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DNA Extraction *of Cynopterus brachyotis* of Labuhan Ratu VII Based on Column Method in Supporting its Molecular Species Confirmation

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Abstract
Background. Bats (Chiroptera) are true flying mammals and nocturnal. Ecologically, bats play important roles as pollinators, seed dispersers, guano producers, biocontrol, and hosts a range of viruses. *Cynopterus brachyotis* belongs to fruit-eating bats and is considered morphologically cryptic with *C. sphinx*.
Aims. To support morphological identification, molecular species confirmation needed to be conducted. DNA extraction, as an initial step, plays a crucial role in molecular analysis.
Methods. The quality and quantity of extracted DNA determine the success rate of sequencing for species confirmation in bats. In this study, oropharyngeal samples were used.
Result. The research procedures included bat capture and DNA oropharyngeal swab sample collection, sample preparation, DNA extraction using a silica column-based commercial kit, DNA concentration measurement using a Qubit assay, and electrophoresis.
Conclusion. The Qubit assay showed DNA concentrations of >1 ng/µL, while electrophoresis did not reveal bright DNA bands.

Keyword: *Cynopterus brachyotis*, DNA extraction, oropharyngeal samples, species, confirmation

INTRODUCTION

Bats (Chiroptera) are the only flying mammals and are nocturnal. (Manek *et al*., 2020). Based on morphological characters such as body size and diet, bats are divided into fruit-eating

and insect-eating species (Ransaleleh *et al*., 2024). *Cynopterus brachyotis* belongs to the group of fruit-eating bats.

Cynopterus brachyotis belongs to the Pteropodidae, a frugivorous family of fruit, flower, and nectar eaters (Sapphire et al., 2020), and can be found in almost all regions of Indonesia, especially Sumatra, Java, Kalimantan, and Sulawesi, except West Papua (Marlinda *et al*., 2019). Its habitats include lowland forests, primary and secondary forests, urban areas, and areas with food resources (Ramona *et al*., 2019). Ecologically, bats act as pollinators for flowering plants, seed dispersers in fruiting plants, and insect controls, especially insect-eating bats (Ransaleleh *et al*., 2024). The faeces and urine produced by bats can also be used as guano fertilizer (Tangguda *et al.*, 2022). *C. brachyotis* also acts as a reservoir for pathogens such as the Nipah virus and *betacoronavirus* (bCOV) (Morcatty *et al*., 2022).

Based on its morphological characteristics, C. brachyotis and C. sphinx are phylogenetically similar (Mubarok *et al*., 2023). Morphological and morphometric characters are used in detail to identify *C. brachyotis*. In the identification of *C. brachyotis* the observed characteristics include the shape of the lower incisors, body colour, and the colour of the hair around the neck, the length of the forearms, the length of the tail, the ears, the tibia, the hind legs, and the weight of the body. In supporting the results of morphological identification, molecular species confirmation is carried out (Sapphire *et al*., 2020).

Genes commonly used for species confirmation include mitochondrial genes such as Cytochrome Oxidase I (COI), the D-loop, and *Cytochrome* b (Cyt b) (Mubarok & Sandika, 2023). The The cytochrome b gene consists of 1,140 bp and has a high degree of variability at the species (intraspecific) and inter-species (interspecific) levels. The cytochrome b gene can confirm cryptic species and their degree of kinship, making it useful for population studies and phylogenetic tree construction (Lam *et al*., 2024).

DNA extraction aims to obtain pure DNA from organisms' tissues as the basic material for genetic identification. The results of DNA extraction greatly determine the success rate of subsequent molecular analysis stages, such as amplification, electrophoresis, and sequencing (Triasih *et al.*, 2020). Factors that affect the quality of extracted DNA include the type and condition of the initial sample, the extraction method used, the contaminant removal technique, and the storage process (Pearce *et al*., 2024). To obtain DNA of optimal quality, this research applied C. brachyotis DNA sampling procedures and DNA extraction protocols.

Molecular identification has become an essential approach for accurate species confirmation in bats, particularly for taxa with cryptic morphological characteristics, such as

fruit bats within the genus *Cynopterus*. Traditional identification based on morphometric traits—such as forearm length, body coloration, and dentition—often leads to misidentification because phenotypic characters overlap among closely related species.

To overcome these limitations, mitochondrial DNA markers (e.g., COI, D-loop, and Cytochrome b genes) are widely used for species-level confirmation, phylogenetic reconstruction, and population genetic studies. The success of these molecular approaches depends heavily on the quality and quantity of extracted DNA, which in turn are influenced by sample type, extraction method, and storage conditions.

Current studies predominantly utilize tissue samples (wing membrane, blood) due to their high DNA yield; however, these methods are invasive and raise ethical concerns, especially for small-bodied bats. As a result, non-invasive or minimally invasive samples such as guano, feces, and oropharyngeal swabs have gained increasing attention. Among these, oropharyngeal swabs are considered advantageous due to reduced animal stress and suitability for pathogen surveillance.

Commercial silica column-based extraction kits are widely adopted due to their standardized protocols, high contaminant removal efficiency, and reproducibility. DNA quantification using fluorometric methods (Qubit) is currently considered more accurate than spectrophotometric methods, particularly for low-concentration DNA samples. Nevertheless, studies report inconsistent electrophoretic visualization when working with low-input DNA derived from non-invasive samples.

Overall, while molecular confirmation of bat species is well established, optimizing DNA extraction from minimally invasive samples remains an active area of research, particularly for tropical bat species.

METHODS

This research is under the Innovation and Collaboration Batch 3 scheme for the domestic year 2025 at Universitas Lampung, in collaboration with the Lampung Disease Investigation Centre. Bat life trapping by mist net and oropharyngeal swab sampling was carried out at Labuhan Ratu VII, East Lampung. At the same time, molecular analysis was performed at the Biotechnology Laboratory of the Lampung Disease Investigation Centre.

Bat sampling was carried out using mist nets, which were set up 4-6 meters high from 18.00 to 22.00. The oropharyngeal swab was collected using a sterile cotton swab, which was

inserted into the bat's oral cavity and rotated slowly. The cotton swabs are then inserted into a VTM tube containing physiological NaCl. VTM tubes were labelled and stored in a cool box.

Sample preparation for molecular analysis was performed using a VTM tube containing a homogenized sample, which was vortexed. DNA extraction was performed using silica columns with the Invitrogen Pure LinkTM Viral RNA/DNA Mini Kit (Catalog #12280050) in four stages: lysis, binding, washing, and elution. The process began with breaking down the cell structure (lysis), which was performed by adding 200 µL of lysis buffer, 25 µL of proteinase K, and 200 µL of the sample to a 1.5 mL microcentrifuge tube. The solution was then homogenized using a vortex and was incubated at 56 °C for 15 minutes. It was then added to 250 µL of absolute alcohol, vortexed, and incubated at room temperature for 5 minutes. The binding stage was performed by transferring the incubated samples to a spin column and centrifuging at 10000 rpm, 4 °C for 1 minute.

The washing stage was performed twice by replacing the collection tube and adding 500 µL of wash buffer, then centrifuging at 10000 rpm at 4 °C for 1 minute. In the second wash, the collection tube containing the supernatant was discarded, and 500 µL of wash buffer was added, then centrifuged again at 10000 rpm at 4 °C for 1 minute.

In the elution stage, the collection tube was replaced with a 1.5 ml microcentrifuge tube, then 50 µL of Nuclease Free Water (NFW) was added and incubated at room temperature for 1 minute. The sample was then centrifuged at 12000 rpm and 4 °C for 1 minute. The spin column on the microtube was discarded, and then the microcentrifuge tube was closed, labelled, and the DNA was stored in the freezer at -20 °C.

The DNA concentration was measured using a Qubit fluorometer to check the quantity of extracted DNA. The reagents used were 199 µL Qubit™ dsDNA BR Buffer and 1 µL Qubit™ reagent. Both reagents are put in a 0.5 ml microtube. The extracted DNA was added to a total of 10 µL to the 190 µL test reagent, resulting in a final volume of 200 µL. The sample is slowly inverted by repeatedly sucking and dispensing the liquid with a micropipette to avoid bubbles or liquid from sticking to the tube walls. The mixture was incubated for 2 minutes at room temperature, then placed in a dark place. The DNA sample is read using a Qubit fluorometer on a program that corresponds to the size expressed in ng/µL.

Electrophoresis is performed using an electrophoresis set, which provides an electrical current through the agarose gel containing DNA in the chamber. Agarose gel 1% is made by dissolving 1 g of agarose powder into 100 mL of TAE 1X buffer in an Erlenmeyer flask. The solution is homogenized by whisking, then heated in the microwave for 3 minutes. The

fluorescent dye SYBR® safe DNA gel stain was added at up to 12 µL, and the mixture was homogenized. The agarose gel mold, after being combed, poured with an agarose solution, and allowed to harden, is ready to use. The solidified agarose gel is transferred into a chamber containing 1X TAE buffer until it is submerged. For comparison, a 100 bp DNA marker was inserted as much as 6 µL. The electrophoresis process is run for 35 minutes at 100 V and 300 A.

RESULTS AND DISCUSSION

The novelty of this research lies in several key aspects:

1. Application of oropharyngeal swab samples for molecular preparation of *Cynopterus brachyotis* in Indonesia, where tissue-based sampling still dominates bat genetic studies.
2. Empirical evaluation of silica column-based DNA extraction specifically for bat oropharyngeal samples, rather than pathogen-focused applications.
3. Combined assessment of DNA quantity (Qubit fluorometry) and quality (electrophoresis) to highlight discrepancies between measurable DNA concentration and visible DNA bands.
4. Context-specific contribution by providing baseline molecular preparation data for *C. brachyotis* populations in Lampung, supporting future genetic and phylogenetic research.
5. Ethical relevance, as the study supports minimally invasive sampling strategies that reduce harm to wildlife.

This study, therefore, contributes methodologically rather than taxonomically, emphasizing sample feasibility and laboratory workflow validation for future sequencing-based confirmation. The results of the DNA concentration measurement obtained showed a value of < 1 ng/µL. (Table 1).

Table 1. DNA concentration measurement results

No	Sample	DNA concentration ng/µL
1.	1	0.698 ng/µL
2.	2	0.668 ng/µL
3.	3	0.700 ng/µL

The Qubit assay results showed the total DNA concentration per microliter of sample. Higher values indicate higher DNA concentrations in the sample (Bruijns *et al*., 2022). The sample with the highest DNA concentration was sample 3, with a value of 0.700 ng/µl. The DNA concentration can be increased through amplification to at least 1 ng/µLor higher so that it can be used for sequencing (Socea *et al*., 2023).

The Sanger method is commonly used in DNA sequencing to determine the nucleotide sequence of DNA. This method is based on amplifying the target DNA fragment, followed by the synthesis of a new DNA strand using the DNA polymerase enzyme—the addition of fluorescently labeled nucleotides that terminate DNA elongation results in DNA fragments of varying lengths. Sanger sequencing is capable of generating sequence reads of up to approximately ±1000 base pairs with very high accuracy, and therefore is often regarded as the gold standard for sequence confirmation and genetic analysis (Al-Shuhaib & Hashim, 2023).

The electrophoresis test showed a thin DNA band luminescence (Figure 1). The low sample concentration may be due to its storage period.

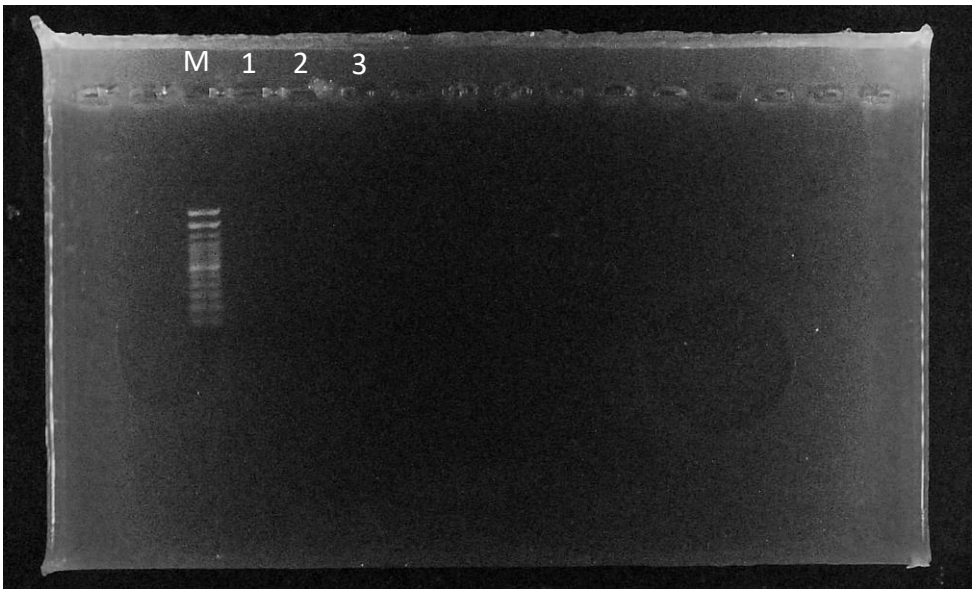

Figure 1. Electrophoresis results on bats' oropharyngeal swab samples (M: marker).

The advantage of oropharyngeal samples is their minimal invasiveness; however, the DNA concentration obtained may be relatively low. The oropharyngeal swab method has been successfully used in previous studies to sequence DNA to the species level in bats of the Vespertilionidae and Rhinolophidae in the UK (Huges *et al*., 2024). Other types of bat samples

that can be used include tissue samples (wing membranes/uropathageum), blood samples, and guano or fecal samples (Arnaout *et al*., 2022).

Factors that affect the quality and quantity of extracted DNA include sample type, the sample's initial condition, and storage period (Walker *et al*., 2019). Other types of samples, such as guano/fecal samples, are more easily degraded and susceptible to contamination because they are mixed with food waste and microbes (Guan *et al*., 2020). Tissue samples, such as wing membrane or blood, can be used to obtain higher DNA concentrations. However, the level of invasiveness is higher, given that C. brachyotis is a small animal; tissue sampling increases the risk of injury if not performed very carefully (Arnaout *et al*., 2022).

Despite advances in bat molecular identification, several research gaps remain evident:

1. Limited optimization studies focusing on increasing DNA yield from oropharyngeal swabs in fruit bats, particularly under tropical field conditions.
2. Lack of comparative analysis between different non-invasive sample types (oropharyngeal swab vs. guano vs. wing membrane) using identical extraction and quantification protocols.
3. Insufficient evaluation of downstream success, such as PCR amplification efficiency and sequencing outcomes, when DNA concentrations are below 1 ng/μL.
4. Minimal reporting on storage duration effects on DNA degradation for swab-based bat samples prior to extraction.
5. Scarcity of region-specific molecular baseline data for Indonesian bat species to support biodiversity monitoring and zoonotic surveillance.

Addressing these gaps would improve methodological reliability and expand the applicability of non-invasive molecular techniques in chiropteran research.

CONCLUSION

The electrophoresis results showed a thin DNA band, and the Qubit assay indicated a DNA concentration of 0.6-0.7 ng/μL. The quality and quantity of DNA can be optimized through amplification before proceeding to further molecular analysis stages.

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