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Phylogenetic relationships of Malaysian monkeys, Cercopithecidae, based on mitochondrial cytochrome c sequences

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ABSTRACT. Mitochondrial DNA cytochrome c oxidase II (COII) gene sequences of Malaysian Cercopithecidae were examined to ascertain their phylogenetic relationships. Colobinae were represented by the genera *Presbytis*, *Trachypithecus* and *Nasalis*, while the genus *Macaca* represented Cercopithecinae. DNA amplification and sequencing of the COII gene was performed on 16 samples. *Symphalangus syndactylus* (Hylobatidae) was used as the outgroup. Data were analyzed using both character (maximum parsimony) and distance (neighbor-joining) methods. Tree topologies indicated that Colobinae and Cercopithecinae have their own distinct monophyletic clade. This result was well supported by bootstrap values and genetic distances derived from the Kimura-2-parameter algorithm. Separation of *Macaca nemestrina* from *M. fascicularis* was also well supported by bootstrap values. In addition, tree topologies indicate a good resolution of the Colobinae phylogenetic relationships at the intergeneric level, but with low bootstrap support. The position of *Nasalis* remained problematic in both trees. Overall, COII is a good gene candidate for portraying the phylogenetic relationships of Malaysian primates at the inter- and intra-subfamily levels.

Key words: Malaysian primates; COII gene; Molecular phylogeny; Cercopithecinae; Colobinae

INTRODUCTION

Malaysian cercopithecids consist of two groups, the cercopithecine (subfamily Cercopithecinae) and colobine (subfamily Colobinae) (Md-Zain, 2001). Members of these subfamilies live sympatrically but are separated by their life styles, either arboreal or terrestrial, and their diets (Marsh and Wilson, 1981). *Macaca*, the only genus in cercopithecine representing the omnivorous group, includes three species: *Macaca fascicularis*, *M. nemestrina* and *M. arctoides*, whereas the wholly Malaysian vegetarian colobines, known as langurs or leaf monkeys, are represented by three genera: *Presbytis*, *Trachypithecus* and *Nasalis* (Oates et al., 1994). *Trachypithecus* is represented by two species (*T. obscurus* and *T. cristatus*) while *Nasalis* by a single species, *Nasalis larvatus* (Md-Zain et al., 2008). The number of species in the genus *Presbytis* varies according to primatologist lists (Groves, 2001; Brandon-Jones et al., 2004; Md-Zain et al., 2008). *P. hosei*, *P. rubicunda*, and *P. frontata* remain as the valid species. However, various classifications have been made on the *P. melalophos* groups involving *femoralis siamensis*, *robinsoni*, *chrysomelas*, and *cruciger*. Many primatologists still favor the retention of *P. melalophos* as a single polytypic species with numerous subspecies (Oates et al., 1994; Md-Zain, 2001). However, some subspecies have been assigned at the species level (Groves, 2001; Brandon-Jones et al., 2004). The differences in the classification of these species may cause difficulty in conservation efforts.

Cytochrome c oxidase subunit II (COII) is the third largest component of the cytochrome c oxidase complex. The evolution rates of the COII gene in both nucleotide and amino acid sequences are believed to be significantly higher than for COI and COIII (Ramharack and Deeley, 1987). Analysis of sequence data based on the COII gene has been successfully conducted with mammals (Adkins et al., 1996; Shevchuk and Allard, 2001). However, in many phylogenetic relationship studies, other genes of mitochondrial DNA are still favored as gene candidates (Shahrom et al., 2005; Khan et al., 2008; Lim et al., 2010; Md-Zain et al., 2010). In primate evolutionary studies, several COII gene data analyses have focused on hominoids and the New World Monkeys (Ruvolo et al., 1991, 1993). For New World Monkeys, the sequence of the COII gene has been studied on Platyrrhini including both Atelidae and Cebidae families (Ascunce et al., 2002): owl monkeys, genus *Aotus* (Ashley and Vaughn, 1995); spider monkeys, genus *Ateles* (Collins and Dubach, 2000) and marmoset species group (Sena et al., 2002).

For Old World Monkeys, molecular phylogeny studies have been focusing mainly on Africa. For example, the phylogenetic studies of the tribe Papionini, composed of baboons, mandrills, drills, and mangabeys were conducted on the African continent (Ruvolo et al., 1991; Disotell et al., 1992; Harris, 2000). Phylogenetic inference using COII gene in the Asian region remains less pronounced than in the African region. These studies include the macaques