# Rediscovery of *Nycticebus coucang insularis* Robinson, 1917 (Primates: Lorisidae) at Tioman Island and its Mitochondrial Genetic Assessment

### (Penemuan Semula *Nycticebus coucang insularis* Robinson, 1917 (Primate: Lorisidae) di Pulau Tioman dan Penilaian Genetik Mitokondrianya)

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ABSTRACT

*Slow lorises (*Nycticebus*) consist of eight species native to Southeast Asia while three species are recognised in Malaysia -* N. coucang, N. menagensis *and* N. kayan. *This study reports on the rediscovery of the subspecies* N. coucang insularis *Robinson, 1917 in Tioman Island and the genetic assessment of its mitochondrial DNA variation. Morphological measurements conform the specimen as the putative* N. coucang *but with distinct colour and markings. Two mitochondrial DNA segments (cytochrome* b *and control region) were produced from the subspecies representing their first registered sequences in GenBank. Genetically, the subspecies showed 99% of nucleotide similarity to* N. coucang *species type for both the DNA segments and constitute its own unique haplotype. Phylogenetic trees constructed using three methods (neighbour joining, maximum likelihood and Bayesian inference) showed two major groups within* Nycticebus; *the basal group was formed by* N. pygmaeus *while the second group consisted of the remaining* Nycticebus *species. The phylogenetic position of the subspecies, however, remains unresolved due to the observed mixing between* N. coucang *and* N. bengalensis. *Several reasons could lead to this condition including the lack of well documented voucher specimens and the short DNA fragments used. In addition, the possibility of hybridisation event between* N. coucang *and* N. bengalensis *could not be excluded as a possible explanation since both species occur sympatrically at the Isthmus of Kra region until the Thailand-Malaysia border. The rediscovery of this subspecies displays the unique faunal diversity that justifies the importance of Tioman Island as a protected area.*

*Keywords: mtDNA;* Nycticebus coucang insularis; *phylogenetic relationships; rediscovery; subspecies*

ABSTRAK

*Kongkang (*Nycticebus*) terdiri daripada lapan spesies yang berasal dari Asia Tenggara dengan tiga spesies dijumpai di Malaysia -* N. coucang, N. menagensis *dan* N. kayan. *Kajian ini melaporkan penemuan semula subspesies* N. coucang insularis *Robinson, 1917 di Pulau Tioman dan penilaian genetik DNA mitokondrianya. Ukuran morfologi yang diambil pada individu tersebut menunjukkan ia menyerupai spesies putatif* N. coucang *namun terdapat perbezaan warna dan tanda pada badan. Dua segmen DNA mitokondria (sitokrom* b *dan* rantau kawalan) *dihasilkan daripada subspesies tersebut merupakan jujukan DNA pertama yang didaftarkan dalam GenBank. Secara genetik, subspesies ini menunjukkan 99% persamaan nukleotida kepada jenis spesies* N. coucang *pada kedua-dua segmen DNA dan membentuk haplotip tersendiri. Pohon filogeni yang dibina menggunakan tiga kaedah (jiran sambung, kebolehjadian maksimum dan pentakbiran* Bayesian) *mendedahkan dua kumpulan utama dalam* Nycticebus; *kumpulan dasar dibentuk oleh* N. pygmaeus *manakala kumpulan kedua terdiri daripada spesies* Nycticebus *yang lain. Kedudukan filogenetik subspesies ini bagaimanapun masih tidak dapat diuraikan kerana pencampuran yang diperhatikan antara* N. coucang *dan* N. bengalensis. *Beberapa sebab boleh membawa kepada keadaan ini termasuk kekurangan dokumentasi baucer spesimen yang betul dan jujukan DNA pendek yang digunakan dalam analisis. Selain itu, kemungkinan kejadian hibridisasi antara* N. coucang *dan* N. bengalensis *tidak boleh dikecualikan sebagai penjelasan kerana kedua-dua spesies wujud secara simpatri dari kawasan Isthmus of Kra hingga sempadan Thailand-Malaysia. Penemuan semula subspesies ini memaparkan kepelbagaian fauna unik di Pulau Tioman yang menunjukkan kepentingannya sebagai kawasan perlindungan.*

*Kata kunci: Hubungan filogenetik; mtDNA;* Nycticebus coucang insularis; *penemuan semula; subspesies*

## INTRODUCTION

Slow lorises (*Nycticebus* spp.) are small nocturnal primates belonging to the family Lorisidae which has a wide distribution occupying 14 countries in the Southeast Asia (SEA) and adjacent areas (Nekaris & Starr 2015). They are arboreal and can be found in various forest types including primary and secondary forests and even agricultural lands. *Nycticebus* species are heavily exploited in the wildlife trade and are popular as pets (Nekaris & Jaffe 2007). In 2007, the Convention on International Trade in Endangered

Species of Wild Fauna and Flora (CITES) elevated their status to Appendix I prohibiting all international trade of the species, their parts, or their derivatives (Nekaris & Nijman 2007). In Peninsular Malaysia, under the new Wildlife Act 2010 [Act 716], all *Nycticebus* species are totally protected.

Currently, eight species within the genus are recognised; *Nycticebus bancanus*, *N. bengalensis*, *N. borneanus*, *N. coucang*, *N. javanicus*, *N. kayan*, *N. menagensis* and *N. pygmaeus* (Nekaris & Starr 2015; Roos et al. 2014). However, only five species have been assessed by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Figure 1) while the remaining three species from Borneo (*N. bancanus*, *N. kayan* and *N. borneanus*) which were recently described remains not assessed. In Malaysia, at least three species

of *Nycticebus* exist; *N. coucang* in the peninsula, while *N. menagensis* and *N. kayan* are found in Borneo. In Tioman Island, Robinson (1917) recognised a subspecies unique to the island and classified it as *N. coucang insularis*. Medway (1966) reported a specimen from the island in 1958 but since then no observations or collections were made on the subspecies which led to Lim et al. (1999) to speculate that the subspecies has gone extinct.

Recently, during the 2016 Biodiversity Inventory Programme held by the Department of Wildlife and National Parks (PERHILITAN) at Tioman Island, a *Nycticebus* individual was caught which could possibly represent one of the only two available specimens of the subspecies *N. coucang insularis* Robinson, 1917. Thus, this study documents the rediscovery of the subspecies and assess its mitochondrial genetic variation.
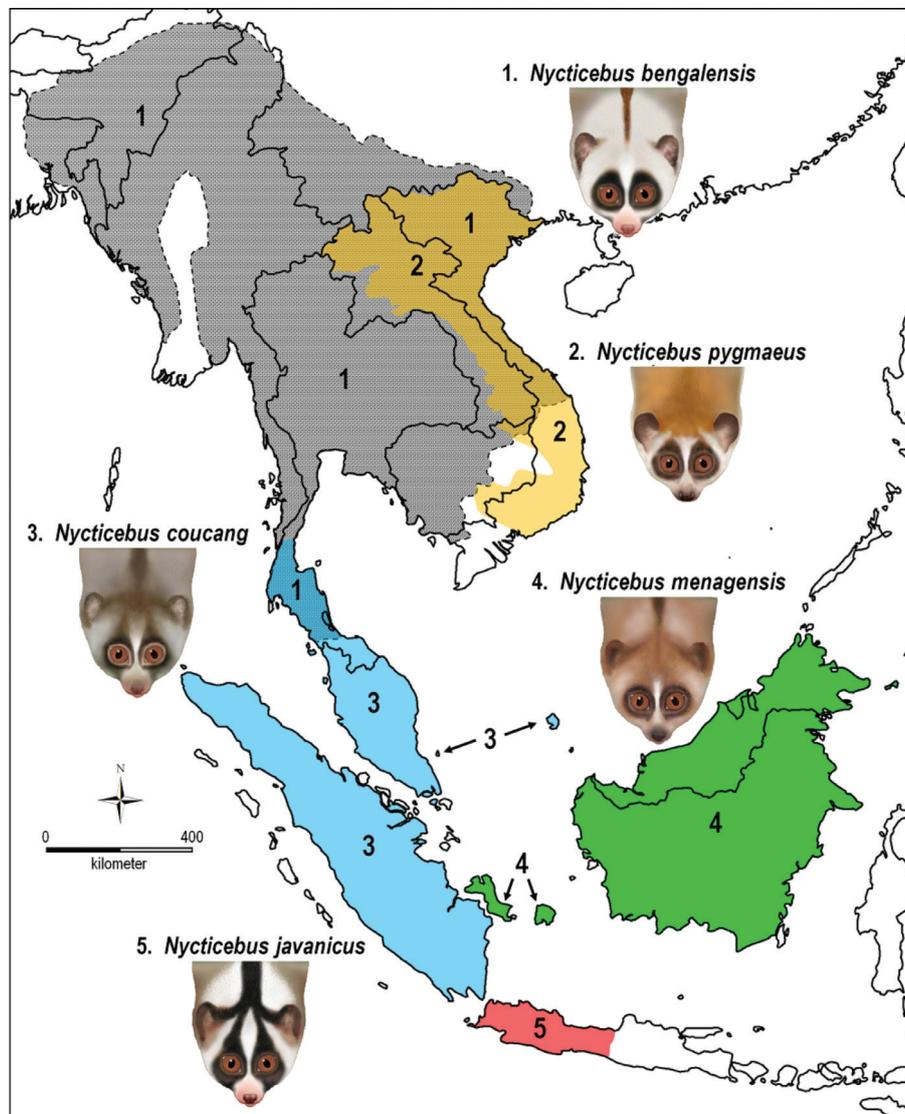


FIGURE 1. Distribution map of five *Nycticebus* species in Southeast Asia as assessed by IUCN (Nekaris & Streicher 2008a, 2008b; Nekaris et al. 2013; Streicher et al. 2008a, 2008b). The remaining three recently recognised species (*N. bancanus*, *N. kayan* and *N. borneanus*) are found in Borneo. Note: *N. bengalensis* occurs sympatrically with *N. pygmaeus* and *N. coucang*. Illustration of *Nycticebus* were taken from Schulze & Groves (2004)

## MATERIALS AND METHODS

### SAMPLE AND MORPHOMETRICS

On 18th April 2016, a male slow loris was caught at Kampung (Kpg.) Sungai Asah, Tioman Island using a scoop net at around 2000 h (Figure 2). Photographs and morphological measurements were taken according to the identification keys provided by Khan (1992) and Payne and Francis (2007) and buccal swabs were taken for DNA analysis. The animal was brought back alive and is currently housed at a captive facility maintained by the PERHILITAN.



FIGURE 2. *Nycticebus coucang insularis* caught at Kpg. Sungai Asah, Tioman Island during the recent 2016 Biodiversity Inventory Programme. Photograph by Tan Cheng Cheng

### MOLECULAR ANALYSIS

Genomic DNA extraction was conducted on the swab samples using the buccal swab protocol provided by the QIAamp® DNA Mini Kit (Qiagen, Germany). PCR amplifications were conducted on two mitochondrial DNA segments: the partial cytochrome *b* gene (cyt *b*) using the universal primer pair of CYTB1/CYTB2 (Kocher et al. 1989); and the control region segment (CR) using the primer pair L15996/H16498 (Chen et al. 2006). Amplifications on both the mtDNA segments were conducted using the PCR mixtures described in Rovie-Ryan et al. (2014) and the thermal profiles as the following; initial denaturation at 95 ℃ for 1 min and then 30 cycles each consisting of denaturation at 94 ℃ for 1 min, annealing at 50 ℃ for 1 min and extending at 72 ℃ for 1 min, followed by a finally extension at 72 ℃ for 3 min. Cycle sequencing was carried out on the PCR products for both directions using the forward and reverse PCR primers on the ABI 3130*xl* DNA Analyzer (Applied Biosystems, USA) as provided by a service provider (1st Base Laboratories Sdn. Bhd., Selangor, Malaysia).

### SEQUENCE AND PHYLOGENETIC ANALYSES

The DNA sequences obtained were examined using the software Geneious v5.6 (Drummond et al. 2012) and BLAST analysis was conducted to check for sequence similarity (Altschul et al. 1990). The sequences obtained from the individual were registered with GenBank with the accession numbers KY680654 and KY680655 for cyt *b* and CR segments, respectively. Other available sequences of *Nycticebus* from GenBank were downloaded for further analyses as listed in Appendix 1. Sequence characterisations including variable sites (VS), conserved sites (CS) and parsimony-informative sites (PIS) were done by using MEGA v7 (Kumar et al. 2016). Standard molecular diversity indices including number of haplotypes (NHap), haplotype diversity (*Hd*) (Nei 1987) and nucleotide diversity (π) (Nei 1987) were calculated by using DnaSP v5 (Librado et al. 2009). In MEGA v7, genetic distances were calculated by using the Kimura two-parameter model (Kimura 1980).

To clarify the phylogenetic position of the subspecies within the genus *Nycticebus*, phylogenetic analyses were conducted on both the mtDNA segments separately using the neighbour-joining (NJ), maximum likelihood (ML) and the Bayesian inference (BI). The NJ and ML analyses were conducted in MEGA v7 with 2,000 bootstrap replicates (Felsenstein 1985). For both the mtDNA segments NJ analysis was performed by using the Kimura 2-Parameter distance model (Kimura 1980) with pairwise deletion option. For ML analysis, the trees were constructed using the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985) and Tamura 3-parameter model (Tamura 1992) for the cyt *b* and CR segment, respectively, as calculated in MEGA v7. BI was conducted by using the software BEAST v2.0 (Bouckaert et al. 2014). In BEAST v2.0, the following settings were enforced; four independent runs each with 50 million MCMC generations and sub-sampled every 50,000 generations, HKY85 substitution model with five gamma category counts (Hasegawa et al. 1985), Relaxed Clock Log Normal and Birth Death Model (Stadler 2010). Gray Slender Loris (*Loris lydekkerianus*), Red Slender Loris (*Loris tardigradus*) and West African Potto (*Perodicticus potto*) were used as the outgroup species in phylogenetic analyses following Somura et al. (2012) as these species are representatives from the major branches within the family Lorisidae.

## RESULTS AND DISCUSSION

Morphological measurements taken on the individual (total length = 279 mm, head body = 263 mm, tail = 16 mm, ear = 18 mm, hind foot = 45 mm and weight = 500 g) conform to the putative *N. coucang* with distinct colours and markings. Based on the facial colouration and markings, the individual showed close resemblance to one of the illustrations by Schulze and Groves (2004) whom described several types of variations occurring in *N. coucang* (Figure 3). This valuable specimen represent the only second available specimen of the subspecies *N. coucang insularis* after Medway (1966) caught the first type specimen from Kpg. Mukut, Tioman Island in 1958. Groves and Maryanto (2008) worked on the craniometrics
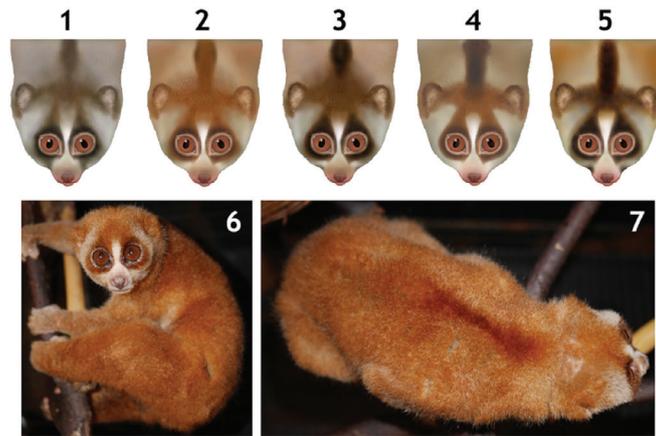
FIGURE 3. Colour and marking variation of *N. coucang*. 1-5 show variation of facial colourations and markings based on Schulze and Groves (2004). 6-7 (photographed by Cheng T.C.) represent the specimen caught at Tioman Island showing close resemblance to illustration 2

of the first type specimen and compared it to several *Nycticebus* skull specimens from the insular region of SEA and suggested that it belongs to the *N. coucang* type from Peninsular Malaysia and Sumatra although they noted that it falls at the edge of the range. Therefore, the rediscovery of this individual from Tioman Island marked an important finding after the gap of almost 60 years between the first and current specimens that can add more information and knowledge on the subspecies. Currently, the individual is kept at a captive facility maintained by the PERHILITAN and monitored for behaviour study.

DNA sequences with the length of 402 base pairs (bp) and 380 bp were obtained from the cyt *b* and CR segments, respectively. Both the DNA sequences represent the first registered sequences of the subspecies in the GenBank database. BLAST result showed 99% of nucleotide similarity to the *N. coucang* species type for both the DNA sequences. Three transitional nucleotides were observed from both the mtDNA segments that distinguished the subspecies *insularis* from the other *N. coucang* forms (Table 1). Within *N. coucang*, eight haplotypes of *N. coucang* were observed for the cyt *b* segment (*n* = 10) while six were observed for the CR segment (*n* = 7). For both segments, the subspecies *insularis* constituted its own unique haplotype. The sequence characteristics (CS, VS and PIS) and diversity indices (NHap, *Hd* and π) within *Nycticebus* are shown in Table 2. For both DNA segments, *N. coucang* demonstrated the highest nucleotide diversity (π) compared to the other species. Table 3 summarises the genetic distances among the haplotypes of *N. coucang* for both the mtDNA segments. The *insularis* subspecies differed from the other *N. coucang* haplotypes by 0.8-8.3% for the cyt *b* and 0.5-10.3% for the CR segment.

Phylogenetic trees constructed using the NJ, ML, and BI on cyt *b* and CR segments produced contrasting topologies. The cyt *b* segment produced a poorly resolved topology (data not shown) by which an admixture between the species *N. bengalensis, N. coucang, N. javanicus* and *N.*

*menagensis* was observed, thus, it was not used for further discussion. The CR segment on the other hand provided a well resolved topology to clarify the relationships within the *Nycticebus*. The NJ, ML and BI trees based on CR segment had similar topologies, thus, the phylogeny was summarised using the NJ tree (Figure 4). In general, two major groups were observed. The basal group was formed by *N. pygmaeus* that diverged earlier from the other *Nycticebus* species, corresponding to previous studies (Chen et al. 2006, 2004; Somura et al. 2012). The second group consisted of the remaining *Nycticebus* species (*N. bengalensis, N. coucang, N. javanicus* and *N. menagensis*) which were previously ranked at subspecies level within *N. coucang*.

Within the second group, *N. javanicus* and *N. menagensis* formed their own clades with strong support (>90% of bootstrap values). However, mixing between *N. bengalensis* and *N. coucang* were observed. Specifically, a single sequence of *N. coucang* from GenBank (AY875955; Figure 4) was clustered within the *N. bengalensis* species group. Further investigation showed that all available *N. coucang* sequences from GenBank (AY875955 - AY875957, AY687887 - AY687888 and GQ449388) were obtained from either confiscated specimens or zoo animals thus raising doubt on the exact origin of the specimens. Therefore, due to the unknown exact locality of the specimens, we believe that the previous phylogenetic relationships constructed on *Nycticebus* (Chen et al. 2006, 2004) were erroneous, especially regarding the relationship between *N. coucang* and *N. bengalensis*. Evidently, this study points out the importance of using well documented voucher specimens or reference samples in phylogenetic studies.

In this study, we attempted to clarify the phylogenetic positioning of the subspecies *N. c. insularis* within *Nycticebus*. However, due to the mixing between *N. coucang* and *N. bengalensis*, the phylogenetic position of the subspecies however remains unresolved. Several reasons could lead to this condition including the doubtful

TABLE 1. Variable sites observed within the *N. coucang* for the cytochrome *b* and control region segment. Three transitional mutations were observed as indicated by the red and italic fonts

**Segment: Cytochrome *b***

| Haplotypes | Nucleotide position | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 21 | 24 | 28 | 29 | 34 | 45 | 46 | 50 | 63 | 66 | 68 | 81 | 84 | 89 | 90 | 95 | 102 | 115 | 135 | 136 | 137 | 138 | 145 | 150 | 167 | 178 | 180 | 196 | 219 | 234 | 236 | 241 | 246 | 261 | 270 | 278 | 285 |
| Hap 1 (AY687889, AY687890) | T | T | C | C | A | A | T | C | T | C | A | A | T | T | C | C | T | G | C | C | A | T | T | A | C | C | C | C | T | T | T | C | T | T | T | A | T |
| Hap 2 (KP410591, KP410592) | C | . | . | T | T | G | G | C | T | C | . | . | T | G | . | . | T | . | . | . | C | . | . | C | . | . | . | . | . | . | . | . | . | . | . | A | T |
| Hap 3 (KP410655) | C | . | . | T | T | G | G | C | T | C | . | . | T | G | . | . | T | . | . | . | C | . | . | C | . | . | . | . | . | . | . | . | . | . | . | A | T |
| Hap 4 (NCU53580) | . | . | C | T | . | . | . | . | . | . | . | . | G | . | . | . | T | . | . | . | A | . | C | . | . | G | . | . | . | . | T | . | . | . | . | A | A |
| Hap 5 (AY878362) | . | . | C | T | . | . | . | . | . | . | . | . | G | . | . | . | T | . | . | . | A | . | C | . | . | G | . | . | . | . | T | . | . | . | . | A | A |
| Hap 6 (AY878363) | . | . | C | T | T | . | . | . | . | T | . | . | G | . | . | . | T | . | . | . | C | . | . | . | . | . | . | . | G | . | T | . | . | . | . | A | A |
| Hap 7 (AY878364) | . | C | C | . | T | . | . | . | . | . | . | . | G | A | T | A | C | T | . | A | C | C | . | C | . | . | T | . | . | . | T | . | . | G | . | A | T |
| Hap 8 (*N. c. insularis*) | . | C | T | T | . | . | . | . | . | T | . | . | G | C | T | . | C | . | . | . | C | . | . | C | . | . | T | . | . | . | T | . | . | *G* | . | A | A |

**Segment: Control region**

| Haplotypes | Nucleotide position | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | 34 | 45 | 47 | 80 | 102 | 104 | 145 | 157 | 184 | 196 | 245 | 268 | 289 | 300 | 304 | 389 | 395 | 189 | 197 | 200 | 204 | 216 | 247 | 245 | 257 | 270 | 278 | 280 | 287 | 340 |
| Hap 1 (GQ449388) | A | C | A | C | A | T | T | A | T | T | C | C | A | A | T | C | T | C | A | G | G | A | G | T | C | G | T | C | C | G | A |
| Hap 2 (AY687887, AY687888) | T | G | C | A | C | C | A | T | G | . | . | . | A | A | A | T | A | . | . | A | A | T | A | . | . | G | C | C | T | T | . |
| Hap 3 (AY875955) | . | . | . | . | . | T | . | . | C | . | . | C | T | C | G | . | C | T | C | G | A | G | A | C | T | C | . | A | . | T | . |
| Hap 4 (AY875956) | . | . | . | . | . | T | . | C | C | . | . | C | T | C | . | C | . | . | C | T | A | A | A | C | T | T | C | . | C | C | . |
| Hap 5 (AY875957) | . | . | . | . | . | . | . | . | . | . | . | . | T | G | . | A | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . |
| Hap 6 (*N. c. insularis*) | . | . | . | T | . | C | C | . | C | . | . | C | T | *G* | . | C | T | C | . | A | C | T | T | C | . | G | . | G | . | . | . |

TABLE 2. Sequence characteristics (conserved sites, variable sites and parsimony informative sites) and diversity indices (number of haplotypes, haplotype diversity and nucleotide diversity) for *Nycticebus* species used in this study

| Genus/ Species | Cytochrome *b* | | | | | | | Control region | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | CS | VS | PIS | Nhap | *Hd* | $\pi$ | N | CS | VS | PIS | Nhap | *Hd* | $\pi$ |
| *Nycticebus bengalensis* | 10 | 388 | 14 | 7 | 7 | 0.93 | 0.012 | 10 | 361 | 18 | 0 | 10 | 1.00 | 0.014 |
| *N. coucang* | 10 | 353 | 49 | 38 | 8 | 0.96 | 0.050 | 7 | 333 | 50 | 42 | 6 | 0.95 | 0.062 |
| *N. javanicus* | 5 | 370 | 32 | 0 | 3 | 0.70 | 0.032 | 2 | 377 | 4 | 0 | 2 | 1.00 | 0.011 |
| *N. menagensis* | 4 | 389 | 13 | 0 | 3 | 0.83 | 0.016 | 6 | 372 | 9 | 3 | 4 | 0.80 | 0.010 |
| *N. pygmaeus* | 17 | 391 | 11 | 3 | 8 | 0.73 | 0.005 | 23 | 368 | 14 | 2 | 13 | 0.88 | 0.005 |
| Total *Nycticebus* | 46 | 314 | 88 | 77 | 25 | 0.95 | 0.076 | 48 | 297 | 86 | 70 | 34 | 0.97 | 0.064 |

TABLE 3. Genetic distances calculated among the *N. coucang* haplotypes for the cytochrome *b* (below the diagonal) and control region (above the diagonal, italics) segments of mtDNA

| Haplotype No. | Hap1 | Hap 2 | Hap 3 | Hap 4 | Hap 5 | Hap 6* | Hap7 |
|---|---|---|---|---|---|---|---|
| Hap1 | | *9.1* | *7.3* | *6.5* | *1.1* | *7.1* | |
| Hap2 | 8.9 | | *10.3* | *8.5* | *8.8* | *9.1* | |
| Hap3 | 9.2 | 0.2 | | *3.6* | *7.4* | *3.6* | |
| Hap4 | 4.6 | 7.1 | 7.4 | | *5.3* | *0.5* | |
| Hap5 | 4.7 | 6.9 | 7.2 | 0.8 | | *5.9* | |
| Hap6 | 4.4 | 7.5 | 7.8 | 1.8 | 1.5 | | |
| Hap7 | 5.5 | 8.4 | 8.6 | 3.6 | 3.3 | 2.8 | |
| Hap8* | 4.9 | 8.0 | 8.3 | 2.3 | 2.0 | 0.5 | 3.3 |

* subspecies *insularis*

origin of *N. coucang* specimens available from GenBank as described above. Additionally, the short DNA fragments used in this study hindered the discovery of more genetic variation (only two and one variable site observed in the cyt *b* and CR segments, respectively) that could possibly distinguish the different species and subspecies. Nevertheless, the possibility of hybridisation event to occur between *N. coucang* and *N. bengalensis* could not be excluded as a possible explanation of the mixing of the species since both occur sympatrically at the Isthmus of Kra region until the Thailand-Malaysia border (see Figure 1). Therefore, to clarify this matter, efforts are underway to sequence individuals especially from *N. coucang* of known locality from Peninsular Malaysia.

In summary, the rediscovery of this subspecies marked a significant finding and further supports the unique faunal diversity of Tioman Island. This subspecies warrants a significant conservation status and should be managed and monitored as a separate management unit (Moritz 1994). This finding also contributed considerably to the diversity of *Nycticebus* in Malaysia generally which recently have seen at least three new species and possibly a new subspecies being discovered. Specifically, the

accumulating discoveries of unique and endemic faunal species in Tioman Island (Chen & Tan 2005; Lim et al. 1999; Onn et al. 2011; Yan et al. 2014; Yeo & Ng 1999) justifies the importance of Tioman Island as a wildlife reserve. Management authorities should look into the possibility of establishing this protected area as a national park. Currently, further efforts are ongoing to measure the genetic diversity of this subspecies using complete mitochondrial genome.
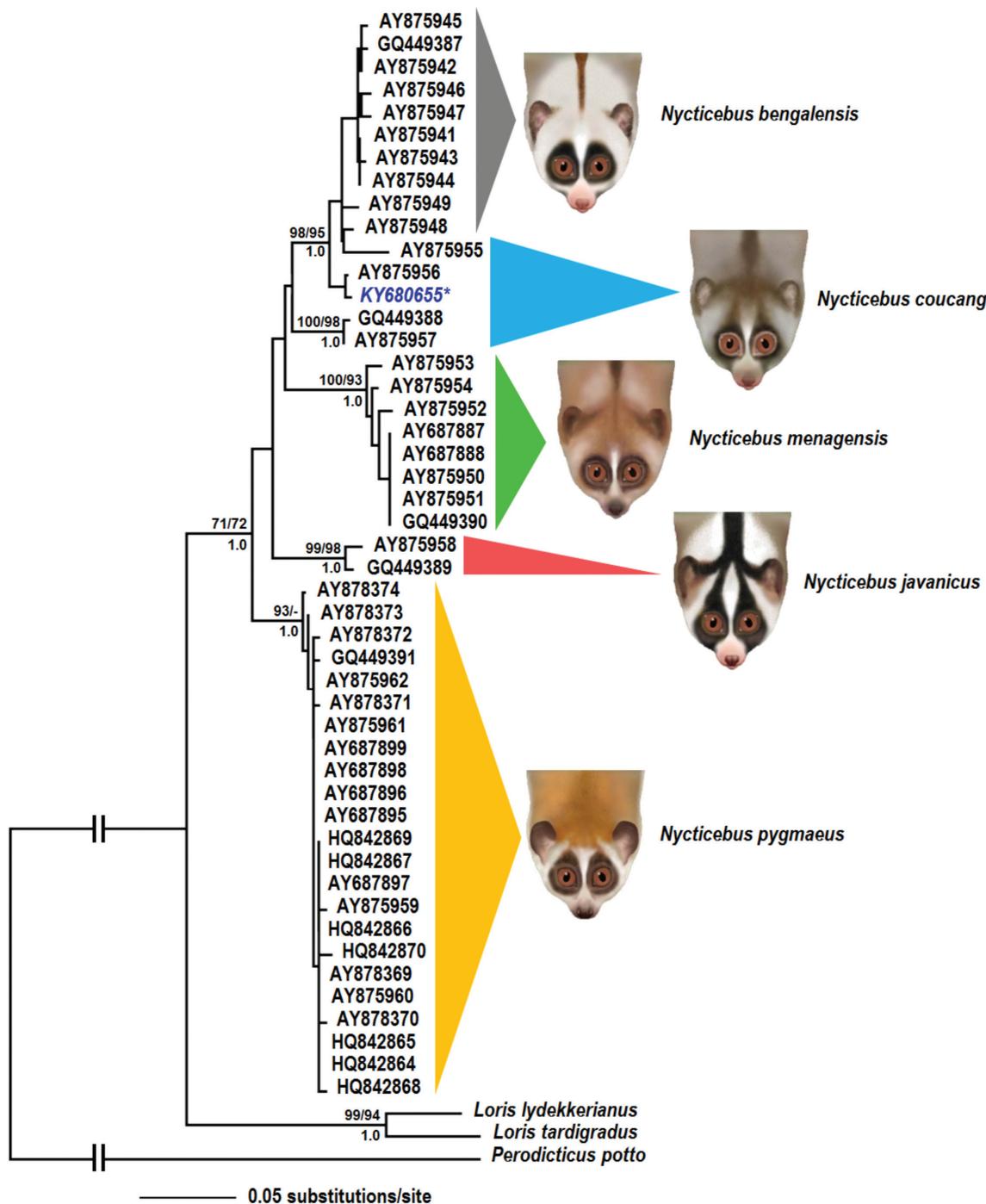
FIGURE 4. Phylogenetic relationships (NJ tree) of the *Nycticebus* species constructed using the control region segment.
Values above the branches represent the bootstrap estimates for NJ and ML, respectively (only values above 50% are shown).
Values below the branches represent BI posterior probability

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APPENDIX 1. List of GenBank sequences used in this study

| No. | Genus/ Species | GenBank Acc No. | |
| --- | --- | --- | --- |
| | | Cyt *b* | CR |
| 1. | *Nycticebus bengalensis* | AY441477 | AY875941 |
| | | AY878360 | AY875942 |
| | | KP410608 | AY875943 |
| | | KP410618 | AY875944 |
| | | KP410627 | AY875945 |
| | | KP410638 | AY875946 |
| | | KP410649 | AY875947 |
| | | KP410652 | AY875948 |
| | | KP410662 | AY875949 |
| | | KP410666 | GQ449387 |
| 2. | *N. coucang* | AY687889 [Hap1] | AY687887 [Hap1] |
| | | AY687890 [Hap1] | AY687888 [Hap1] |
| | | KP410591 [Hap1] | GQ449388 [Hap2] |
| | | KP410592 [Hap1] | AY875955 [Hap3] |
| | | KP410655 [Hap1] | AY875956 [Hap4] |
| | | NCU53580 [Hap1] | AY875957 [Hap5] |
| | | AY878362 [Hap1] | |
| | | AY878363 [Hap1] | |
| | | AY878364 [Hap1] | |
| 3. | *N. javanicus* | AY878365 | AY875958 |
| | | KP410601 | GQ449389 |
| | | KP410612 | |
| | | KP410654 | |
| | | KP410658 | |
| 4. | *N. menagensis* | AY878361 | AY875950 |
| | | KP410604 | AY875951 |
| | | KP410617 | AY875952 |
| | | KP410632 | AY875953 |
| | | | AY875954 |
| | | | GQ449390 |
| 5. | *N. pygmaeus* | AY441476 | AY687895 |
| | | AY687891 | AY687896 |
| | | AY687892 | AY687897 |
| | | AY687893 | AY687898 |
| | | AY687894 | AY687899 |
| | | AY687900 | AY875959 |
| | | AY878366 | AY875960 |
| | | AY878367 | AY875961 |
| | | AY878368 | AY875962 |
| | | KP410590 | AY878369 |
| | | KP410606 | AY878370 |
| | | KP410607 | AY878371 |
| | | KP410614 | AY878372 |
| | | KP410624 | AY878373 |
| | | KP410626 | AY878374 |
| | | KP410648 | GQ449391 |
| | | KP410664 | HQ842864 |
| | | | HQ842865 |
| | | | HQ842866 |
| | | | HQ842867 |
| | | | HQ842868 |
| | | | HQ842869 |
| | | | HQ842870 |
| 6. | *Loris lydekkerianus* | KC757402 | KC757402 |
| 7. | *L. tardigradus* | AB371094 | AB371094 |
| 8. | *Perodicticus potto* | AB371095 | AB371095 |