Commensalism in Necrophagous Arthropods May Lead to the Discovery of Clandestine Burials

Aidan Holman[1*], Adrienne Brundage[1], Kevin Conway[2], Ryan Mohammed[3]

1. Department of Entomology, Texas A&M University, College Station, TX 77843, USA

2. Department of Ecology and Conservation Biology, Texas A&M University, College Station, TX 77843, USA

3. Department of Life Sciences, The University of the West Indies, St. Augustine, TT

*E-mail: crownboxco@tamu.edu

Abstract: Today, uncovering illicitly buried persons in homicide cases usually involves ground-penetrating radar and other electronic resistivity methods, and for emergency situations that is unlikely to change. However, taking advantage of what is visible above ground could reveal probative information regarding reduced fields of search of suspected areas. Insects are the most species-rich group of animals on the planet and many species of insect have the ability to dwell above and below ground. Ants, for example, can build nests that are sizeable enough to be noticed above ground but may also extend deep beneath the surface. In this study, insects were surveyed from sites that were designed to mimic clandestine burials related to homicide. Chicken bait was buried over a foot deep and left to decay for several days in a warm, forested area, near areas of noticeable ant activity. Daily observations and identification of insects on the ground surface directly over the burial site were conducted. Ants were the most prevalent at each site, however, they were also the most difficult to discern from far distances. Flies were easily discernable from up to distances of 27 ft, on average, but were only available after colonization of the carrion done by ants. Given these results, it is likely that necrophagous arthropods can be used to aid in the discovery of clandestine burials.

Keywords: Forensic entomology, necrophagous arthropods, clandestine burials, homicide

---

Introduction: It's easily understandable that objects visible to our eyes are less difficult to imagine than objects that are hidden. And yet, forensic scientists have made it their mission to imagine both scenarios, equally, as it often requires the interpretation of evidence to snag a perpetrator. One of the many examples is the very real entombment of victims of homicide. Clandestine burials are typically shallow, most often found no more than one meter below the ground, yet methods for finding corpses prove tedious (Pringle et al. 2021). Currently, methods to locate corpses associated with clandestine burials are limited to ground-penetrating radar, electrical resistivity tomography (ERT), fixed probe resistivity (FPR), and observational analysis of surface characteristics (or geophysical monitoring) (Berezowski et al. 2021; de Castro & de Cunha 2021; Solla et al. 2012; Schultz 2007; Barone & Di Maggio 2019; Pringle et al. 2021).

Ground-penetrating radar (GPR), the most efficient against the other resistivity methods (FPR and ERT), uses a device that transmits electromagnetic energy into the ground and detects the reflected waves that reach back to its antennae, due to the changes in dielectric permittivity in the subsurface (Berezowski et al. 2021; Pringle et al. 2021). Despite the preference for tools, all of these methods have one thing in common: they only tell us if the body is below the targeted area, not where to target. Presently, the only method for giving an accurate and precise area of targeting for those devices are Scent-detection canines or cadaver dogs (Perrault et al. 2015). These dogs are trained to locate the odor source that may be leaking organic volatile compounds unique to decaying flesh. Such canines are well-trained and show high efficacy in narrowing search areas for up to 6 weeks post-mortem (Perrault et al. 2015). Training these dogs is costly (upwards of $4,000 per dog) and only a handful of facilities in the United States offer this type of training (Wisner 2021).

Funding might be better spent elsewhere if a crime lab already possesses the appropriate equipment for a specific crime. For example, nearly all (98%) crime labs in the United States employed standard protocols for DNA analysis–the most praised method for victim identification (Peterson & Hickman 2005). The volatile leakage from corpses underground aren't only identifiable by Canids, but they will also attract necrophagous insects and other arthropods like ants, beetles, flies, and spiders (Payne 1965). Of which ants and beetles are some of the most species-rich group of insects that are known to dominate the "underworld" and the biosphere (Fenton 1947). And because of the already profound studies involving ants and their interactions with buried carrion (and their commonness), they've become the inimitable focus of study for possible clandestine burial-discovery methods (Eubanks et al. 2019).

Ants are eusocial insects that build large complex underground structures, maximizing at about 4 meters below ground (well within the one-meter average for most buried bodies) (Tschinkel 2003). With these large underground systems, ants can essentially "see" a greater deal of things that occur below the surface that are out of our plane of vision. And if these tunnels extend to the carrion, they open the possibility for other small necrophagous animal traffic like spiders, flies, beetles, and more. Consequently, looking for trace evidence among these insects can reveal what happens below the surface, specifically through diet tests in the examination and analysis of bile and fecal content. For example, fecal analysis of insects, like flies, can occur after purposely allowing the colonization of a febrile person for a diagnosis, known as Xenodiagnosis (Schenone 1999). Other studies that have proven the direct transmission of human DNA from insects also exist (Durdle 2020).

The goal of the present study was to determine which arthropods are attracted to and able to reach buried carrion and about how long until they're visible above ground, including how well they are at being visible. Because insects are proven capable of containing human DNA (Durdle 2020), this experimentation expresses how difficult finding the insects or other arthropods that might contain the DNA would be. The results may assist forensic scientists and crime scene search teams by introducing new avenues of study to aid in the discovery of clandestine burials.

Materials & Methods:

Preparation of Carrion. Two whole 3-3.4 lb, food-grade chickens (*Arawak Ltd., Tunapuna-Piarco, Trinidad and Tobago*) were used to simulate carrion. Numerous 1.5"-deep slits, resembling stab wounds in homicide victims, were introduced onto the surface of the chicken using a chef's knife.

Preparation of Burial Site. Following previous work (Pringle et al. 2021), two different sites were selected for carrion burial based on proximity to ant activity. Ants were collected near both would-be sites. These ants were identified as *Ectatomma* sp., species of ponerine ants that are known for their food-robbing behavior. This indicated the species was necrophagous and should be found near other ant nests, if not their own. Their diet confirmed the areas of choice for (burial) site placement where these ants were discovered.

Figure 1. *Left: Burial Site 1 depth, surface-level aligned with the 13" mark from the bottom of the hole. Right: Burial Site 2 depth, surface-level aligned with 13.8" from the bottom of the hole.*

Each burial site was positioned approximately 5-6 feet away from the observed activity, one towards an area with large amounts of deciduous trees (Site 1) and the other in an area with little to no tree or grass cover (Site 2). Both holes were dug to be deeper than 1 foot deep (Site 1 to a maximum depth of 13" deep; Site 2 to a maximum depth of 13.8"; see Figure 1), without paying close attention to uniformity in depth across the burial site, as is common with clandestine burial sites (Pringle et al. 2021). Each carrion was carefully placed into each burial site. Using the dirt dug to create the site, the site was then re-covered. A pink ribbon was tied around a branch above the burial site to indicate the experimental area (see Figure 2). The geographic coordinates of each site were obtained using Google Maps (see Appendix A).

Figure 2. *Black arrow points towards the indicative pink ribbons tied to each branch. Burial sites are circled in red.*

Post-burial Activity. During the daytime (between 8:30 a.m. and no later than 4:30 p.m.), daily and prior to any rain, representative samples of observed insects were collected for identification. The collection of flies during daily observations was done using a sweep-net, as were larger ants. Insect activity was visually observed at maximum distances.

Carrion was exhumed 10 days after the initial burial and all insects that were present in the uprooted soil were collected. Larvae were first killed in hot water and then placed into 75% ethanol for preservation, while adults were placed directly into 75% ethanol for curation. Arthropods were identified, with a 50X-1300X Digital Microscope (*TOMLOV, Shenzhen, Guangdong*), to the lowest taxonomic level using available keys (Bolton 1994; Carvalho & Mello-Patiu 2008; e Vairo et al. 2015; Seago 1953; Wegner 2011).

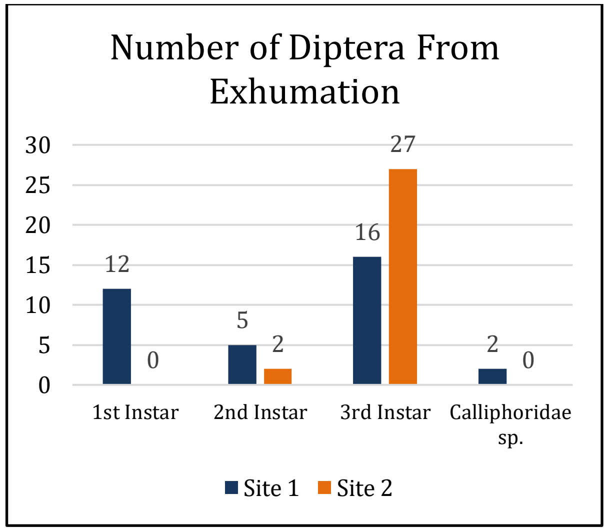

Figure 3. *Number of Diptera larvae identified from each site after exhumation. 1st-3rd instars are for Sarcophigidae spp.*

Results: After the first day, observations were made at each site yielding no ant or other insect activity. After two days, ants were observed, however, it was difficult to do so standing without leaning very closely toward the site. The ants observed were very small, and upon microscopic examination, were revealed to be fire ants (Genus *Solenopsis*). After three days, flies and ants (Genera: *Solenopsis* and *Ectatomma*–see Appendix B) could be found over Site 2, and the same for Site 1 on Day 4. Flies were identified to be from the Family Sarcophigidae (see Appendix B), with *Sarcophaga africa* being the most common species present from collection above ground. Flies were also observed going inside tunnels in the soil directly above each burial site, which may have been built by ants.

On Day 5, before sampling, another student (5’9” or 1.75 m tall) was asked to determine how far away he could stand before insect activity was indiscernible. The only insect found to be most noticeable and discernible to the student were the flesh flies that consistently circled above the site during the observation event. At Site 1, this distance was 24.42 ft (7.44 m). At Site 2, this distance was 29.58 ft (9.02 m), thus the average being 27 ft (8.23 m).

On Day 6, there were only two discernable flies (*Sarcophaga africa*) at Site 1 and zero at Site 2. At Site 2, instead, there was a non-uniform hole of about one foot in length, 6 inches in width, and 3.5 inches deep at its deepest part, which contained 4 ghost spiders (Araneae: Anyphaenidae). These were the most common spiders found with sweep-netting and visual analyses forms of collection of spiders done in the area. *Solenopsis* sp. showed similar activity as reported for previous days at both sites.

Days 7 and 8 consisted of heavy rainfall. No insect activity appeared directly after heavy rainfall (0-2 hours) for either site. However, hours after light rainfall, *Sarcophaga africa* and *Solenopsis* sp. returned to Site 2. By Day 8, The hole from Day 6 had become deep enough to expose the surface of the carrion. At this point in the study, the actively decaying carrion and the soil immediately surrounding the carrion were dug up before any more disturbance could occur. The larvae and pupae from exhumation were identified as Sarcophagidae among the two 2nd-instar larvae, twenty-seven 3rd-instar larvae, and three pupae collected (see Figure 3). A mole cricket (Orthoptera: Gryllotalpidae) was also found in the collected soil near the carrion.

On Day 9, Site 1's surface had been re-colonized by Sarcophagidae and *Solenopsis* sp. The surface above the carrion had sunken to almost an inch in depth below surface level. Upon exhumation of Site 1 (Day 10), the carrion was found to be in active decay (almost indistinguishable from Site 2's carrion). Larvae and pupae were identified as Sarcophagidae among the twelve 1st-instar larvae, five 2nd-instar larvae, sixteen 3rd-instar larvae, and one pupa collected (see Figure 3). Also, within the soil and the carrion collected, existed two blowfly larvae (Diptera: Calliphoridae), and four unidentified black Coleopteran larvae.

Discussion: Our results showed that arthropod activity at burials, without protective or inorganic wrappage, can start to show between 24 hours and 48 hours (1-2 days). Discernable insect activity from afar was noticeable beginning between 48 hours and 96 hours (2-4 days) and was most often due to Dipteran presence (specifically Family: Sarcophagidae). The farthest distance away, on average, before discernment of Diptera was unlikely was 27 ft (or 8.23 m).

Problems arose starting on Day 6 when agricultural burning took place around 1 pm and lasted for about 2 hours. This may have affected the amount of Diptera at both sites. As for the hole that appeared at Site 2, upon chatting with the other local residents on the property, the reasons given were consistent with stray dogs that roam the area regularly, some hungry. We know that cadaver dogs can locate buried flesh from the gaseous leakage of the carrion so this explanation has a strong basis (Perrault et al. 2015). The absence of arthropod activity on Days 7 and 8, specifically Dipteran, is the consequence of heavy rainfall. However, ants, Solenoptids, and some Ectatommids were visible at both sites the morning of Day 8, following light rainfall, as were some *Sarcophaga africa* at Site 2. This data is coherent with Krüger and Azevedo (2013) where low humidity and higher temperatures showed the greatest Dipteran presence, with high humidity and higher temperatures showing a lower Dipteran presence (Azevedo & Krüger 2013). But as pointed out earlier, the activity at Site 2 existed when the carrion was bare and unrestricted by inches of soil.

During daily observations, the Diptera were seen using the tunnels the ants may have dug to reach the carrion. This is coherent with the data that supports ants' direct and indirect involvement with the decomposition of carrion through alteration of the colonization of other necrophagous animals (Eubanks et al. 2019). What's more is in common practice (even today), discovering the presence of fly-related material in clandestine graves is indicative of body relocation (Mansegosa et al. 2021) from previous studies showing flies won't tunnel into compact soil as deep as 20cm (Gunn & Bird 2011). Here, however, we show that flies can use tunnels built by ants to reach the carrion even after the body has been buried. Being so, ants were the most (consistently) prevalent insect at both sites throughout the experiment. However, the Solenoptids that were available were less than 1mm in length which made it very difficult to see their presence without a close-up examination of each site. Ectatommids were also present but less consistently. They were more discernable than the Solenoptids due to their larger size but still required close-up (not as close as one would need to get to see the Solenoptids but still close enough that distances beyond 4 foot couldn't discern them) examination for lack of a visible colony.

Another factor that's important to consider when using ants is diet. While most ants are opportunistic (meaning they consume nutrients when and where available), not all are, some are selective. Leaf-cutter ants (Genus: Atta), for example, have a diet that disagrees with decaying flesh, and they won't go near it if at all possible, and therefore would be unlikely to carry conerning human DNA (Eubanks et al. 2019). This would indicate that forensic search teams would need some entomological knowledge surrounding ant biology and identification to avoid collecting ants that wouldn't aid in burial discovery.

During daily observations, there was no clear difference in the number of adult flies (at least more than four) above ground at each site, before rainfall occurred. Upon the quantification of Diptera presence from larval masses identified from each site after exhumation, Site 1 contained the greater amount. Site 1 also had one other family of fly larvae (Calliphoridae) present whereas Site 2 only had Sarcophagidae larvae. Despite previous literature's data (Dietze 2020) representing above-ground carrion-feeding, where flies prefer shaded areas to sunlit ones, it is interesting to see a similar pattern with underground carrion feeding.

Overall, using insects and other arthropods as indications for locating clandestine burials appears graspable from this data. It's suggested that necrophagous arthropods, like the ones listed within this paper, be sampled at different locations within a suspected area for human DNA remnants. A positive DNA result might reduce the field of search within the suspected area, in relation to where the insect (that was positive for human DNA) was collected. Despite DNA's popularity among U.S. crime labs (Peterson & Hickman 2005), current methods for discovering clandestine burials–GPR, ERT, FPR, geophysical monitoring, and the less popular, cadaver dogs–don't use DNA analysis (Berezowski et al. 2021; de Castro & de Cunha 2021; Solla et al. 2012; Schultz 2007; Barone & Di Maggio 2019; Pringle et al. 2021; Perrault et al. 2015; Wisner 2021). Outside of project direction, we observed commensalism between ants and flies (not solely) that opens new avenues for the meaning of fly material found in clandestine burials than was previously understood.

Acknowledgments: Thank you to the Wildlife Section of the Forestry Division of Trinidad for granting permission to conduct scientific collection of arthropods. Thank you to the 2022 Texas A&M Trinidad and Tobago Study Abroad group for everyone's acclamation and passive motivation through the hard work they put into finishing their projects and helping others with theirs. Thank you, Zachary Twomey, for serving as our model for the farthest distance possible before indiscernible insect activity. Thank you to our two wonderful mentors, Drs. Adrienne Brundage and Kevin Conway, for your patience, tolerance, bodies of expertise, and for working with us to make this trip possible through strict precautions and dedication against contracting the infamous COVID-19.

Literature Cited

Azevedo, R. R., & Krüger, R. F. (2013). The influence of temperature and humidity on abundance and richness of Calliphoridae (Diptera). *Iheringia. Série Zoologia*, 103(2), 145-152.

Balme, G. R., Denning, S. S., Cammack, J. A., & Watson, D. W. (2012). Blow flies (Diptera: Calliphoridae) survive burial: Evidence of ascending vertical dispersal. *Forensic Science International*, 216(1-3), e1-e4.

Barone, P. M., & Di Maggio, R. M. (2019). Forensic geophysics: ground penetrating radar (GPR) techniques and missing persons investigations. *Forensic Sciences Research*, 4(4), 337-340.

Berezowski, V., Mallett, X., Ellis, J., & Moffat, I. (2021). Using ground penetrating radar and resistivity methods to locate unmarked graves: a review. *Remote Sensing*, 13(15), 2880.

Bolton, B. (1994). Identification guide to the ant genera of the world (No. C/595.796 B65).

Carvalho, C. J. B. D., and C. A. D. Mello-Patiu. (2008). Key to the adults of the most common forensic species of Diptera in South America. *Revista Brasileira de Entomologia*. 52: 390–406.

de Castro, K. C. P. L., & da Cunha, L. S. (2021). Forensic Investigations With The Identification Of Human Remains With Ground Penetrating Radar (Gpr): A Review Pesquisas Forenses Com A Identificação De Vestígios Humanos Com O Radar De Penetração Do Solo (Gpr): Uma Revisão.

Dietze, K. (2020). Effect of Sunlight Versus Shade on the Magnitude of Presence of Forensically Important Species of Diptera in Northeastern Trinidad. Instars: *A Journal of Student Research*, 6(1).

Durdle, A. (2020). Insects as vectors of DNA in a forensic context. *Wiley Interdisciplinary Reviews: Forensic Science*, 2(2), e1355.

e Vairo, K. P., Moura, M. O., & de Mello-Patiu, C. A. (2015). Comparative morphology and identification key for females of nine Sarcophagidae species (Diptera) with forensic importance in Southern Brazil. *Revista Brasileira de Entomologia*, 59(3), 177-187.

Eubanks, M. D., Lin, C., & Tarone, A. M. (2019). The role of ants in vertebrate carrion decomposition. *Food Webs*, 18, e00109.

Fenton, G. R. (1947). The soil fauna: With special reference to the ecosystem of forest soil. *The Journal of Animal Ecology*, 76-93.

Gunn, A., & Bird, J. (2011). The ability of the blowflies Calliphora vomitoria (Linnaeus), Calliphora vicina (Rob-Desvoidy) and Lucilia sericata (Meigen)(Diptera: Calliphoridae) and the muscid flies Muscina stabulans (Fallén) and Muscina prolapsa (Harris)(Diptera: Muscidae) to colonise buried remains. *Forensic Science International*, 207(1-3), 198-204.

Mansegosa, D. A., Giannotti, P. S., Marchiori, J. I., Jofré, F. N., Aballay, F. H., & Aisa, C. F. (2021). The story of a homicide: The location, exhumation, and multidisciplinary

analysis of a clandestine burial. *Forensic Science International: Reports*, 3, 100165.
Payne, J. A. (1965). A summer carrion study of the baby pig Sus scrofa Linnaeus. *Ecology, 46*(5), 592-602.
Perrault, K. A., Stefanuto, P. H., Stuart, B. H., Rai, T., Focant, J. F., & Forbes, S. L. (2015). Detection of decomposition volatile organic compounds in soil following removal of remains from a surface deposition site. *Forensic science, medicine, and pathology*, 11(3), 376-387.
Peterson, J. L., & Hickman, M. J. (2005). Census of publicly funded forensic crime laboratories, 2002. Washington, DC: *US Department of Justice, Office of Justice Programs, Bureau of Justice Statistics*.
Pringle, J. K., Stimpson, I. G., Wisniewski, K. D., Heaton, V., Davenward, B., Mirosch, N., ... & Jervis, J. R. (2021). Geophysical monitoring of simulated clandestine burials of murder victims to aid forensic investigators. *Geology Today*, 37(2), 63-65.
Schenone, H. (1999). Xenodiagnosis. Memórias do Instituto Oswaldo Cruz, 94, 289-294.
Schultz, J. J. (2007). Using ground-penetrating radar to locate clandestine graves of homicide victims: forming forensic archaeology partnerships with law enforcement. *Homicide Studies*, 11(1), 15-29.
Seago, J. M. (1953). Fly larvae: pictorial key to some common species. US Department of Health, Education and Welfare Public Health Service, Communicable Disease Center, Training Branch, Atlanta, GA.
Solla, M., Riveiro, B., Álvarez, M. X., & Arias, P. (2012). Experimental forensic scenes for the characterization of ground-penetrating radar wave response. *Forensic science international*, 220(1-3), 50-58.
Tschinkel, W. R. (2003). Subterranean ant nests: trace fossils past and future?. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 192(1-4), 321-333.
Wegner, G. S. (2011). Spider Identification Guide. *BASF The Chemical Company*, 1-55.
Wisner, H. R. (2021). It Costs an Arm and a Leg: Cadaver Dog Workability Study (Doctoral dissertation, Prescott College).

Appendix A

Summary: Pages 10 and 11 contain geographic images of the experiment in relation to the beach resort property, ant activity and each site, and Toco, Trinidad, as well as what surrounds it.

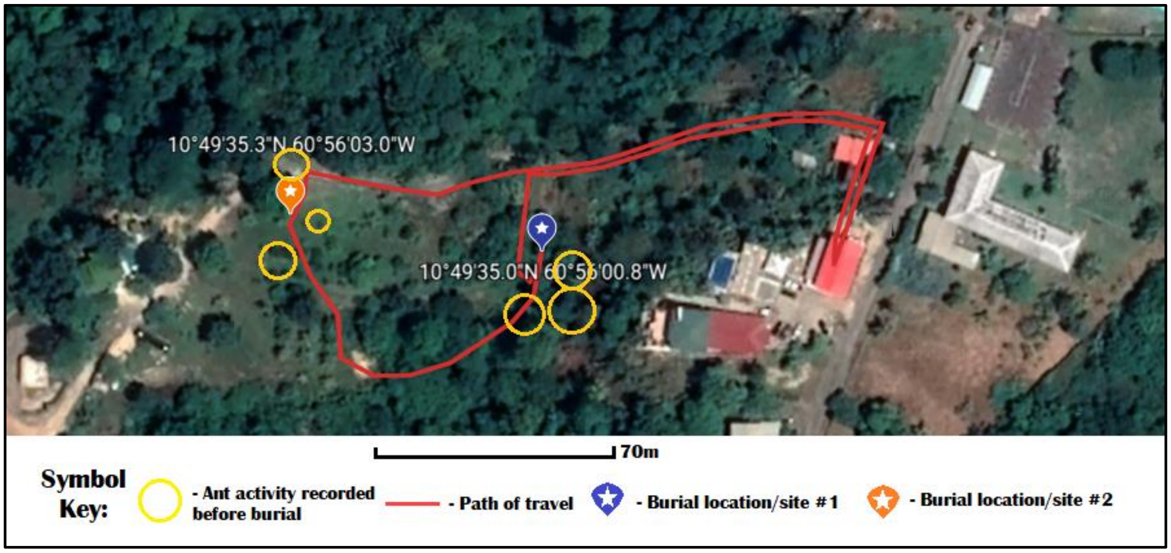

Figure 4. *2-Dimensional satellite map showing forestry and markers for burial sites, ant activity, and travel path in respect to the Jammev Beach Resort in Toco, Trinidad*

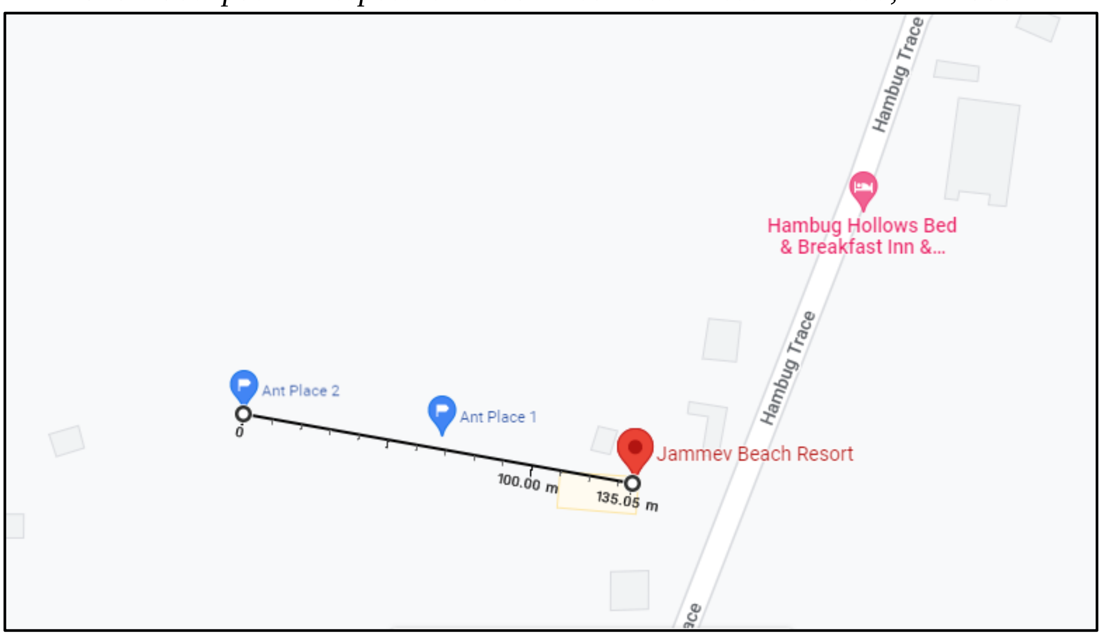

Figure 5. *2-Dimensional satellite view (without terrain mode from Google Maps) of experiment markers in respect to the Jammev Beach Resort in Toco, Trinidad*

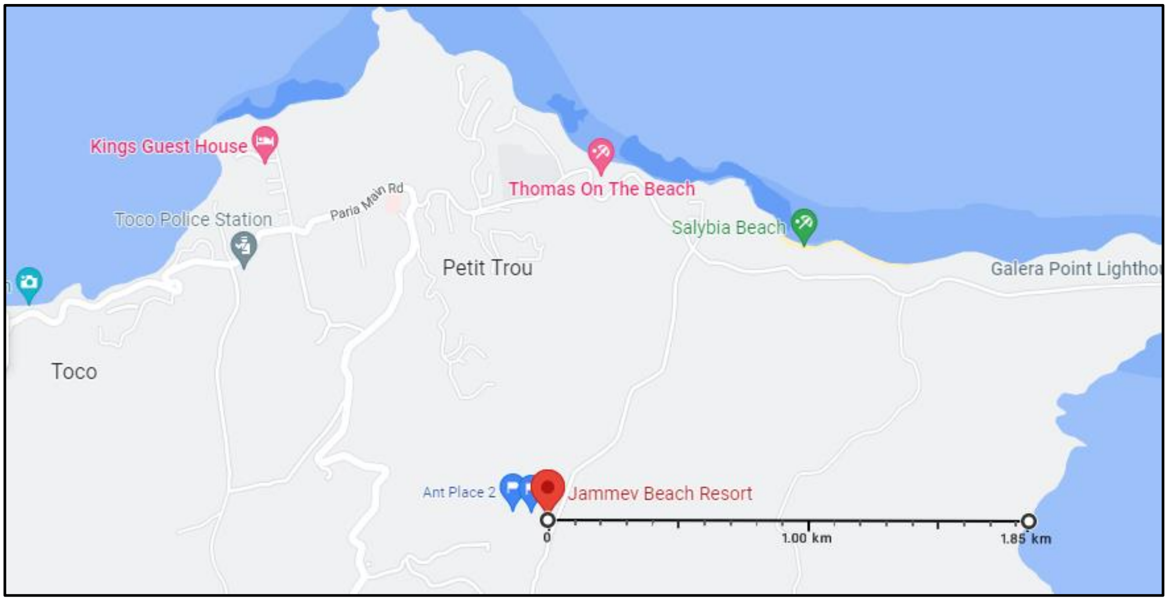

Figure 6. *2-Dimensional satellite view of burial sites and the Jammev Beach Resort in respect to a broader area of Toco and (distance from) the Caribbean Sea*

Appendix B

Summary: Photos of common arthropods collected at each site

Figure 7. Fire ant (Genus: Solenopsis) consistently present at each Site. Left: circled are the red jaws. Right: circled is the characteristic and prominent petiole pattern (two lumps)

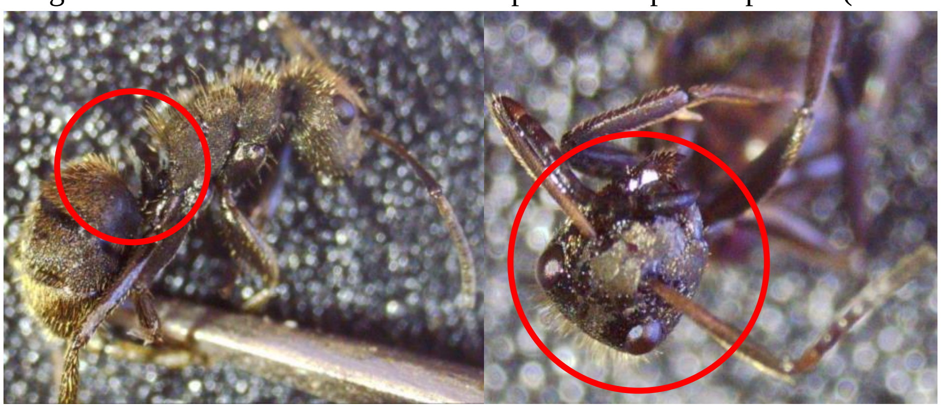

Figure 8. Ponerine ant (Genus: Ectatomma). Left: lateral view, circled is the characteristic prominent petiole pattern (one lump). Right: circled is the head, including large mandibles and anterior head shape

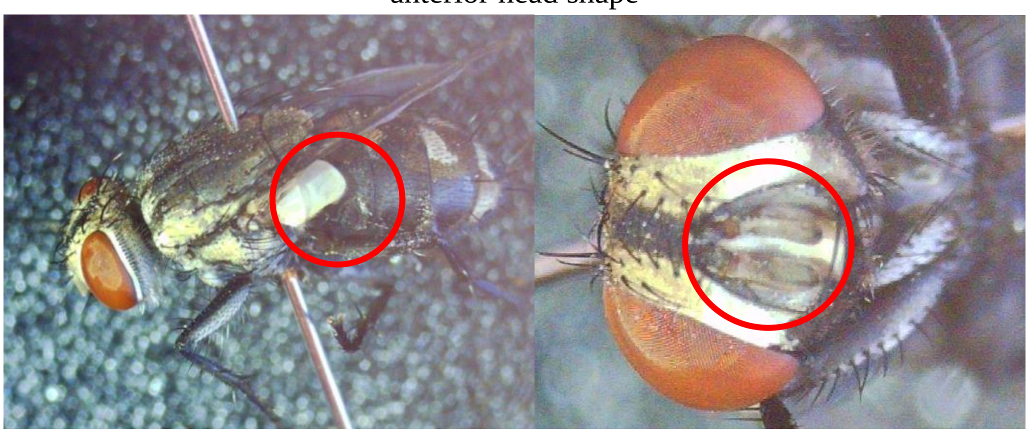

Figure 9. Female adult *Sarcophaga africa* (Diptera: Sarcophagidae). Left: lateral view, circled is the well-developed calypter. Right: anterior view, circle highlights the presence of the ptilinal fissure and lunule