BIOEDUSCIENCE
ISSN: 2614-1558
http://journal.uhamka.ac.id/index.php/bioeduscience

Molecular Characteristics of Geoffroy's Rousette *Rousettus amplexicaudatus* Based on Cytochrome C Oxidase Subunit I and Cytochrome b Genes

Santi Nurul Kamilah [1*], Jarulis [1], Aida Fitriani Sitompul [2], Mifta Mardiah [1], Mira Ervinda [1], Fanni Khoirillah [1], Fitri Dwi Lestari [1], Dinda Zulkani [1]

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Jl. WR. Supratman, Kandang Limun, Bengkulu, Indonesia, 38371
[2] Departement of Biology Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Jl. Williem Iskandar, Psr. V Medan Estate, Medan, Indonesia, 20221
* Correspondence: santi.nurul.kamilah@unib.ac.id

Abstract

Background: *Rousettus amplexicaudatus* is widely distributed across Indonesia, including the Suruman Cave in South Bengkulu. Due to similarities in morphology within the Rousettus group, identification can be challenging. We conducted a molecular analysis using COI and Cytochrome b genes from mitochondrial DNA to explore its genetic traits. DNA was extracted from the blood tissue of seven individuals from the Suruman Cave population, and gene amplification was performed with 20 bp primers. Sequence data were analyzed using MEGA XI software. **Results:** As a result, characteristics of the COI gene, which is 897 bp in length, were characterized by a high frequency of base pairs Adenine-Thymine (55.5%) and Guanine-Cytosine (44.5%), with the majority of the DNA sequence exhibiting a high degree of conservation sites (97.8%). The average intrapopulation genetic distance based on the COI gene was 0.77%, with four specific sites for *R. amplexicaudatus* Suruman Cave. The *Cytochrome* b gene, which is 635 bp long, is characterized by Adenine-Thymine base pairs of 53.7% and Guanine-Cytosine of 46.3%. Cytochrome b is more conserved than the COI (99.1%). The average intrapopulation genetic distance based on the *Cytochrome* b gene is 0.3% and has no population-specific sites. **Conclusions:** Both sequences showed a consistent pattern in phylogenetic tree analysis, which suggests the Suruman population is the group of *R. amplexicaudatus*. Therefore, these sequences can be proposed as molecular markers for *R. amplexicaudatus*, particularly when compared to the whole sequences of the COI and cytochrome b.

Keywords: Chiroptera, Cytochrome b, Cytochrome Oxidase subunit I (COI), Pteropodidae, *Rousettus amplexicaudatus*

Article history
Received: 12 Jun 2024
Accepted: 04 Nov 2024
Published: 31 Mar 2025

Publisher's Note:
BIOEDUSCIENCE stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Citation:
Kamilah et al. 2025. Molecular Characteristics of Geoffroy's Rousette *Rousettus amplexicaudatus* Based on Cytochrome C Oxidase Subunit I and Cytochrome b Genes. BIOEDUSCIENCE, 9(1), 36-48. doi: 10.22263/jbes/15226

Introduction

Bats play a vital role in the ecosystem, serving as insect predators, pollinators, and seed dispersers. As insectivorous bats control insect populations, nectarivorous bats pollinate various plants, and frugivorous bats disperse plant seeds. These organisms are essential in maintaining ecological equilibrium (Ramírez-Fráncel et al., 2022). Bat droppings or guano in caves contain nitrogen and phosphate and can be used as an alternative fertilizer (Afa, 2016; Lukman, 2022; Tangguda et al., 2022). However, the community has not widely recognized the importance of bats (Medellin et al., 2017; Kemp et al., 2019). The nectarivorous and frugivorous bats are often misunderstood as destroying crops of high

economic value, leading to the destruction of fruit yields. Most bat species are classified as unprotected in the Indonesian Legislation (KLHK-LIPI, 2019) and as Data Deficient and Least Concern species on the IUCN Red List of Threatened Species. Some species are reported to be in decline, while others have been declared extinct (IUCN, 2021). Among the principal causes of bat population decline is habitat loss or destruction (Laurindo et al., 2019; Frick et al., 2020).

Bat identification is typically conducted through the examination of visible morphological characteristics. A variety of traits may serve as distinguishing features between species. These include facial shape, ears, tail morphology, body color, body weight, and body morphometry such as upper arm, head length, tail length, and total length (Ceballos, 2014; Juste et al., 2018; Thanh et al., 2019). Some species exhibit characteristics that facilitate their identification. Other species demonstrate a high degree of morphological similarity, particularly species from the same genera (Ceballos, 2014). For example, species of the genus *Rousettus* display considerable morphological similarity (Waldien et al., 2019).

Mitochondrial DNA (mtDNA) comprises circular genes with heavy and light strands of DNA and encodes 13 protein-encoding genes, 22 tRNA genes, two rRNA genes, and a control region (Yue et al., 2019; Jahari et al., 2020; Zhang et al., 2021; Luo et al., 2022). In contrast to nuclear DNA, mitochondrial DNA (mtDNA) exhibits several distinctive characteristics. For instance, it comprises numerous identical copies, is inherited uniparentally (from the female parent), does not undergo recombination, has a relatively small number of genomes, and exhibits a high evolutionary rate compared to nuclear DNA. A notable feature is that a proportion of the genes are more highly conserved than others. The COI gene (Cytochrome Oxidase Subunit I) provides a case in point. Consequently, mitochondrial DNA is an optimal choice for molecular taxonomic studies, analysis of genetic diversity, and evolutionary rates (Ladoukakis & Zouros, 2017).

One of the advantages of COI genes is that they are stable. In the mammal group, this gene is stable with minimal deletions, insertions, and substitutions. However, its characteristics can distinguish taxon levels, thus representing a valuable tool for studying the genetic characteristics of a species or population. Furthermore, it can be employed as a species DNA barcode (Hebert et al., 2003). Meanwhile, the Cytochrome b gene tends to have a higher mutation rate than the COI gene, making it suitable for studying the genetic diversity in populations (Wright et al., 2018; Park et al., 2019).

The process of confirming bat species using molecular techniques has been initiated for numerous bat species, with the genus *Rousettus* as a notable example. A total of ten species grouped into the genus *Rousettus* have been identified (Francis, 2019), and six of them can be found in Indonesia (Maryanto et al., 2019). Since they are very similar morphologically, the identification process often goes wrong. Some species occur sympatrically (Lengkong et al., 2016). However, morphological misidentifications in many species have been successfully reconfirmed through molecular research then placed them in the correct taxonomic status (Juste et al., 2018; Srinivasulu et al., 2019; Ancillotto et al., 2020; Calahorra-Oliart et al., 2021; Mulvaney et al., 2023). Huang et al. (2014) and Tu et al. (2017) have conducted molecular analysis on another group of cryptic species, *Tylonycteris pachypus,* and *T. robusta*. The results of this study indicated that both consist of several different species.

Our preliminary findings confirm the presence of *R. amplexicaudatus* in Suruman Cave, South Bengkulu, Indonesia. Suruman Cave is a large cave with an entrance at a height of over 10 meters and a length of more than 3 kilometers. The cave is mainly exposed to lightless areas, except the entrance cave. *R. amplexicaudatus* was discovered exclusively in the lightlessness of the cave, about 300–350 meters from the cave entrance. They are typically associated with the *Eonycteris spelaea* colony. The area surrounding the cave entrance still receives dim sunlight and is commonly inhabited by other species from the *Hipposideros* group (Kamilah et al., 2019). This species is usually found in large colonies in the cave. The density of individuals per square meter can exceed more than

400 (Carpenter et al., 2014). In the present study, we examined the characteristics of specific partial segments of the COI and Cytochrome b genes of mitochondrial DNA in *R. amplexicaudatus* from Suruman Cave. Similar studies have been conducted by other researchers on *R. amplexicaudatus*, as exemplified by (Luczon et al., 2019; Stribna et al., 2019; Hassanin et al., 2020). Furthermore, some DNA sequences of the COI and cytochrome b genes are also available in GenBank in the form of whole sequences and partial genes. However, most of the current available partial genes are positioned in the middle of the gene. In this study, we have provided and analyzed the sequence of COI and Cytochrome b from the initial region of the genes. Thus, this research has facilitated the expansion of molecular data related to bats, with a particular focus on *R. amplexicaudatus*. This data may be utilized for taxonomy, ecology, and conservation analyses.

Methods

Collecting Sample

The research plan underwent an ethical review by the Research Ethics Commission of LPPM Bengkulu University, which determined that the research met the scientific standards and ethical guidelines (ethical acceptable No. 13 /KER-LPPM/EC/2023). Hand nets were used to capture the bats in the Suruman cave. Personal protective equipment such as safety gloves and masks were used during animal capturing, handling, and sample collection to reduce the potential risk of pathogen transmission. Species identification is based on the analysis of morphological characteristics, such as facial structure, wing shape, body dimensions (forearm length, body length, tail length, ear length), and other physical traits. Species identification has followed the descriptions outlined in the mammal guidebooks developed by Payne et al. (2000) and (Wilson & Mittermeier, 2019). Blood was collected about 0.1-0.5 ml from the seven individuals of *R. amplexicaudatus*, using the disposable syringe with a needle gently through the interfemoral veins of the bat and preserved in the Eppendorf tube with EDTA at -20 °C. After recording the morphometric data and blood collection, the bats were released immediately into the cave. The molecular analysis was conducted at the Laboratory of Biotechnology, Biology Department, University of Bengkulu. The blood samples are stored at the Laboratory of Zoology, Basic Science, University of Bengkulu, with the names AM1, AM2, AM3, AM4, AM5, E8C, and E9C.

Figure 1. *Rousettus amplexicaudatus* from Suruman Cave, South Bengkulu

DNA Extraction

DNA was extracted according to the Spin-Column Protocol, mediated with DNeasy Tissue Kit ® Blood and Tissue Kit (cat. No. 69504 (50) procured from Qiagen.

DNA Amplification and Sequencing

The COI and Cytochrome b genes were amplified through polymerase chain reaction (PCR) using specific DNA primers. The DNA primers were designed online at the Primer3 program (http://bio-info.ut.ee/primer3-0.4.0/primer3). The DNA primers were designed using the sequence of *R. amplexicaudatus* from the Philippines, available at https://www.ncbi.nlm.nih.gov/ with the accession number NC_045044. The designed DNA primers were a forward primer, CORAF 5'-aatcggagccccagacatag-3', and a reverse primer, CORAR 5'-cggctcccatagataggacg-3', for the COI gene with a length of 20 bp, with an amplification target length of approximately 899 bp (the sequence is located at nucleotide number 258-1156). The DNA primers for the amplification of the Cytochrome b gene are the forward primer CYRAF (5'-cccctcaagcatctcctac-3') and the reverse primer CYRAR (5'-gaggggtgttcagtgggtta-3'). Each primer has a length of 20 bp, while the amplification target length was approximately 728 bp (sequence position is located at nucleotide number 69-796).

The COI and cytochrome b genes were amplified using the Polymerase Chain Reaction (PCR) technique. The mixture of materials for DNA amplification consisted of 50 µl, with the following composition: 25 µl of MyTaq™ HS Red Mix-Bioline 2x, 1 µl of DNA primer forward, 1 µl of DNA primer reverse, 3 µl of DNA template, and 20 µl of nuclease-free water. The target DNA sequence was amplified using the following protocol: pre-denaturation at 95^0C for 5 minutes, denaturation at 94^0C for 1 minute, annealing at 60^0C for 45 seconds, elongation at 72^0C for 1 minute, post-elongation at 72^0C for 8 minutes, and cooling at 4^0C for 10 minutes. A polymerase chain reaction (PCR) machine was used at a pre-denaturation temperature of 95^0C for 5 minutes, denaturation at 94^0C for 1 minute, annealing at 60^0C for 45 seconds, elongation at 72^0C for 1 minute, post-elongation at 72^0C for 8 minutes, and cooling at 4^0C for 10 minutes. DNA amplification was conducted for 35 cycles. The quality of the DNA was evaluated using an electrophoresis machine with a 1.2% agarose gel and TAE 1x buffer solution. PCR products with an acceptable quality threshold indicate that the amplification was successful. The PCR products were sent to the First Base Sdn. Bhd. Laboratory in Malaysia for DNA sequencing stage.

Data Analysis

DNA sequencing results in the ABI files were analyzed and edited using the BioEdit version 7.2 program. The sequences obtained were then validated using BLASTn (Basic Local Alignment Search Tool- nucleotide) to identify sequence similarities. The COI and Cytochrome b gene sequences were aligned using Clustal W in the MEGA 11 program (Tamura et al., 2021). The sequences were compared with those of similar genes from the same or closely related species using data from the GenBank library. Phylogenetic tree reconstruction was performed using the Neighbor-Joining (NJ), Bootstrap method with 1000 repetitions, and the genetic distance analysis based on Kimura 2 parameters (K2P) (Tamura et al., 2021). The molecular characteristics of each gene are displayed in the form of a DNA barcode created by a DNA barcode generator (https://biorad-ads.com/DNABarcodeWeb/).

Result

We successfully obtained an analyzable COI gene sequence of 897 bp in length, as presented in Table 1. This analysis revealed that 878 (97.9%) are noted as conserved sites (C), while 19 (2.1%) are variation sites (V), which are divided into six parsimony-informative sites (0.67%) and thirteen singleton sites (1.45%). The conserved region identified in these sequences is part of the sequence that remains similar, stable, and persistent within the COI gene of the seven individuals of *R. amplexicaudatus* Suruman Cave. A Parsimony-informative site refers to a minimal number of substitutions; it contains at least two types of nucleotides, and at least two occur with a minimum frequency of two. On the other hand, the singleton site contains at least two types of nucleotides, with, at most, one occurring multiple times.

Table 1. Molecular characteristics of the partial gene COI (897 bp) of *R. amplexicaudatus* Suruman Cave, South Bengkulu

Individual	*Number of Conserved Sites*	Number of Variable Sites			Composition of Nucleotide (%)			
		V	Pi	S	A	T	G	C
Individual 1					26.2	29.3	17.1	27.4
Individual 2					26.3	29.3	16.9	27.4
Individual 3					26.3	29.3	16.9	27.4
Individual 4	878	19	6	13	26.2	29.3	17.1	27.4
Individual 5					26.2	29.3	17.2	27.3
Individual 6					26.0	29.1	17.6	27.2
Individual 7					26.2	29.7	17.1	27.3

Description: Conserved site = number of nucleotides that are maintained, V = number of nucleotides that have changed, Pi = number of nucleotides that are Parsimony-informative, S = number of Singleton nucleotides, A = Adenine, T = Thymine, G = Guanine, C = Cytosine

Table 2. Molecular characteristics of the partial gene Cytochrome b (635 bp) of *R. amplexicaudatus* Suruman Cave, South Bengkulu

Individual	*Number of Conserved Sites*	Number of Variable Sites			Composition of Nucleotide (%)			
		V	Pi	S	A	T	G	C
Individual 1					26.0	27.6	14.8	31.7
Individual 2					26.1	27.9	14.6	31.3
Individual 3					26.0	27.6	14.8	31.7
Individual 4	629	6	1	5	26.0	27.9	14.8	31.3
Individual 5					26.0	27.7	14.8	31.5
Individual 6					26.0	27.9	14.8	31.3
Individual 7					26.1	27.7	14.6	31.5

Description: Conserved site = number of nucleotides that are maintained, V = number of nucleotides that have changed, Pi = number of nucleotides that are Parsimony-informative, S = number of Singleton nucleotides, A = Adenine, T = Thymine, G = Guanine, C = Cytosine

Sequence analysis of the Cytochrome b gene, 635 bp in length, revealed 629 conservative sites (C) (99.1%), six variation sites (V) (0.9%), one parsimony-informative site (0.16%), and five singleton sites (0.78%). The only variations present in the Suruman population of the cytochrome b gene were transitional substitutions. The nucleotide changes occurred exclusively between purine (adenine-guanine) and pyrimidine (thymine-cytosine).

Table 3. The percentages of genetic distances of intrapopulation, interpopulation of the *R. amplexicaudatus*, and outgroup based on COI genes with 897 bp in length

No	**Species**	**1**	**2**	**3**	**4**	**5**
1	*R. amplexicaudatus* (Suruman-Indonesia)	0 - 1.58				
2	*R. amplexicaudatus* (Philippines)	0.56 - 1.92	0.00			
3	*R. amplexicaudatus* (Malay Peninsular)	11.05 - 12.26	10.65 - 10.66	0.90		
4	*R. amplexicaudatus* (*Unknown*)	10.66 - 11.99	10.26 - 10.27	1.93 - 2.28	0.34	
5	*Outgroup*	15.19 - 17.88	14.92 - 16.79	13.95 - 16.19	13.82 - 15.92	0.00 - 8.57
Intrapopulation *R. amplexicaudatus* (Suruman-Indonesia)				0.00 - 1.58	$\bar{x}$: 0.77	

Interpopulation *R. amplexicaudatus*	0.56 - 12.26	$\bar{x}$: 7.84
Interspecies within genus *Rousettus* (*Outgroup*)	15.19 - 17.88	$\bar{x}$: 16.67

Table 4. The percentages of genetic distances of intrapopulation, interpopulation of the *R. amplexicaudatus*, and outgroup based on Cytochrome b genes with 635 bp in length

No	Species	1	2	3	4	5
1	*R. amplexicaudatus* (Suruman-Indonesia)	0.00 -0,47				
2	*R. amplexicaudatus* (Philippines)	0.32 - 1.11	0.00 -1.27			
3	*R. amplexicaudatus* (Malay Peninsular)	7.43 - 8.54	6.89 - 8.51	1.28		
4	*R. amplexicaudatus* (*Unknown*)	6.71 - 7.25	6.18 - 7.23	0.79 - 1.28	0,16	
5	*Outgroup*	12.84 - 14.88	13.01 - 14.88	14.03 - 14.86	13.63 - 14.47	0.63 - 6.90

Intrapopulation *R. amplexicaudatus* (Suruman-Indonesia)	0.00 - 0.47	$\bar{x}$: 0.3
Interpopulation *R. amplexicaudatus*	0.32 - 8.54	$\bar{x}$: 3.39
Interspecies within genus *Rousettus* (*Outgroup*)	12.84 - 14.88	$\bar{x}$: 13.86

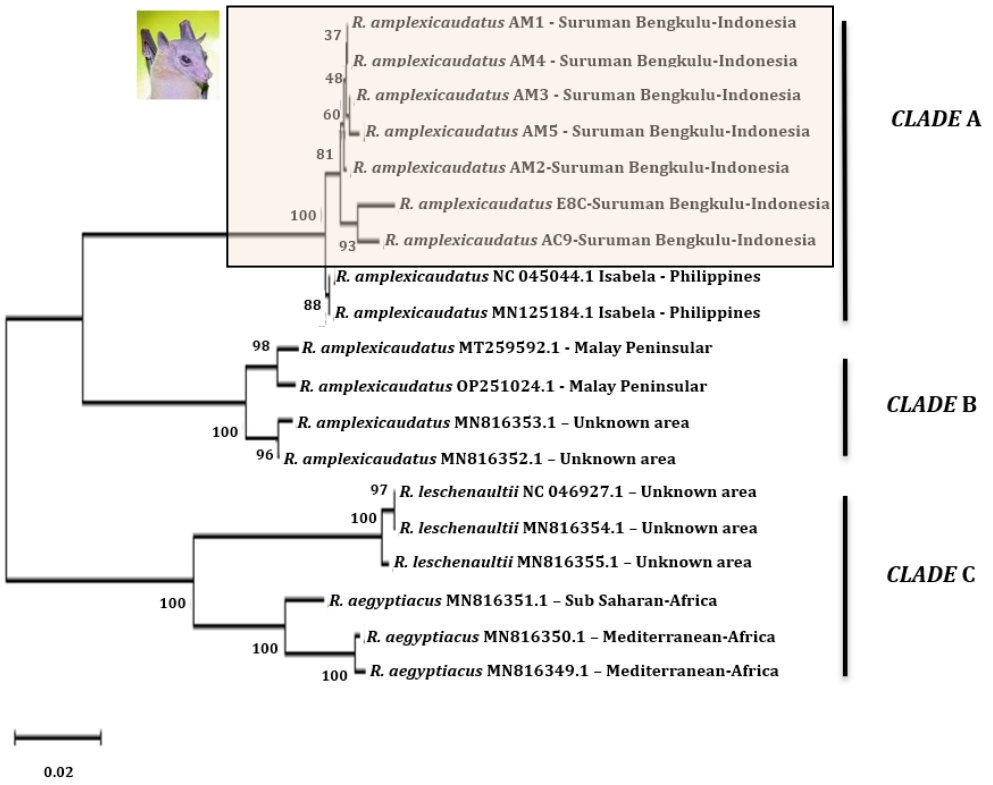

Figure 2. The phylogenetic tree presents a genetic relationship of the *Rousettus amplexicaudatus* Suruman Cave. The illustration tree is based on the 897 bp of the COI sequence, which was analyzed by Neighbor-Joining (NJ)

The phylogenetic tree illustrates the intraspecies and interspecies relationships of *R. amplexicaudatus* based on the COI and Cytochrome b genes. Suruman Cave population forms a monophyletic group distinct from other populations (Figures 1 and 2). The phylogenetic tree is divided into three parts: Clade A, Clade B, and Clade C. Clade A and B comprise the group of *R. amplexicaudatus* species. Clade A consists of the Suruman populations and the Philippines, with a 100% bootstrap value supporting this conclusion.

However, the phylogenetic tree branches indicate they were separated into two different populations.

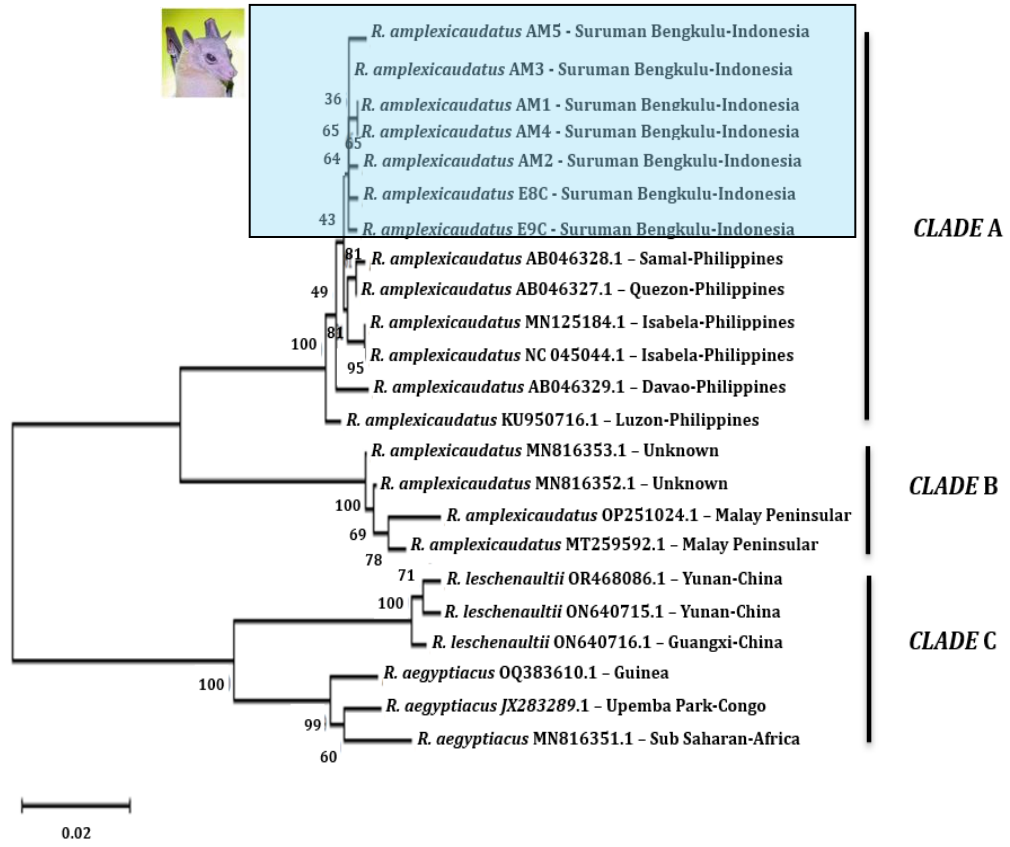

Figure 3. The phylogenetic tree presents a genetic relationship of the *Rousettus amplexicaudatus* Suruman Cave. The illustration tree is based on the 635 bp of the Cytochrome b sequence, which Neighbor-Joining (NJ) analyzed.

Discussion

Molecular Characteristics of COI and *Cytochrome* b Gene of *R. amplexicaudatus* Suruman Cave, South Bengkulu

The partial genes of the COI of *R. amplexicaudatus* Suruman Cave were aligned with the sequence of the intact COI genes of *R. amplexicaudatus* from other regions available in the NCBI molecular library. The percentage of conserved sites was higher in the Cytochrome b (99.1%), while the COI exhibited 97.9%. Demos et al. (2019) state that the COI gene is more conserved. The genes are typically more conserved at the initial region of the gene (Hebert et al., 2003; Lopez-Oceja et al., 2016). The effect of the high variation in the area under analysis is likely responsible for the observed discrepancy in the percentage of conservative sites within the COI and cytochrome b genes. The Cytochrome b sequences amplified and analyzed were positioned on the initial region of the gene, compared to the COI segments.

A comparative analysis of the COI gene sequence revealed the presence of four nucleotide sites that exhibited specific characteristics unique to the *R. amplexicaudatus* Suruman Cave population. These sites were identified as 59, 116, 229, and 625. The population of the Suruman Cave at site number 59 has a thymine base, while other populations have a cytosine base. Similarly, site number 116 is occupied by thymine bases in the Suruman Cave population, whereas other populations exhibit cytosine and guanine bases. At site 229, all individuals from the Suruman Cave population are found to be conserved on the cytosine base, while other populations are conserved on the thymine base. Finally, at site 625, the Suruman population has an adenine base, while other populations have a guanine base. The mean nucleotide composition of the COI gene

(Table 1) is highest in the thymine base at 29.3% and lowest in the guanine base at 17.1%. In a sequential order, the base composition of Thymine (T) is the highest, followed by cytosine (C), adenine (A), and Guanine (G). The base pair composition of adenine-thymine (AT) was 55.5%, while guanine-cytosine (GC) constituted 44.5%. The composition of base pairs within a species is relatively consistent, although it can vary between species. For instance, in the species *Pteropus giganteus*, the AT content is 52.4%, while the GC content is 47.6% (Karamat et al., 2021). In the intact COI gene sequence of *R. amplexicaudatus*, Thymine is the most prevalent base (28.8%), while Guanine is the least prevalent (16.8%) (Mendoza & Fontanilla, 2019). In the mitogenome, however, Adenine is the most abundant base composition, followed by Cytosine, Thymine, and Guanine (Mendoza & Fontanilla, 2019; Jahari et al., 2020). Similar to other species, such as *Cynopterus brachyotis* (Yoon et al., 2016), *Rhinolophus pusillus* (Wang et al., 2020), and *R. affinis* (Ding et al., 2021).

In contrast to the COI sequence, the characteristics of the Cytochrome b of the Suruman population are characterized by the highest average base composition on Cytosine (31.5%). Cytosine (29.1 - 29.7 bp) is the most prevalent base, followed by Thymine, Adenine, and Guanine. The adenine-thymine content (AT) was 53.7%, while guanine-cytosine (GC) content accounted for 46.3%. The number of conserved sites is considerably higher in the Cytochrome b sequences, while the variation sites are notably lower (6 sites or 0.94%). A comparison of the two species, *R. aegyptiacus* and *R. leschenaultii*, revealed that the percentage of variation sites was significantly higher than conserved sites, even when considering a relatively small sequence of 1001 bp.

Interpopulation polymorphism analysis based on Cytochrome b of the Suruman population characterized by 574 (90.39%) conserved sites (C), 61 (9.61%) variation sites (V) which comprised 44 (6.93%) Parsimony-informative sites, 17 (2.68%) Singleton sites. The conservative sites of *R. amplexicaudatus* between the Suruman, Philippines, Malay Peninsular, and Unknown populations indicate molecular differences geographically. Species that are geographically separated do not experience gene flow in between. Each population has adapted to the area in which it lives, thus causing them to undergo molecular variation. Interpopulation polymorphism in the cytochrome b sequences occurs as transitional substitutions and transversions. However, substitutions are more common than transversions. Of the 61 variation sites, 4 had transversion changes, and 57 had transition changes.

Genetic Distance

A genetic distance analysis of the COI gene of *R. amplexicaudatus* was conducted based on intrapopulation, interpopulation, and interspecies comparisons within the genus *Rousettus* (outgroup). As illustrated in Table 3, the genetic distance of the Suruman population exhibits a range of values between 0 and 1.58%, with an average of 0.77%. The interpopulation value is 7.84%. In contrast to the findings of Luczon et al. (2019), the genetic distance between *R. amplexicaudatus* populations in the Philippines and those from mainland Southeast Asia is notably high, with a value of 12.70%. With this considerable genetic distance, it is questionable whether the population of *R. amplexicaudatus* from Suruman (Sumatra) and the Philippines are different species from the Malaysian population. The genetic distance between the Suruman population and the outgroup is quite far (16.67%), not surprisingly because the outgroup consists of another two species, namely *R. leschenaultii* and *R. aegyptiacus*. Luczon et al. (2019) reported that the overall genetic distance within the Philippines Pteropodidae species was 19.87%. Based on the interpopulation genetic distance of R. amplexicaudatus, it is known that the lowest distance is between the Suruman population and the Philippines, with a value of 0.56-1.92%. (Table 2). This indicates that the Suruman population is more genetically related to the Philippines population than others. This is reflected in the topology of the phylogenetic tree, where the Philippines and Suruman populations are in the same

branch, with sister taxon being populations from Malaysia and the Unknown area (Figure 3). The most significant genetic distance observed after the Philippines is to the Unknown population, with a value of 10.66–11.99%. The most significant distance is to the population from Malay Peninsular, with a value of 11.05–12.26%. Therefore, these two populations are situated in a distinct subclade, separate from populations from Suruman and the Philippines.

The genetic distance of *R. amplexicaudatus* based on the Cytochrome b gene exhibits a lower interval value than the COI gene within the population of Suruman Cave; it is 0–0.47% (average 0.3%). The interpopulation genetic distance value is 3.39%. The interspecies genetic distance with the outgroups *R. leschenaultii* and *R. aegyptiacus* is 13.86%. The outcomes of the Cytochrome b gene analysis were consistent with those of the COI gene analysis. The Suruman population exhibited the lowest genetic distance from the Philippines population (0.32-1.11%), while the Malay Peninsular population exhibited the most significant genetic distance (7.43-8.54%). As in all living organisms, the genetic diversity of bats is influenced by various environmental factors. The greater the number of environmental factors impacting a population, the more significant changes may occur. The quantity of external environmental stressors to which a given species is subjected can influence its capacity for adaptive response (Cruz-Salazar et al., 2018; Peixoto et al., 2018; Soto-Centeno & Simmons, 2022; Festa et al., 2023).

Two phylogenetic trees were constructed based on the genetic variation of the COI and Cytochrome b. This analysis revealed the relationship between the *R. amplexicaudatus* Suruman population and other populations. The study demonstrated that the Suruman population constituted a discrete entity, distinct from the Philippines, Malay Peninsular, and Unknown populations. The population was divided into three clades: A, B, and C. Clades A and B are Clades of the *R. amplexicaudatus* species, as evidenced by the phylogenetic tree constructed using the COI gene and the Cytochrome b gene. Clade A, which exhibits the closest genetic distance (Table 3), comprises the Suruman and the Philippines populations. A 100% bootstrap value constructs this. Nevertheless, these two populations were found to constitute discrete groups.

A phylogenetic analysis using the COI gene yielded the following results: The bootstrap value for the Suruman population was 81%, while that for the Philippines population was 88%. The Malay Peninsular and Unknown populations are classified within Clade B, exhibiting a greater genetic distance than populations within Clade A, with a bootstrap value of 100%. Although the genetic distance is considerable, it does not exceed 3%. This suggests that clades A and B populations are considered a group of the same species. Clade C is a well-supported outgroup with a bootstrap value of 100%. This outgroup clade, which differs from the *R. amplexicaudatus* species, indicates that the outgroup species differs from the sample and comparison individuals despite being of the same genus. The bootstrap values displayed in the phylogenetic tree reconstruction results are predominantly at a high or strong level. However, some values are still considered low or below the significance threshold in the phylogenetic tree. This study's COI gene phylogenetic tree exhibits the lowest bootstrap value of 37% and the highest of 100%. The bootstrap method for assessing phylogenetic trees is a standard technique for inferring confidence values in reconstructed phylogenetic trees of multiple taxa. It refers to the reliability of the resulting sequence-based phylogeny trees. The expected bootstrap value is more than 95% replication, which means the similarity value of the proposed branching form in phylogenetic tree reconstruction. However, the value we get may be much lower than expected, which is caused by several obstacles during the tree reconstruction process, such as the limited comparative sequence data available in GenBank and the sequences that have been used. This can occur due to the limited number of characters supporting each node. This phenomenon is particularly prevalent in recently divergent groups (Soltis & Soltis, 2003; Zaharias et al., 2023).

The phylogenetic analysis of the Cytochrome b gene indicates that the Suruman population constitutes a discrete group with a bootstrap value of 64%. The Philippines

population also forms its group, with a bootstrap value of 31%. Some individuals appear to be members of lineages not part of the Philippines population group. This occurs because the data for the Philippines population are individuals from five different regions within the Philippines. This has implications for genetic variation and the appearance of the phylogenetic tree. The populations of *R. amplexicaudatus* from Malay Peninsular and Unknown are found in clade B, with a bootstrap value of 100%. Clade C is an outgroup with a bootstrap value of 100%. Phylogenetic reconstruction from research by (Luczon et al., 2019; Stribna et al., 2019) demonstrates a clear separation between *R. amplexicaudatus* and other species.

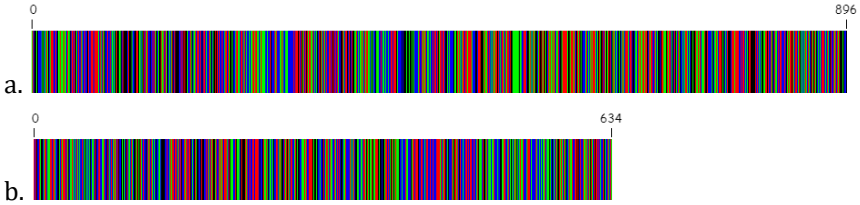

Figure 4. The characteristics sequence of the COI and Cytochrome b genes of *R. amplexicaudatus* Suruman Cave population, (a) COI gene 897 bp, (b) Cytochrome b gene (635 bp). The differences in bar color indicate the nucleotide difference. Black is for Guanine, blue is for Cytosine, green is for Adenine, and red is for Thymine.

A DNA sequence analysis at the initial region of the genes revealed that Cytochrome b exhibited the largest conserved sites compared to the COI gene, with minimal polymorphism intrapopulation. Both genes demonstrated a minimal intrapopulation variation in the composition of nucleotides. This suggests that these sequences have a high degree of genetic stability. Additionally, the sequences showed a consistent pattern in phylogenetic tree analysis, which indicates the Suruman population is the group of *R. amplexicaudatus*. Therefore, these sequences can be proposed as molecular markers for the *R. amplexicaudatus* species, particularly when compared to the whole sequences of the COI gene and cytochrome b.

Conclusions

The COI gene sequence of 897 bp was obtained from research on *R. amplexicaudatus* from Suruman Cave, South Bengkulu. COI gene sequence is characterized by 878 conserved sites (97.8%) and 19 variation sites (2.2%). The variation site consists of 6 parsimony-informative sites and 13 singleton sites. Thymine base composition was 29.3%, the cytosine 27.4%, the adenine 26.2%, and the guanine 17.1%. The base pair composition of adenine-thymine (55.5%) was higher than that of guanine-cytosine (44.5%). Cytochrome b (635 bp) was characterized by 629 conserved sites (99.1%) and six variation sites (0.9%), of which one was parsimony-informative, and 5 were singleton sites. The base composition of the Cytochrome b sequence was Cytosine (31.5%), Thymine (27.7%), Adenine (26.0%), and Guanine (14.8%). Adenine-thymine base pairs (53.7%) were more common than Guanine-Cytosine (46.3%). The mean intrapopulation genetic distance of the Suruman population in the COI gene (0.77%) was more significant than the Cytochrome b gene (0.3%). Phylogenetic reconstruction based on both genes revealed that the Suruman population is monophyletic, forming a distinct lineage within the broader *R. amplexicaudatus* population. Both sequences, COI (897 bp) and Cytochrome b (635 bp), can be utilized as *R. amplexicaudatus* molecular species markers.

Declaration statement

We want to express our gratitude to UPP and the Dean of the Faculty of Mathematics and Natural Sciences, University of Bengkulu, for their assistance in facilitating the smooth running of this research. Their contributions have enabled us to obtain Research Grants in Research Collaboration with Other Universities (No. 2033/UN30.12/HK/2022), allowing us to conduct this research appropriately. Additionally, gratitude is extended to

Dr. Sipriyadi, Vestidhia Yunisya Atmaja, M.Sc., Ahmad Fakhri, M.Si., Aprira, S.Si., and all contributors who made this research successful.

References

Afa, M. (2016). The effect of natural guano organic fertilizer on growth and yield of spring onion (Allium Fistulosum L.). *Agrotech Journal*, *1*(1), 26–32. https://doi.org/10.31327/atj.v1i1.206

Ancillotto, L., Bosso, L., Smeraldo, S., Mori, E., Mazza, G., Herkt, M., Galimberti, A., Ramazzotti, F., & Russo, D. (2020). An African bat in Europe, Plecotus gaisleri: Biogeographic and ecological insights from molecular taxonomy and Species Distribution Models. *Ecology and Evolution*, *10*(12), 5785–5800. https://doi.org/10.1002/ece3.6317

Calahorra-Oliart, A., Ospina-Garcés, S. M., & León-Paniagua, L. (2021). Cryptic species in Glossophaga soricina (Chiroptera: Phyllostomidae): do morphological data support molecular evidence? *Journal of Mammalogy*, *102*(1), 54–68. https://doi.org/10.1093/jmammal/gyaa116

Carpenter, E. S., Gomez, R., Waldien, D. L., & Sherwin, R. E. (2014). Photographic estimation of roosting density of Geoffroy ' 's Rousette Fruit Bat Rousettus amplexicaudatus (Chiroptera : Pteropodidae) at Monfort Bat Cave, Philippines. *Journal of Threatened Taxa*, *6*(6), 5838–5844. http://dx.doi.org/10.11609/JoTT.o3522.5838-44

Ceballos, G. (2014). *Mammals of Mexico*. Johns Hopkins University Press. https://doi.org/https://books.google.co.id/books?hl=id&lr=&id=UrvxBQAAQBAJ&oi=fnd&pg=PP1&ots=nhNgNv-x9A&sig=4G5qJ3EfjhoYuakDXU2SQPGdQKc&redir_esc=y#v=onepage&q&f=false

Cruz-Salazar, B., Ruiz-Montoya, L., Mendoza-Sáenz, V. H., Riechers-Pérez, A., & García-Bautista, M. (2018). Genetic diversity of tropical bats and its relationship with ecological role in a tropical semievergreen rain forest in EL ocote biosphere reserve, Chiapas, Mexico. *Tropical Conservation Science*, *11*. https://doi.org/10.1177/1940082917752473

Demos, T. C., Webala, P. W., Peterhans, J. C. K., Goodman, S. M., Bartonjo, M., & Patterson, B. D. (2019). Molecular Phylogenetics of Slit-Faced Bats (Chiroptera: Nycteridae) Reveal Deeply Divergent African Lineages. *Journal of Zoological Systematics and Evolutionary Research*, *57*(4), 1019–1038. https://doi.org/10.1111/jzs.12313

Ding, Y., Chen, W., & Mao, X. (2021). The Complete Mitochondrial Genome of Rhinolophus affinis himalayanus. *Mitochondrial DNA Part B*, *6*(1), 164–165. https://doi.org/10.1080/23802359.2020.1856011

Festa, F., Ancillotto, L., Santini, L., Pacifici, M., Rocha, R., Toshkova, N., Amorim, F., Benítez-López, A., Domer, A., Hamidović, D., Kramer-Schadt, S., Mathews, F., Radchuk, V., Rebelo, H., Ruczynski, I., Solem, E., Tsoar, A., Russo, D., & Razgour, O. (2023). Bat responses to climate change: a systematic review. *Biological Reviews*, *98*(1), 19–33. https://doi.org/10.1111/brv.12893

Francis, C. (2019). *Field guide to the mammals of Southeast Asia* (2nd ed.). Bloomsbury Publishing.

Frick, W. F., Kingston, T., & Flanders, J. (2020). A Review of the Major Threats and Challenges to Global Bat Conservation. *Annals of the New York Academy of Sciences*, *1469*(1), 5–25. https://doi.org/10.1111/nyas.14045

Hassanin, A., Bonillo, C., Tshikung, D., Pongombo Shongo, C., Pourrut, X., Kadjo, B., Nakouné, E., Tu, V. T., Prié, V., & Goodman, S. M. (2020). Phylogeny of African fruit bats (Chiroptera, Pteropodidae) based on complete mitochondrial genomes. *Journal of Zoological Systematics and Evolutionary Research*, *58*(4), 1395–1410. https://doi.org/10.1111/jzs.12373

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological Identifications Through DNA Barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Huang, C., Yu, W., Xu, Z., Qiu, Y., Chen, M., Qiu, B., Motokawa, M., Harada, M., Li, Y., & Wu, Y. (2014). A Cryptic Species of the Tylonycteris pachypus Complex (Chiroptera: Vespertilionidae) and Its Population Genetic Structure in Southern China and Nearby Regions. *International Journal of Biological Sciences*, *10*(2), 200–211. https://doi.org/10.7150/ijbs.7301

IUCN. (2021). *IUCN Red List for Chiroptera.* IUCN. https://doi.org/https://www.iucnredlist.org/search/list?taxonomies=100265&searchType=species

Jahari, P. N. S., Azman, S. M., Munian, K., Fauzi, N. F. M., Shamsir, M. S., Richter, S. R., & Salleh, F. M. (2020). The First Complete Mitochondrial Genome Data of Geoffroy's Rousette, Rousettus amplexicaudatus Originating From Malaysia. *Mitochondrial DNA Part B: Resources*, *5*(3), 3280–3282. https://doi.org/10.1080/23802359.2020.1812449

Juste, J., Ruedi, M., Puechmaille, S. J., Salicini, I., & Ibáñez, C. (2018). Two New Cryptic Bat Species within the Myotis nattereri Species Complex (Vespertilionidae, Chiroptera) from the Western Palaearctic. *Acta Chiropterologica*, *20*(2), 285–300. https://doi.org/10.3161/15081109ACC2018.20.2.001

Kamilah, S. N., Manaf, S., Darwis, W., Duya, N., Harmolis, D., & Meriana. (2019). Perilaku Pemilihan Lokasi Roosting Site Pada Kelelawar di Gua Suruman Bengkulu Selatan. *Prosiding Semirata BKS PTN Wilayah Barat Bidang MIPA*, 830–835. https://doi.org/10.5281/zenodo.6832566

Karamat, S., Ashraf, N., Akhtar, T., Rahim, F., Shafi, N., Khalid, S., Shahid, B., Khawaja, S., Rahim, J., Majeed, Z., Z., L., & Menmood, M. (2021). CO1-based DNA barcoding for assessing diversity of Pteropus giganteus from the State of Azad Jammu Kashmir, Pakistan. *Braz. J. Biol.*, *81*(3), 584–591. https://doi.org/10.1590/1519-6984.226466

Kemp, J., López-Baucells, A., Rocha, R., Wangensteen, O. S., Andriatafika, Z., Nair, A., & Cabeza, M. (2019). Bats as potential suppressors of multiple agricultural pests: A case study from Madagascar. *Agriculture, Ecosystems and Environment*, *269*, 88–96. https://doi.org/10.1016/j.agee.2018.09.027

KLHK-LIPI. (2019). *Panduan Identifikasi Jenis Satwa Liar di Lindungi; Mamalia*. KLHK-LIPI. https://doi.org/http://ksdae.menlhk.go.id/assets/publikasi/BUKU PANDUAN IDENTIFIKASI MAMALIA DILINDUNGI_020819.pdf

Ladoukakis, E. D., & Zouros, E. (2017). Evolution and inheritance of animal mitochondrial DNA: Rules and exceptions. *Journal of Biological Research (Greece)*, *24*(1), 1–7. https://doi.org/10.1186/s40709-017-0060-4

Laurindo, R. S., Novaes, R. L. M., Vizentin-Bugoni, J., & Gregorin, R. (2019). The effects of habitat loss on bat-fruit networks. *Biodiversity and Conservation*, *28*(3), 589–601. https://doi.org/10.1007/s10531-018-1676-x

Lengkong, H. J., Arisoesilaningsih, E., Hakim, L., & Sudarto. (2016). Morphological Variations and New Species Description of Genus Rousettus Bat from Gunung Duasudara Sanctuary, North Sulawesi, Indonesia. *OnLine Journal of Biological Sciences*, *16*(2), 90–101. https://doi.org/10.3844/ojbsci.2016.90.101

Lopez-Oceja, A., Gamarra, D., Borragan, S., Jiménez-Moreno, S., & De Pancorbo, M. M. (2016). A new cyt b gene universal primer is set for forensic analysis. *Forensic Science International: Genetics*, *23*, 159–165. https://doi.org/10.1016/j.fsigen.2016.05.001

Luczon, A. U., Ampo, S. A. M. M., Roño, J. G. A., Duya, M. R. M., Ong, P. S., & Fontanilla, I. K. C. (2019). DNA barcodes reveal high genetic diversity in Philippine fruit bats. *Philippine Journal of Science*, *148*(Special Issue 1), 133–152. https://doi.org/DOI: 10.1016/j.fsigen.2016.05.001

Lukman. (2022). Pemanfaatan Pupuk Guano dalam Sistem Pertanian Berkelanjutan dan Dampaknya pada Pertumbuhan dan Hasil Tanaman Jagung Manis (Zea mays saccharata L). *Jurnal Ilmu Pertanian Indonesia*, *27*(4), 590–595. https://doi.org/10.18343/jipi.27.4.590

Luo, P. F., Wang, W. F., Wang, X. L., Wang, Y. L., Wang, S. W., Yan, S. S., He, Q. Q., & Zhou, J. (2022). The complete mitochondrial genome of the Great evening bat Ia io (Chiroptera: Vespertiilionidae) from karst area, Southwestern China. *Mitochondrial DNA Part B: Resources*, *7*(4), 587–589. https://doi.org/10.1080/23802359.2022.2057247

Maryanto, I., Maharadatunkamsi, Achmadi, A. S., Wiantoro, S., Sulistyadi, E., Yoneda, M., Suyanto, A., & Sugardjito, J. (2019). *Checklist of the Mammals of Indonesia* (Third Edit). LIPI Press. Bogor. https://doi.org/https://www.researchgate.net/publication/338687953_CHECKLIST_OF_THE_MAMMALS_OF_INDONESIA_Scientific_English_Indonesia_Name_and_Distribution_Area_Table_in_Indonesia_Including_CITES_IUCN_and_Indonesian_Category_for_Conservation

Medellin, R. A., Wiederholt, R., & Lopez-Hoffman, L. (2017). Conservation relevance of bat caves for biodiversity and ecosystem services. *Biological Conservation*, *211*(Part B), 45–50. https://doi.org/10.1016/j.biocon.2017.01.012

Mendoza, R. V. D., & Fontanilla, I. K. C. (2019). Whole Mitochondrial Genome of a Geoffroy's Rousette, Rousettus amplexicaudatus (Pteropodidae). *Mitochondrial DNA Part B*, *4*(2), 3546–3548. https://doi.org/10.1080/23802359.2019.1676671

Mulvaney, J., Moir, M., & Cherry, M. I. (2023). DNA barcoding reveals cryptic diversification and taxonomic discordance among bats and birds within Sub-Saharan Africa. *Biodiversity and Conservation*, *32*(14), 4895–4914. https://doi.org/10.1007/s10531-023-02737-1

Park, S., Noh, P., Choi, Y. S., Joo, S., Jeong, G., & Kim, S. S. (2019). Population genetic structure based on mitochondrial DNA analysis of Ikonnikov's whiskered bat (Myotis ikonnikovi - Chiroptera: Vespertilionidae) from Korea. *Journal of Ecology and Environment*, *43*(1), 1–8. https://doi.org/10.1186/s41610-019-0140-5

Payne, J., Francis, C. M., Philipps, K., & Kartikasari, S. N. (2000). *Panduan Lapangan Mamalia di Kalimantan, Sabah, Serawak, dan Brunai Darussalam*. The Sabah Society dan WWF. Kuala Lumpur.

Peixoto, F. P., Braga, P. H. P., & Mendes, P. (2018). A synthesis of ecological and evolutionary determinants of bat diversity across spatial scales. *BMC Ecology*, *18*(1), 1–14. https://doi.org/10.1186/s12898-018-0174-z

Ramírez-Fráncel LA, García-Herrera LV, Losada-Prado S, Reinoso-Flórez G, Sánchez-Hernández, A, Estrada-Villegas, S, Lim BK, G. G. (2022). Bats and their vital ecosystem services: a global review. *Integrative Zoology*, *17*(1), 2–23. https://doi.org/10.1111/1749-4877.12552

Soltis, P. S., & Soltis, D. E. (2003). Applying the Bootstrap in Phylogeny Reconstruction. *Statistical Science*, *18*(2), 256–267. https://doi.org/10.1214/ss/1063994980

Soto-Centeno, J. A., & Simmons, N. B. (2022). Environmentally driven phenotypic convergence and niche conservatism accompany speciation in hoary bats. *Scientific Reports*, *12*(1), 1–13. https://doi.org/10.1038/s41598-022-26453-y

Srinivasulu, C., Srinivasulu, A., Srinivasulu, B., & Jones, G. (2019). Integrated approaches to identifying cryptic bat species in areas of high endemism: The case of Rhinolophus andamanensis in the Andaman Islands. *PLoS ONE*, *14*(10), 1–15. https://doi.org/10.1371/journal.pone.0213562

Stribna, T., Romportl, D., Demjanovič, J., Vogeler, A., Tschapka, M., Benda, P., Horáček, I., Juste, J., Goodman, S. M., & Hulva, P. (2019). Pan African phylogeography and palaeodistribution of rousettine fruit bats: Ecogeographic correlation with Pleistocene climate vegetation cycles. *Journal of Biogeography*, *46*(10), 2336–2349. https://doi.org/10.1111/jbi.13651

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Tangguda, S., Valentine, R. T., Hariyadi, D. R., & Sudiarsa, I. N. (2022). Pemanfaatan Kotoran Kelelawar sebagai Pupuk Guano di Desa Bolok, Kupang Barat, Nusa Tenggara Timur. *Agrikultura*, *33*(3), 289–295. https://doi.org/10.24198/agrikultura.v33i3.40690

Thanh, H. T., Son, N. T., Duong, V. T., Luong, N. T., Loi, D. N., & Thong, V. D. (2019). New Records and Morphological Assessments of Long-Nosed Fruit Bats (Chiroptera: Pteropodidae: Macroglossus spp.) from Vietnam. *Tap Chi Sinh Hoc*, *41*(4), 117–124. https://doi.org/10.15625/0866-7160/v41n4.14695

Tu, V. T., Csorba, G., Ruedi, M., Furey, N. M., Son, N. T., Thong, V. D., Bonillo, C., & Hassanin, A. (2017). Comparative Phylogeography of Bamboo Bats of the Genus Tylonycteris (Chiroptera, Vespertilionidae) in Southeast Asia. *European Journal of Taxonomy*, *2017*(274), 1–38. https://doi.org/10.5852/ejt.2017.274

Waldien, D. L., Wilson, Z., Adleson, S., Aziz, A. S., Bates, P. J. J., Bumrungsri, S., Furey, N., Ingle, N. R., Mildenstein, T., Phelps, K., Tanalgo, K., Soisook, P., Thong, V. D., Wiantoro, S., & Tsang, S. M. (2019). Rousettus amplexicaudatus. In *The IUCN Red List of Threatened Species 2019: e.T19754A22001514*. http://dx.doi.org/10.2305/IUCN.UK.2019- 3.RLTS.T19754A22001514.en

Wang, J., Zhao, A., & Sun, H. (2020). The Complete Mitochondrial Genome of the Least Horseshoe Bat (Rhinolophus pusillus). *Mitochondrial DNA Part B*, *5*(1), 881–882. https://doi.org/10.1080/23802359.2020.1717389

Wilson, D. E., & Mittermeier, R. A. (2019). *Handbook of the Mammals of the World Volume 9 - Bats*. Lynx Edicions. https://doi.org/10.5281/zenodo.6448815

Wright, P. G. R., Hamilton, P. B., Schofield, H., Glover, A., Damant, C., Davidson-Watts, I., & Mathews, F. (2018). Genetic structure and diversity of a rare woodland bat, Myotis bechsteinii: comparison of continental Europe and Britain. *Conservation Genetics*, *19*(4), 777–787. https://doi.org/10.1007/s10592-018-1053-z

Yoon, K. B., Kim, J. Y., & Park, Y. C. (2016). Characteristics of Complete Mitogenome of the Lesser Short-Nosed Fruit Bat Cynopterus brachyotis (Chiroptera: Pteropodidae) in Malaysia. *Mitochondrial DNA Part A Resources*, *27*(3), 2091–2092. https://doi.org/10.3109/19401736.2014.982571

Yue, Y., Huang, Z., Li, F., Thapa, S., Hu, Y., Wu, Y., & Yu, W. (2019). In China, the complete mitochondrial genome of the tube-nosed bat Murina cyclotis (Chiroptera: Vespertilionidae). *Mitochondrial DNA Part B: Resources*, *4*(2), 2248–2250. https://doi.org/10.1080/23802359.2019.1623125

Zaharias, P., Lemoine, F., & Gascuel, O. (2023). Robustness of Felsenstein's Versus Transfer Bootstrap Supports With Respect to Taxon Sampling. *Systematic Biology*, *72*(6), 1280–1295. https://doi.org/10.1093/sysbio/syad052

Zhang, L., Sun, K., Csorba, G., Hughes, A. C., Jin, L., Xiao, Y., & Feng, J. (2021). Complete Mitochondrial Genomes Reveal Robust Phylogenetic Signals and Evidence of Positive Selection in Horseshoe Bats. *BMC Ecology and Evolution*, *21*(199), 1–15. https://doi.org/10.1186/s12862-021-01926-2