsula. This study aims to confirm the presence of the Bengal Slow Loris in Malaysia by sequencing the mitochondrial COI gene from samples collected from Langkawi Island, Peninsular Malaysia, and Borneo. Phylogenetic analyses produced tree topologies that support the grouping of slow loris samples by their localities. The tree topologies further show that slow loris samples from Sarawak and Peninsular Malaysia form two distinct clades. The clade from Peninsular Malaysia was divided into two subclades, Langkawi and Selangor. The Langkawi slow loris subclade includes sequences from GenBank representing *N. bengalensis*, supported by a high bootstrap value. This mitochondrial DNA finding has a significant contribution to indicate the presence of the Bengal Slow Loris in Malaysia.

Keywords: Biogeography, Malaysian primates, *Nycticebus bengalensis*, *Nycticebus coucang*, phylogeny, slow loris

INTRODUCTION

The slow loris (Genus *Nycticebus*, family Lorisidae) is a nocturnal primate found in South and Southeast Asian regions (Roos et al. 2014). Species distributions range from eastern India to Indochina and southern China south to the Malay Peninsula and Java, Borneo to the western Philippines (Groves 2001; Brandon-Jones et al. 2004). There are eight species currently recognized under this genus *Nycticebus coucang*, *Nycticebus javanicus*, *Nycticebus pygmaeus*, *Nycticebus bengalensis*, *Nycticebus menagensis*, *Nycticebus kayan*, *Nycticebus bancanus* and *Nycticebus borneanus* (Munds et al. 2013; Nekarlis and Starr 2015). *N. bengalensis* has the largest range of any species in the genus because it is found in Myanmar, Cambodia, southern China, northeast India, Laos, Thailand, Vietnam and Bangladesh (Brandon-Jones et al. 2004; Roos et al. 2014).

Most *Nycticebus* classifications were based on morphological data while few molecular studies have been performed (Chen et al. 2006; Cao et al. 2017). Similar morphological characteristics between species make the identification process difficult at the species or subspecies level (Blair et al. 2011). Previous molecular studies were conducted by Chen et al. (2006) and Md-Zain et al. (2009) to study the taxonomy of *Nycticebus*. Using the

mitochondrial gene Cytochrome *b* (Cyt *b*), Md-Zain et al. (2009) showed separation between samples from Peninsular Malaysia and Borneo that were later considered as different species (Roos et al. 2014). In addition, Chen et al. (2006) found that *N. c. coucang* and *N. bengalensis* could not be distinguished, probably due to the limited geographic sampling of this study or misidentification of specimens. While eight species are currently recognized in the genus (Nekarlis & Starr 2015), many more molecular systematic studies need to be carried out to improve our understanding of *Nycticebus* genetic identity and current distribution.

Previously, only *N. coucang* has been described as distributed in the Malay Peninsula, with several subspecies (Brandon-Jones et al. 2004). Recently, Rovie-Ryan et al. (2018) rediscovered *N. c. insularis* in Tioman Island using two mitochondrial loci, the Cyt *b* and D-loop region. The presence of *N. bengalensis* has been hypothesized in the Northern part of the Malay Peninsula; however, no scientific evidence is available to confirm the presence. This paper presents the first-ever molecular scientific study to confirm the presence of *N. bengalensis* in Malaysia using the Cytochrome Oxidase I (COI) mitochondrial region as a candidate locus. The COI gene has been widely used in systematic, population and phylogeography studies in primates (Abdul-Latiff et al. 2017) and other mammals