







The first complete mitochondrial genome data of Geoffroy's rousette, *Rousettus amplexicaudatus* originating from Malaysia

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ABSTRACT

The increasing interest in understanding the evolutionary relationship between members of the Pteropodidae family has been greatly aided by genomic data from the Old World fruit bats. Here we present the complete mitogenome of Geoffroy's rousette, *Rousettus amplexicaudatus* found in Peninsular Malaysia. The mitogenome constructed is 16,511bp in length containing 37 genes; 13 protein-coding genes (PCGs), 22 tRNA genes, two rRNA genes, and a D-loop region. The overall base composition is estimated to be 32.28% for A, 25.64% for T, 14.09% for G and 27.98% for C, indicating a slightly AT rich feature (57.93%). A phylogenetic and BLASTn analysis against other available mitogenomes showed Malaysian *R. amplexicaudatus* matched 98% similarity to the same species in Cambodia and Vietnam. However, it differed considerably (92.53% similarity) with the same species in the Philippines. This suggests flexibility in *Rousettus* sp. with regards to adapting to mesic and dry habitats, ability for long-distance dispersal and remarkably precise lingual echolocation thus supporting its wide-range distribution and colonization. Further taxonomical and mitogenomic comparatives are required in resolving the evolutionary relationship between *Rousettus* spp.

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In tropical and subtropical regions of the Old World, fruit bats are an important seed dispersal agents that provide vital ecological services (Chan et al. 2020). Amidst fruit bats, the echolocating bats of the genus *Rousettus* in the Pteropodidae family are widely distributed from Asia to Africa (Hassanin et al. 2020). There are ten *Rousettus* sp. reported worldwide and six of these species occurring within Southeast Asia (Francis 2019). *Rousettus* spp. are selected as the best candidate amongst the Pteropodidae family to study evolutionary relationships as it is the only genus that has the capability of long-distance dispersal and has showed remarkable ecological flexibility in characteristics such as a well-developed echolocation system (Almeida et al. 2016; Stribna et al. 2019; Hassanin et al. 2020). However, lack of genomic data of Asian *Rousettus* sp. could hinder the extensive study of the genus. In this study, we sequenced and provided the whole mitochondrial genome of *R. amplexicaudatus*, which is the first mitogenome of *Rousettus* species from Malaysia.

Muscle tissue was obtained from Gading Forest Reserve, Selangor, Malaysia (Latitude: 3° 21' 0" N Longitude: 101° 15' 0" E) (Munian et al. 2020). The specimen voucher number MZF1072 was deposited in the Zoological Collection of Forest Research Institute Malaysia (FRIM). The methodologies used for DNA isolation, library construction, read assembly, and gene annotation are described in (Mohd

Salleh et al. 2017; Jahari et al. 2020). The mitogenome of *R. amplexicaudatus* from this study (MT259592) is a circular molecule with 16,511 bp in length. Similar to other volant mammals, it contained 13 protein-coding genes (PCGs), 22 transfer RNA genes, 2 ribosomal RNA genes and 1 D-loop region (Yoon et al. 2016; Hassanin et al. 2020). The overall base composition of *R. amplexicaudatus* is estimated to be 32.28% for A, 25.64% for T, 14.09% for G and 27.98% for C, indicating a slightly AT rich feature (57.93%). The total length of the protein-coding gene sequences (PCGs) is 11,405 bp. The total length of the 22 tRNA genes is 1509 bp, ranging from 57 bp (tRNA^{Ser}) to 74 bp (tRNA^{Leu}). The 12S rRNA gene length is 969 bp and the 16S rRNA gene length is 1571 bp, and are located between the tRNA^{Phe} and tRNA^{Leu}, and are separated by the tRNA^{Val} gene. The control region is located between tRNA^{Pro} and tRNA^{Phe} genes. The genes mostly located on the heavy (H) strand except for NAD6 and eight tRNAs genes (tRNA^{Gln}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys}, tRNA^{Tyr}, tRNA^{Ser}, tRNA^{Glu}, tRNA^{Pro}), which were found to be located on the lower (L) strand.

The Malaysian *R. amplexicaudatus* mitogenome shows 98% similarity to the same species from Cambodia (MN816352.1) and Vietnam (MN816353.1) (Hassanin et al. 2020) available in Genbank. Interestingly, it diverged by nearly 8% (92.53% match) for the same species originating from the Philippines

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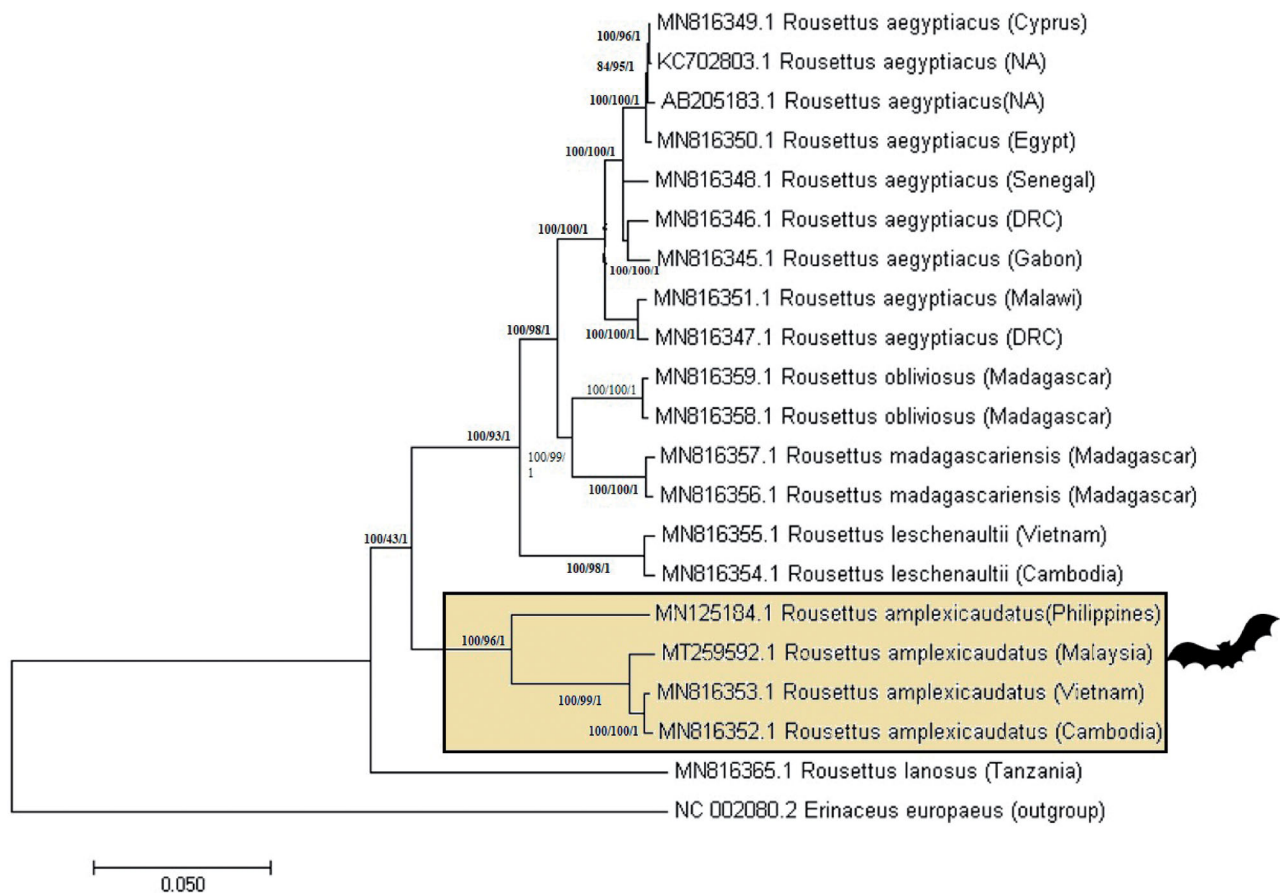


Figure 1. Phylogenetic tree constructed using the whole mitogenome of Malaysian *R. amplexicaudatus* (MT259592) and other *Roussettus* sp. from different countries. The tree was generated from NJ/ML/Bayesian method using hedgehog as an outgroup. Bootstrap values generated from 1000 replicates for NJ/ML/Bayesian analysis. The number at each node indicated the bootstrap probability of NJ/ML/Bayesian analysis. (NA: not available; DRC: Democratic Republic of the Congo).

(MN125184.1) (Mendoza & Fontanilla 2019). In Figure 1, the phylogenetic analysis of this relationship of the *Roussettus* sp. mitogenomes is depicted. The phylogenetic tree generated a monophyletic clade of *R. amplexicaudatus* from Malaysia, Cambodia and Vietnam meanwhile; Philippines *R. amplexicaudatus* formed another branch. Unlike other fruit bats, *Roussettus* sp. are reported to be more adaptive to certain biogeographic barriers. Their flexibility to adapting in mesic and dry habitats, ability for long-distance dispersal and remarkably precise lingual echolocation support the species wide-range distribution and colonization (Happold & Happold 2013; Stribna et al. 2019; Hassanin et al. 2020). Therefore, further taxonomical and mitogenome comparative studies are essential to resolve the evolutionary relationships of this widely distributed *Roussettus* genus especially within Southeast Asia region.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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Data availability statement

The data that support the findings of this study are openly available in the National Center for Biotechnology Information (NCBI) at <https://www.ncbi.nlm.nih.gov>, accession number MT259592.

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