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Molecular identification of the ant *Pheidole nodus* in the diet of *Indotyphlops braminus* (Daudin, 1803) in Taiwan

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Typhlopids are known to forage on termites and ants, including their eggs, larvae, and adults (Punzo, 1974; Shine and Webb, 1990; Torres et al., 2000). However, few studies have identified the prey of the typhlopids to species level (Torres et al., 2000; Mizuno and Kojima, 2015; Jono et al., 2019). The Brahminy Blindsnake, *Indotyphlops braminus*, is a common blindsnake species inhabiting leaf litter or soil that has become widely distributed globally through transport in the trade of potted plants (Sidharthan et al., 2023). In Taiwan, *I. braminus* is widely distributed from low to mid-elevation. The diet of *I. braminus*, however, remains poorly known due to its fossorial habits. Here, we report the molecular identification of *Pheidole nodus* ants from the stomach contents of two blindsnakes from Taroko National Park, Taiwan.

On 23 May 2024, we conducted an amphibian and reptile night survey at Xibao, Taroko National Park, Taiwan (24.2069°N, 121.4822°E). Just after 20:00 h, we spotted two *I. braminus* within 100 m of each other. Both individuals were found on the trail next to short grass. To measure the snakes, we gently captured them by hand. Both individuals immediately regurgitated their stomach contents, which were transparent, elongated, and viscous to the touch (Fig. 1). The stomach contents also included some black remnants. To identify the prey items consumed, we preserved the stomach contents of the two snakes separately in 75% ethanol.

The stomach contents were first studied under a compound microscope. Both were found to contain ant body parts, including mandibles, legs, and setae (Fig. 2). Based on the number of remnants it appears that both snakes had fed on several ants within a short time period. Since the ant material was too fragmented for identification to species level, we used a sequencing-based approach. Part of the stomach contents was placed in a sterile Eppendorf tube and genomic DNA was extracted using the QIAamp DNA Mini Kit (Hilden, Germany). The partial mitochondrial cytochrome c oxidase subunit I gene (*COX1*) was amplified using the primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). The PCR conditions were: 2 min at 95°C

Figure 1. Stomach content regurgitated from *Indotyphlops braminus* found in Xibao, Taroko National Park, Taiwan on 23 May 2024.

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for initial denaturation followed by 35 cycles of 60 s at 95°C for denaturation, 60 s at 40°C for annealing, 90 s at 72°C for extension, and a final extension at 72°C for 7 min. The PCR product was confirmed by gel electrophoresis, purified, and then sent for sequencing. The *COX1* sequences from the stomach content showed > 99% similarity to sequences of Pheidole nodus deposited in GenBank (e.g., PQ349834.1).

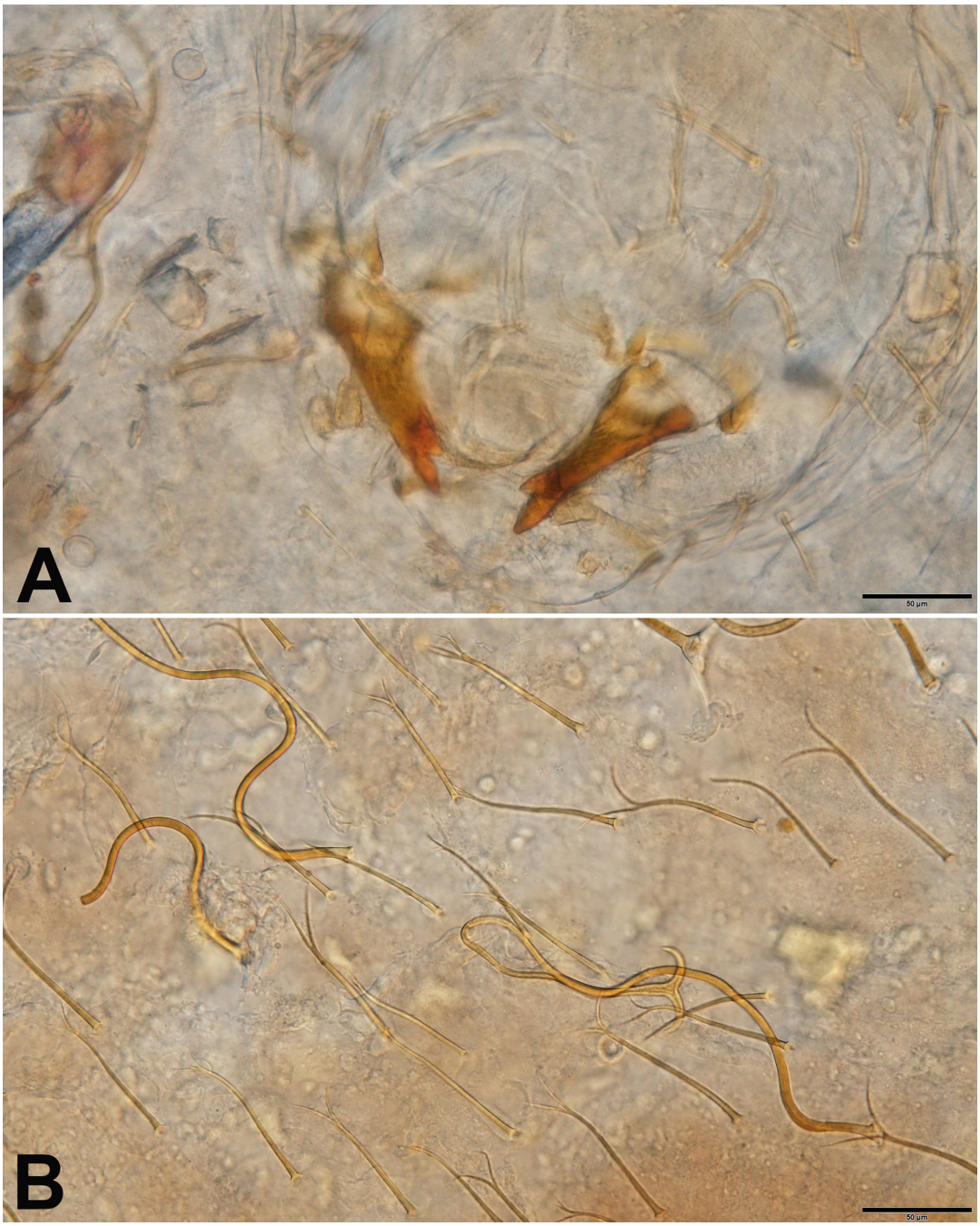

Figure 2. Remnants of *Pheidole nodus* from the stomach content of *Indotyphlops braminus* found in Xibao, Taroko National Park, Taiwan. (A) Mandibles. (B) Legs and setae. Images taken at 400x magnification, scale bar = 50 µm.

Prey selection can be limited by gape size of typhlopids (Webb and Shine, 1993). With their small body size and wide global distribution, *Pheidole* species often serve as an important food resource for ant specialists, including typhlopids (Shine and Webb, 1990; Torres et al., 2000; Sun et al., 2025). In addition, Watkins et al. (1967) and Webb and Shine (1992) indicated that typhlopids can differentiate among ant species. *Pheidole nodus* is a common species that can be found in forests or cultivated lands (Sarnat et al., 2015). Small body size and large populations in Taiwan can provide stable food resources for *I. braminus*. The diet of *I. braminus*, however, is often difficult to track due to its small body size and fossorial habits. In this report, we found that DNA sequencing is an ideal method for identifying the stomach contents of small snakes.

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