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Oral Swab-Based DNA Extraction Of *Cynopterus Brachyotis:* An Initial Step For Species Confirmation

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Abstract

Background. Identifying bat species based on morphological characteristics often faces challenges due to character overlap among species, especially within cryptic species groups. *Cynopterus brachyotis* is one of the fruit bats with high genetic diversity but relatively uniform morphology.

Aims. Therefore, a molecular approach is needed to support accurate species confirmation. Under the HETI Research Grant of Innovation and Collaboration Batch 3- II Year 2025, this study aims to obtain genomic DNA from *C. brachyotis* bats caught at the Lampung Disease Investigation Center as the initial step of molecular species confirmation.

Methods. Samples were obtained by taking oral swabs on bat individuals caught using mist nets. DNA extraction is performed using silica membrane-based methods with commercial kits. Quantitative evaluation of DNA was performed using a Qubit Fluorometer, while qualitative evaluation was performed by agarose gel electrophoresis.

Conclusion. The results showed that the DNA concentration was in the low range (0.1–0.7 ng/µL), with a DNA band appearing very thin on electrophoresis.

Implementation. Nevertheless, the quantification results confirm that DNA was successfully extracted and remains suitable for advanced molecular analysis, with optimization at the DNA amplification stage.

Keywords: *Cynopterus brachyotis*, DNA extraction, Lampung Disease Investigation Center, oral swabs,

INTRODUCTION

Bats are a group of mammals with high diversity and have an important ecological role, especially as pollinators and seed dispersers (Marlinda *et al*., 2021; Schultes, 2003). One of the species of fruit bats that is commonly found in Indonesia is *Cynopterus brachyotis*. However, morphologically, the identification of this species is often limited by the similarity of physical characteristics between species in the genus Cynopterus, even including the *cryptic species* group (Campbell *et al*., 2004; Chattopadhyay *et al*., 2016).

DNA-based molecular approaches have been widely used to overcome the limitations of morphological identification because they provide more objective and accurate genetic information (Hebert *et al*., 2003). DNA analysis allows the identification of species based on differences in genetic sequences that are not always reflected in morphological characters, especially in groups of organisms with a high degree of phenotypic similarity. The molecular approach is considered a reliable method for more precise species confirmation. One of the genetic markers commonly used in taxonomic and phylogenetic studies is the cytochrome b gene (Farag *et al*., 2020). The cytochrome b gene shows a balanced degree of conservation and variation, making it effective for distinguishing closely related species (Kamagi *et al*., 2021).

The quality and quantity of the extracted DNA largely determine the success of molecular analysis. The DNA extraction is a crucial step in species confirmation research. This study is focused on DNA extraction from *Cynopterus. Brachyotis bats were* found in the Lampung Disease Investigation Center area using oral swab samples. The Lampung Disease Investigation Center is located in a semi-urban environment that supports wildlife, including bats. This habitat condition makes it a relevant research location, especially for studying the existence and molecular identification of Cynopterus brachyotis to complement morphological identification in groups of species with similar physical characteristics.

Identifying bat species, especially in the fruit bat group, remains challenging due to morphological similarities and the presence of *cryptic species*. *Cynopterus brachyotis* is known to have high genetic diversity but relatively uniform morphological variation, so morphological approaches often lead to identification uncertainty. To overcome these limitations, DNA-based molecular approaches have advanced significantly. They are now standard in modern taxonomy, primarily through the use of mitochondrial gene markers such as *cytochrome b* and DNA barcoding.

Several previous studies have shown that the quality and quantity of the extracted DNA mainly determine the success of molecular analysis. Generally, invasive tissue samples (e.g. wing or muscle tissue) are used to obtain high-quality DNA. However, this approach has ethical and conservation limitations, especially in wildlife. Therefore, non-invasive methods such as oral swabs are gaining attention, although they often yield DNA at low concentrations. This research is in the early stages of developing non-invasive methods to support the molecular identification of bats in Indonesia, particularly in semi-urban areas and for disease surveillance.

METHODS

Research under the Research Grant of Innovation and Collaboration Batch 3- II Year 2025, carried out in December 2025, in collaboration with the Lampung Disease Investigation Center. Bat life sampling using the mist-net method was conducted in the Lampung Disease Investigation Center area, Bandar Lampung. An oral swab sample was collected with a cotton swab, and DNA extraction was performed at the Biotechnology Laboratory of the Lampung Disease Investigation Center.

Oral swab samples are vortexed to ensure homogenization and release of biological material from the cotton swab into the medium. The sample is then prepared for DNA extraction.

DNA extraction was performed using the PureLink™ Viral DNA Mini Kit according to the manufacturer's silica membrane-based protocol. The sample was mixed with lysis buffers and proteinase K, then incubated to lyse the cells. After ethanol addition, the suspension is transferred to the spin column to bind the DNA to the silica membrane. The washing stage is performed to remove contaminants, followed by DNA elution with *RNase-free water*. The extracted DNA is stored at −20°C until further analysis.

DNA evaluation was performed quantitatively using a Qubit™ Fluorometer and qualitatively by agarose gel electrophoresis. DNA quantity was measured using 10 µL of extracted DNA mixed with 190 µL qubit™ reagent (199 µL dsDNA BR *Buffer* + 1 µL Qubit™ Reagent), homogenized slowly, incubated for 2 min at room temperature, and read using a Qubit™ Fluorometer (ng/µL). Quality analysis was performed using a 1% agarose gel prepared from 1 g of agarose in 100 mL of 1× TAE buffer, with the addition of SYBR® Safe DNA Gel Stain, and loaded into a chamber containing 1× TAE buffer. The DNA sample and DNA marker (6 µL) were inserted into the gel well, then electrophoresed for 35 minutes at 100 V and 300 mA. The results of electrophoresis were visualized under bluelight and documented using a camera connected to a computer via the EOS Utility app to capture DNA band images.

Although molecular approaches to bat identification have been widely developed, some research gaps remain unanswered:

1. There is a lack of studies that specifically evaluate the effectiveness of oral swabs as a source of DNA in *Cynopterus brachyotis* in Indonesia.

2. Lack of empirical data on the quantity and quality of DNA from oral swab extraction and its implications for advanced stages, such as PCR and sequencing.
3. There have not been many studies that link the local ecological context (semi-urban, disease investigation centers) to the success of non-invasive DNA extraction in bats.
4. Previous research has focused more on the final results of molecular identification, rather than on the early stages of DNA extraction as the foundation of successful follow-up analysis.

DISCUSSION

The main novelty of this research lies in:

1. **The use of oral swabs as a non-invasive method** for DNA extraction of *Cynopterus brachyotis* in Indonesia, which is still rarely reported.
2. **Focus on the early stages of molecular identification**, i.e. quantitative and qualitative evaluation of extracted DNA, as an important basis before PCR analysis and species confirmation.
3. **The context of the research location** at the Lampung Disease Investigation Center, which is relevant for the integration of biodiversity studies with disease surveillance and environmental health.
4. Provision of **baseline data** on the range of oral swab DNA concentration (0.1–0.7 ng/μL) which can be used as a reference for the optimization of DNA amplification methods in future studies.

The results of DNA quantification using the Qubit Fluorometer showed that the DNA concentration in the oral swab of Cynopterus brachyotis ranged from 0.1 to 0.7 ng/μL and remained detectable in all samples. This is consistent with the principle of Qubit fluorometry, which uses a specific dye for double-stranded DNA (dsDNA), resulting in high sensitivity for detecting DNA even at low concentrations and greater accuracy than conventional spectrophotometry methods (Nakayama *et al.*, 2016).

Qualitative analysis through agarose gel electrophoresis showed thin DNA bands (faint bands) (Figure 1). This thin band reflects a low amount of DNA, so the fluorescent intensity of the intercalated dye is not strong enough to be clearly visualized. The intensity of the bands in the electrophoresis gel is affected by the amount of DNA and the integrity of the fragments; samples with little or fragmented DNA produce faint bands (Wittmeier & Hummel, 2022).

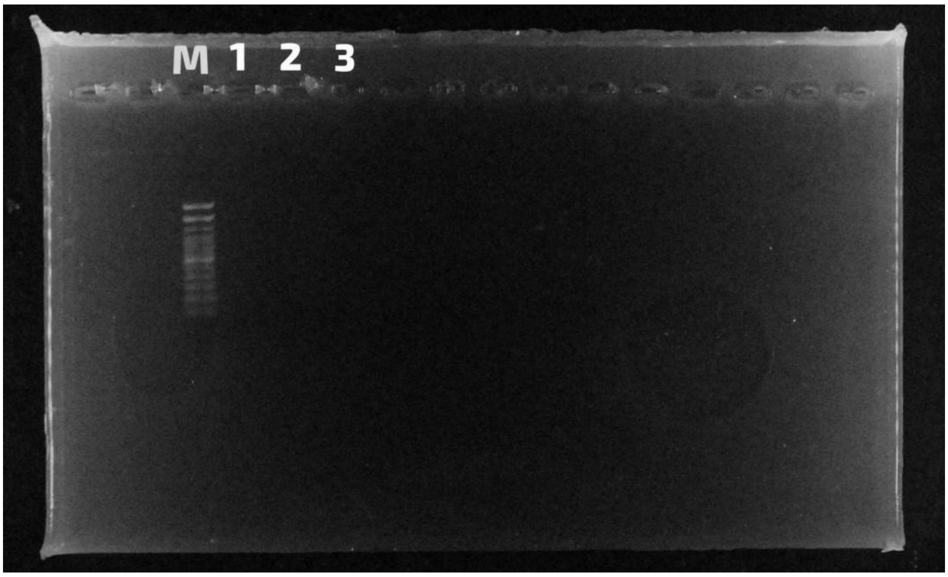

Figure 1. Results of electrophoresis of C. *brachyotis* oral swab samples

Fluorometry can detect small amounts of DNA despite its high fragmentation, while visualization of bands on gels requires larger amounts of DNA to produce clear signals. The two methods complement each other in assessing the success of DNA extraction (Cotes-Perdomo *et al*., 2025). Although the electrophoresis band is thin, the presence of DNA is still quantitatively confirmed. This suggests that the extracted DNA could still potentially be used in advanced molecular analysis, such as PCR for species confirmation. The success of PCR amplification in low-concentration samples depends on proper primer design, polymerase enzyme sensitivity, and optimal reaction conditions. Optimizing the number of PCR cycles, annealing temperature, or DNA concentration can increase the likelihood of amplifying the target DNA from small initial samples (Cotes-Perdomo *et al*., 2025).

CONCLUSION

DNA extraction from *Cynopterus brachyotis* oral swab samples obtained at the Lampung Disease Investigation Center was successful; the DNA concentration was relatively low (0.1–0.7 ng/μL), and the DNA band on electrophoresis appeared to be very thin. These results suggest that DNA from oral swab samples can still be obtained and potentially used for advanced molecular analysis, including species confirmation. This study confirms the importance of DNA extraction as the first step in molecular species identification, especially for species with similar morphologies.

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