

# The application of an eDNA metabarcoding method for bat studies コウモリ研究への環境 DNA メタバーコーディング手法の応用

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## 1. Introduction

Bat species play an important role in ecosystems, especially as efficient predators and pollinators. While bat populations are declining worldwide by anthropogenic effects, the scientific knowledge about their distribution and ecological information is limited partly because conventional methods such as capture by a mist net and harp trap are often not efficient enough.

I used an environmental DNA (eDNA) metabarcoding technique for understanding the distribution and ecology of bat species in caves. eDNA is genetic materials that are found in an environment and derived from organisms living in focusing habitat. Bats also use river water for drinking, and cave-dwelling bats shed their feces including their tissues into water flow inside of the cave. I hypothesized that those water provides a source for researchers to collect bats eDNA by which bat fauna inside a cave can be estimated without entering the cave.

## 2. Materials and Methods

I collected 45 water samples in four caves (Otomi dai-ichi cave, Otomi dai-ni cave, Yamadano dai-ichi cave, Yamadano dai-ni cave) in Iriomote-jima Island, Okinawa, Japan, in March in 2018 to August in 2019. I directly collected 500 ml of water from the water coming out of caves, with a syringe and a filter on site. After transported to the lab, I conducted DNA extraction, DNA amplification by MiMammal (mammal universal primer), and sequencing with a Next-generation sequencer (iSeq, Illumina). In addition, I conducted visual observation and used bat detector inside of caves with water sampling to compare the results from conventional and eDNA method.

## 3. Results and Discussion

I detected eDNA originated from three cave-dwelling bat species (Yaeyama little horseshoe bat *Rhinolophus perditus*, lesser leaf-nosed bat *Hipposideros turpis* and East-Asian little bent-winged bat *Miniopterus fuscus*) from 24 of the 45 water samples. Observed (visual and bat detector) bats inside the caves were all detected by eDNA metabarcoding on water samples which were collected at the outlet of cave rivers. In addition, eDNA was detected for two bat species without observation in seven points. It suggests that eDNA metabarcoding is a better tool for estimating their distribution.

## 4. Conclusion

This study suggests that eDNA metabarcoding has an advantage to investigate cave bat distributions. Given that we cannot observe their behavior or status inside caves by this method alone, however, we need to combine eDNA metabarcoding and other research tools with minimum disturbance for better understanding cave bat ecology and conservation of these species.