# Evaluating Turtle Ant (Hymenoptera: Formicidae) Survey Techniques in Northeast Trinidad

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**Abstract:** Methods of specimen collection can vary in effectiveness across taxa and geographic distribution. For the turtle ant of the genus *Cephalotes* (Hymenoptera: Formicidae), their attraction to urine and arboreal habitat make collection methods unique for this genus. Turtle ants have a widespread Neotropical distribution and are present in numerous environments. While most studies have been conducted in the Brazilian savannah, this study aims to test these methods in the coastal rainforest of northeast Trinidad. Altering the environment in which these survey methods are used has the potential to drastically change the effectiveness of these tools. With urine as the primary bait source, arboreal pitfall traps were placed, and bait was sprayed at the base of trees. Four species were collected, with a few individuals using both methods. Potentially due to differences in canopy height and nutritional preference, these collection methods proved ineffective in this environment.

*Keywords: Collection methods, turtle ants, Trinidad, urine bait, rainforest*

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*Cephalotes* is a genus of arboreal ants commonly known as turtle ants. The name turtle ant refers to their shield-like heads used by the soldier caste to prevent entry into their nests, a behavior known as phragmosis. (Gordon, Zelaya et al. 2019). The differences in morphology and behavior between and within species present a unique genus for evolutionary biologists to use as a model for diversification and ecological specialization. (Powell 2008, Powell, Price et al. 2020). Beyond this role in evolutionary and ecological research, the genus is known to have a symbiotic relationship with gut bacteria that can access nitrogen found in vertebrate urine. (Hu, Sanders et al. 2018). Vertebrate urine may also play a role in providing sodium to turtle ants in addition to nitrogen. (Kaspari, Yanoviak et al. 2009). It is assumed that these factors make urine an ideal attractant for turtle ants in field settings, and researchers have observed that urine results in a high degree of foraging. (Powell 2008).

Many studies have involved collecting and observing turtle ants using this method, but most have been restricted to the Brazilian Cerrado (Powell 2008, Powell, Costa et al. 2011, Powell and Peretz 2021). This environment consists of a shrub-covered inland savannah with low canopy woodlands

between 3-8 meters in height (Powell, Costa et al. 2011). Contrasting with the field site for this study, Toco, Trinidad, contains a coastal tropical rainforest with a canopy up to 50 meters in height (Shrivastava 2003). This study aims to evaluate commonly used turtle ant survey methods outlined in (Powell, Costa, et al. 2011) to see if they can be effectively used when studying turtle ants of the Caribbean. General protocols include arboreal pitfall traps wired to tree canopies to catch foraging ants. These pitfall traps consist of urine as bait mixed with water and detergent to kill foragers (Powell, Costa et al. 2011). An alternative method consists of spraying undiluted urine on the trees' bases, waiting for foragers to arrive, and then collecting them via aspirator (Powell and Peretz 2021). With differences in both ecosystem and climate, it is expected that these methods will not be as effective as collecting for surveys or experiments as in the Cerrado.

## Materials and Methods

**Pitfall Traps Setup.** Arboreal pitfall traps made from plastic urinalysis containers wired to trees were used to catch and survey turtle ant species. The containers were filled a quarter full of 1:1 urine and water with a drop of odorless dish soap (HEB, San Antonio, TX) to act as a killing agent. The traps were wired to 10 trees within a secondary forest in the Jammev Beach Resort in Toco, Trinidad. Two traps were placed approximately 2.5-3 meters from the base of the trunk on each tree using mechanic's wire (Harbor Freight, Calabasas, CA). These heights were chosen as they were the highest that could be reached using a ladder in the uneven terrain. Six trees were surveyed: trees along the bank of a local creek, trees with high canopy coverage, palm species, trees with low canopy coverage, fichus, and mahogany trees. Only one mahogany and fichus tree were surveyed, while two trees of the other types were surveyed. Traps were collected and refilled every 48 hours by pouring the pitfalls into a tea strainer and then transferring the specimens, regardless of taxa, into plastic whirl-pak bags (NASCO, Atlanta, GA) for later identification. The traps were then refilled to the same level with the same bait solution. (Powell et al. 2011)

**Spray Bottle Attraction.** A spray bottle with pure urine was used to attract turtle ants to the base of trees. Trees were checked after 20-30 minutes for turtle ant foragers. An aspirator (Bioquip, Compton, CA) collected as many individuals as possible from these foraging sites (Powell and Peretz, 2021). Other ant genera were also collected to see how effective this method was across the family. This method was used at Jammev Beach Resort in the same sampling site as the pitfalls, at trees along the Tompire River, at the shore near the Galera Point Lighthouse, and at La Foret Beach. All locations were in Northeastern Trinidad.

**Identification of Specimens.** All turtle ants collected were identified down to species using the key from *Diversity and adaptation of the ant genus Cephalotes* (Baroni and De Andrade, 1999). All other ants were identified down to genus using the *Identification Guide to Ant Genera of the World* (Bolton, 1994). Any other insects or arthropods caught were identified down to order.

## Results

**Spray Bottle Collection.** *C. minutus* and *C. atratus* were among the turtle ants collected

foraging from sprayed urine. C. atratus was collected from bamboo at the Tompire River, while *C. minutus* was collected from a palm tree at La Foret Beach. Otherwise, spraying at the base of trees at any location resulted in either minimal foraging by other ant genera or no ant activity even after 40-50 minutes. Specimens indicative of those foraging in areas sprayed with urine were collected, if any were found at all.

**Pitfall Traps.** Three turtle ant species were collected from the pitfalls, and four individuals were collected. These included one *C. pallens*, one *C. umbraculatus*, and two *C. minutus* individuals. Flies were the most common insect found in the traps, with most specimens belonging to the Dipteran families Phoridae, Drosophilidae, and Micropezidae. Roaches and termites in the order Blattodea were also common, with Termitidae termites making tunnels into the traps on several occasions (Fig 1). The ant genera Camponotus and Pseudomyrmex were among the most commonly collected in the pitfalls (Fig . 2)

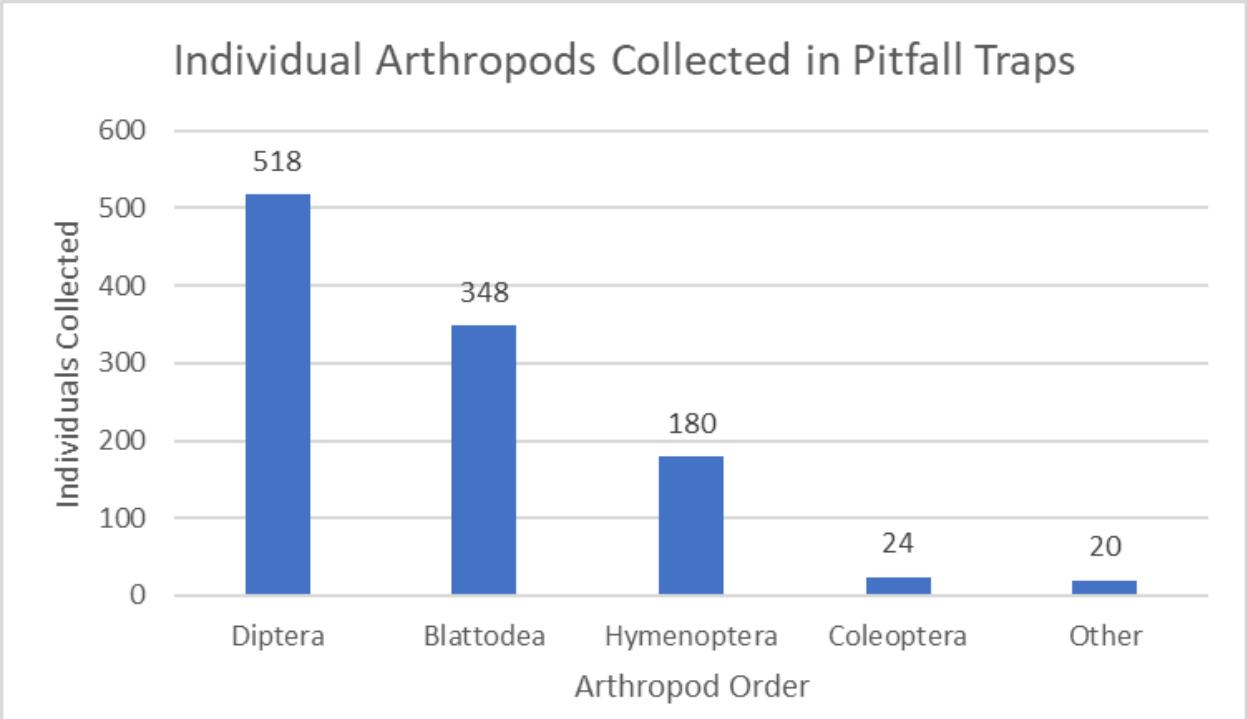


*Fig. 1 Arthropods collected by pitfall traps*

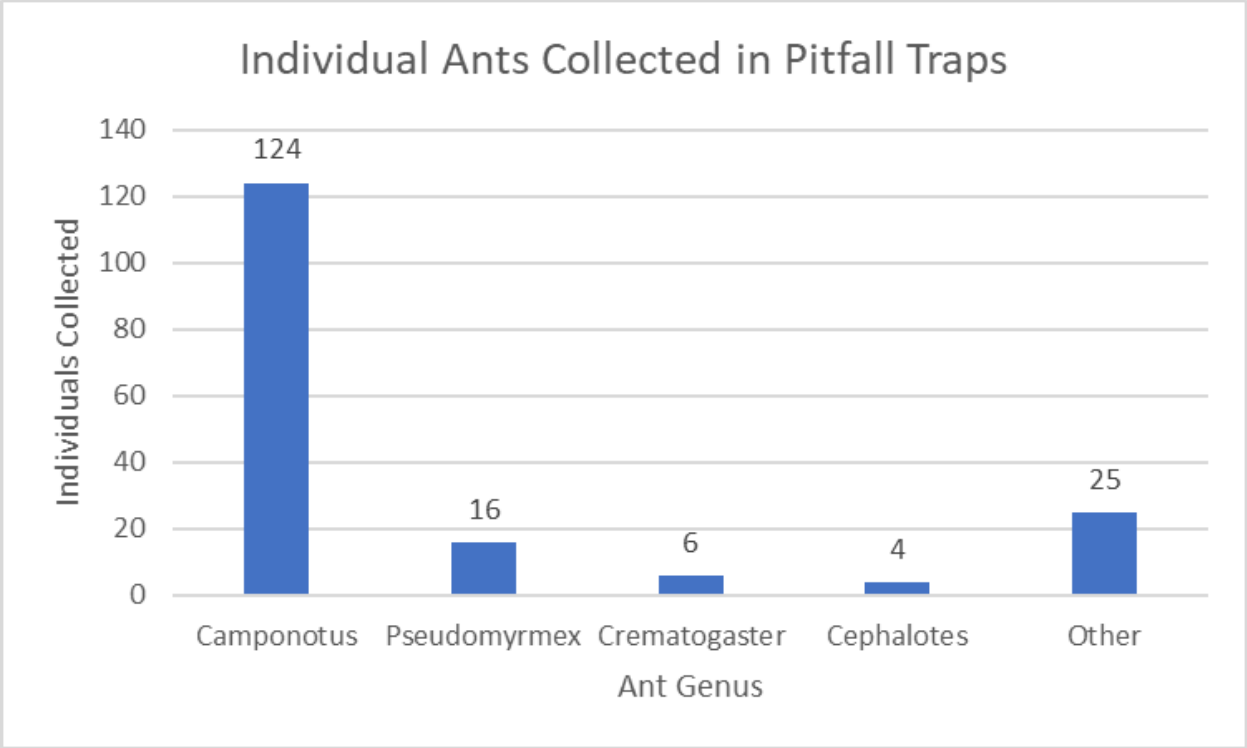


*Fig. 2 Ants collected by pitfall traps*

**Discussion**

The turtle ant genus *Cephalotes* is a diverse taxon of arboreal ants commonly used as models for caste specialization. (Powell 2008, Powell, Price et al. 2020). This can be attributed to the variation in phragmosis behaviors between species and vast distribution across Neotropical ecoregions. (Guénard, Weiser et al. 2017). Turtle ants have unique feeding behaviors, accessing nitrogen and sodium-rich food sources such as urine. (Hu, Sanders et al. 2018). Thus, the most effective bait for turtle ants consists of vertebrate urine (Powell 2008). However, most studies using this method have been restricted to inland savannah and not coastal tropical rainforests (Powell 2008, Powell, Costa et al. 2011, Powell and Peretz 2021).

Modeling after the protocol used in (Powell, Costa et al. 2011) For turtle ant surveys, the same methods were less effective at collecting turtle ants in Toco, Trinidad. Only four individuals comprising three species were collected from pitfall traps. According to the Global Ant Diversity Index (GABI) and personal observations, this does not come close to encompassing the diversity present on the island or in the region (Guénard, Weiser et al. 2017). The arboreal traps proved more effective in collecting individuals of other genera of arboreal ants rather than *Cephalotes*. Additionally, other insect orders were collected in higher abundance than Hymenoptera.

Several factors can be attributed to this lack of success: foraging preference concerning canopy level, urine volatility, dilution from rainwater, and coastal feeding preference. Established turtle ant foraging networks may be far higher than the location of the pitfalls. The presence of other ant genera may also indicate that the location of the pitfalls was dominated by these other genera foraging. If

location preference is not a factor, the urine itself may need to be concentrated enough to attract foragers. Distillation in both the protocol and rainwater may have reduced the attraction of the urine. Additionally, the urine itself may be less attractive to turtle ants living in coastal regions. It is thought that urine is an attractant to ants and other arthropods due to low sodium concentrations in different food sources (Kaspari, Yanoviak et al. 2009). With this forest being relatively close to the coast, these sodium requirements may already be met (Clay, Donoso et al. 2015). The explanation for why this method of surveying was ineffective is potentially caused by one or multiple factors. However, the use of spray baits does suggest that dilution of urine played a role.

The spray baits consisted of undiluted urine and did attract foragers of two turtle ant species with limited foraging from other genera. Despite the low species diversity at these sites, more individuals were seen foraging in less time than the pitfalls. The lack of abundant bycatch also made this method more straightforward to employ. However, this method may need help collecting foragers found in higher canopy levels or trees where other ant genera dominate lower levels.

To improve survey techniques in tropical coastal environments, future studies should test the effectiveness of arboreal pitfalls in both higher canopy levels and higher urine concentrations. It may also benefit surveys to be conducted during the dry season to reduce the amount of rainwater diluting the traps or to add coverings to prevent rainwater from diluting the baits. Ultimately, these methods need to be altered to effectively collect turtle ants in coastal tropical rainforests like that of Northeast Trinidad

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