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Adetimehin, A. D., Mole, C. G., Finaughty, D. A., & Heyns, M. (2022). Caught in the act: impact of *Crematogaster* cf. *liengmei* (Hymenoptera: Formicidae) necrophagous behavior on neonate pigs (*Sus scrofa domesticus* L.) in the Western Cape Province of South Africa. *International journal of legal medicine*. Advance online publication. <https://doi.org/10.1007/s00414-022-02835-9>

[Link to publication record in Ulster University Research Portal](#)

Published in:

International journal of legal medicine

Publication Status:

Published (in print/issue): 07/05/2022

DOI:

[10.1007/s00414-022-02835-9](https://doi.org/10.1007/s00414-022-02835-9)

Document Version

Author Accepted version

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Caught in the act: impact of *Crematogaster cf. liengmei* (Hymenoptera: Formicidae) necrophagous behaviour on neonate pigs (*Sus scrofa domestica* L.) in the Western Cape Province of South Africa

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Abstract

Ants have been documented as part of the entomo-sarcosaprophagous community. They have been known to alter the process of carcass decomposition due to their ability to feed on fly eggs/larvae and create post-mortem skin injuries. However, studies on the impact of ants on decomposing carcasses are scarce, especially within the Western Cape Province of South Africa. This study was part of a research project that utilized two (2) neonate pig carcasses in each month of the year to establish baseline data on the insect species associated with decomposing carcasses in the Western Cape Province of South Africa. In the early spring (September 2020), mid-autumn (May 2021), and mid-winter (July 2021) trials respectively, several individuals of *Crematogaster cf. liengmei* colonized the pig carcasses shortly after deployment. There, they fed on the flesh of the carcasses and further inflicted bite marks and conspicuous post-mortem skin lesions. Following the reduction in the presence of *Crematogaster cf. liengmei* specifically in the mid-winter trial, non-vital bleeding was observed as a consequence of the skin lesions. In the early spring, mid- and late autumn (May 2021), and early (June 2021) and mid-winter trials respectively, *Crematogaster cf. liengmei* prevented the formation of large maggot masses, principally through the predation of fly eggs, larvae, and adults. The observations recorded in this study are of considerable importance in forensic investigations as the effect of the necrophagous behaviour of *Crematogaster cf. liengmei* on decomposing remains can be misinterpreted by inexperienced investigators during crime scene investigations and may alter post-mortem interval estimations.

Keywords: Ants, *Crematogaster cf. liengmei*, Forensic entomology, Decomposition, Post-mortem interval, Season

Introduction

A decomposing human or animal cadaver is a nutrient-rich and energy-filled ephemeral resource that is a key component of nutrient cycling in all ecological systems [1]. This resource is attractive to a wide range of local fauna including vertebrate and invertebrate obligate and facultative scavengers, insectivorous predators, and incidental species, all capable of utilizing it as a source of food, site of reproduction, or a provisional environment for predation and parasitism [1 – 3]. The bulk of local fauna associated with decomposing human and animal remains are members of the class Insecta. Within the class Insecta, insects belonging to the orders Diptera (flies) and Coleoptera (beetles) are by far the most studied groups in forensic entomology due to their ability to utilize decomposing vertebrate remains as a source of food and/or breeding site [4 – 6]. Furthermore, the adults and immature stages of these two insect groups have consistently proven useful in the estimation of the minimum post-mortem interval of an individual or animal [5, 7].

Ants (Hymenoptera: Formicidae) are known to be one of the most ubiquitous and ecologically dominant insect groups found in different terrestrial environments [8, 9]. This insect group possesses the ability to exploit a wide range of nutritional resources ranging from plants to microorganisms (e.g., fungi) and decomposing vertebrate remains [9, 10]. In the literature, several species of ants have been documented to be one of the first responders to decomposing vertebrate remains [9 – 11]. Furthermore, they have been reported to be present in all the stages of decomposition of vertebrate remains [9, 12 – 16]. A recent review study by Eubanks et al. [9] documented a total of 155 ant species to be associated with decomposing vertebrate carcasses in different parts of the world.

Several studies have described the direct and indirect impacts of different ant species on the decomposition processes of human and animal remains [8 – 10,

16, 17]. Different species of ants have been documented to sever and/or remove pieces of flesh away from decomposing vertebrate carrion [9]. This leads to the creation of post-mortem skin lesions on decomposing remains, which may alter existing trauma or mimic trauma [8 – 11, 18]. Ants may also alter the decomposition process by feeding directly on the carrion, or on the immature and/or adult stages of other carrion insects [9]. Furthermore, tissue damage caused by ants can be used as entry or oviposition sites by other insects [8, 9, 11, 18 – 20]. Bleeding as a consequence of the skin lesions caused by the feeding activities of ants has also been reported [21]. All of these could potentially result in an error in both post-mortem interval estimation and the determination of the cause of death [8 – 11, 18].

In South Africa, several ant species have been documented to be associated with the remains of different animal species (i.e., pigs, and impala) and at different stages of decomposition [22 – 24]. However, studies clearly describing the impacts of ants on decomposing vertebrate remains are scarce [25]. For instance, in the Free State Province, Kolver [25] found several ants preying on the eggs and larvae of flies and further inflicting some damages on the skin of a burnt pig carcass. Similarly, Botham [26] observed several *Anoplolepis custodiens* (Hymenoptera: Formicidae) individuals preying on old and newly emerged adult flies close to a decomposing pig carcass. To the best of our knowledge, we are not aware of any published studies describing the impact of ants on decomposing carcasses especially within the Western Cape Province of South Africa. Consequently, we report for the first time in the province, the impact of the necrophagous behaviour of *Crematogaster* cf. *liengmei* (Hymenoptera: Formicidae) on decomposing neonate pigs in different months/seasons of the year.

Materials and methods

Study site

This study formed part of a larger insect successional and decomposition study conducted within the area of Table Mountain National Park, adjacent to the University of Cape Town Upper Campus, Rondebosch, South Africa (S33° 57.164' E; E018° 27.733') between 2020 and 2021. Table Mountain National Park is typically a rocky terrain situated on the Cape Peninsula in a mountainous region located at the south-western tip of the African continent [27]. The Park is dominated by Mediterranean-like shrubland called fynbos (part of the Cape Floristic Kingdom), which is dependent on fire and susceptible to fire [28]. Four seasonal decomposition trials were conducted for approximately one year between 13 August 2020 and 24 September 2021. Seasonal trials commenced as close as practically possible to the winter/summer solstices and spring/autumn equinoxes of the southern hemisphere [29]. The onset and duration of each monthly trial within a specific season can be seen in Table 1.

Table 1 Onset and duration of each monthly trial in each season of the Western Cape Province

Trial	Number of pigs	Mass of pigs in kg (mean (SD))	Duration (days)
Spring (Equinox: 21st Sept)			
<i>Trial 1 (September 2020)</i>	2	1 (0.07)	34
<i>Trial 2 (October 2020)</i>	2	1 (0.35)	24
<i>Trial 3 (November 2020)</i>	2	1.1 (0.07)	16

Summer (Solstice: 21st Dec)			
<i>Trial 1 (December 2020)</i>	2	0.8 (0.00)	10
<i>Trial 2 (January 2021)</i>	2	1.8 (0.21)	13
<i>Trial 3 (February 2021)</i>	2	1.4 (0.14)	13
Autumn (Equinox: 22nd Mar)			
<i>Trial 1 (March 2021)</i>	2	1.1 (0.14)	13
<i>*Trial 2 (May 2021)</i>	2	1.2 (0.00)	29
<i>Trial 3 (May 2021)</i>	2	1.2 (0.14)	35
Winter (Solstice: 22nd June)			
<i>#Trial 1 (August 2020)</i>	2	1.4 (0.07)	48
<i>Trial 2 (June 2021)</i>	2	1 (0.07)	50
<i>Trial 3 (July 2021)</i>	2	1.9 (0.07)	66

*Trial start delayed due to wildfires in the area. #First winter trial conducted in August 2020 but referred to as late winter trial

Animal model and experimental setup

The animal model used for this study were stillborn pigs (*Sus scrofa domesticus* L.). Pigs have been regarded as the most suitable surrogates to a human body due to the similarities in the integumentary, circulatory, and digestive systems [30]. Stillborn pigs were obtained from the Mariendahl Experimental farm, Stellenbosch University, and frozen at -20°C until commencement of the experimental trials. Prior to commencement of the experimental trials, the stillborn pig carcasses were thawed overnight for 12 hours. A total of 24 stillborn pig carcasses were utilized during the study. At each study month, two (2) stillborn pig carcasses were placed individually into a partially open-

bottomed plastic basket positioned inside a rigid steel cage (Fig. 1). The carcasses were in contact with the soil due to the openings in the mesh-like floor of the plastic baskets. The steel cages were a minimum of 25 meters apart to ensure there was no overlapping of crawling insect population within the study site [31].

Documentation of observations, collection, and identification of ants

Daily abundance of ants and their location(s) on, in, and around the carcasses during data collection were documented using detailed field notes and manual photography. Similarly, the characteristic activity of the ants on, in, and around the carcasses and their interaction with other insects present on, around, and/or flying above the carcasses in each of the monthly trials ~~was~~ were documented using detailed field notes and manual photography. Ants were collected from the carcasses with the aid of soft tweezers and micro spoons after undisturbed photographs of the carcasses were taken. After collection, the ants were transferred immediately into a killing jar containing paper towels dampened with ethyl acetate. Following euthanasia, the ants were transferred into screwcap containers after which they were taken to the laboratory and then stored in 70% ethanol until identification. The collected ant specimens were identified using the morphological descriptions in Fisher and Bolton [32].

Results and discussion

Daily abundance and presence

Daily abundance and presence of the ants on the carcasses in each of the monthly trials are provided as a Supplementary Information (Online Resource 1). The ant specimens were identified to be *Crematogaster* cf. *liengmei*.

Necrophagous activity: bite marks, flesh removal, and infliction of skin lesions

On dDay 3 of the early spring trial, a few individuals of *Crematogaster* cf. *liengmei* were observed underneath one of the carcasses (i.e., carcass 2). However, on dDay 4, aggregations of *Crematogaster* cf. *liengmei* were observed around the anus, in and around the oral, nasal, and ocular cavities, around and underneath the right ear, and between the fore- and hindlimbs of the carcass (Fig. 2a₁ and 2b). On dDay 5, a reduction in the abundance of *Crematogaster* cf. *liengmei* on the carcass was observed (Fig. 2b) and we hypothesize that such occurrence may be linked to the rainfall that occurred earlier in the day. While climatic data was recorded, detailed inferential analysis of the effects of climatic conditions (e.g., rainfall) on the presence/absence of *Crematogaster* cf. *liengmei* on the vertebrate carcasses is beyond the scope of this study. Following the ant invasion, several ant-inflicted bite marks and fleshy, conspicuous reddish-brown and irregular shaped lesions were observed on the skin of the carcass especially around the previously mentioned aggregation sites (Fig. 2c₁ and 2d). Similar observations were recorded in the mid-autumn (between dDays 2 and 5) and mid-winter (between dDays 4 and 6) trials (Fig. 3a₁₋₃d; Fig. 4a₁₋₄d). The aggregation of ants around the above-mentioned body parts and the occurrence of ant-inflicted bite marks and fleshy/reddish-brown lesions have been documented in other similar studies on decomposing human and animal remains [9, 11, 18, 21, 33 – 35]. We attribute the irregularly shaped lesions on the skin of the pig carcasses to the secretion and release of formic acid and other glandular chemicals on the epidermal layer of the carcass skin by the ants during feeding [18, 21, 34]. These observations are forensically significant, particularly for unnatural death scene investigations, as the post-mortem bite marks and skin lesions on decomposing vertebrate remains can be misinterpreted by inexperienced investigators as peri- or ante-mortem excoriations [8, 34].

Haemorrhage: blood discharge from the ant-inflicted skin lesions

Three days (i.e., ~~d~~Day 7) after the arrival of several individuals of *Crematogaster* cf. *liengmei* on one of the carcasses (i.e., carcass 2) in the mid-winter trial (July 2021), bleeding was observed from one of the lesions created between the forelimbs (Fig. 4d). To the best of our knowledge, this has never been reported in any decomposition study utilizing animal carcasses in Africa. Our observation is consistent with the findings of Ventura et al. [21] in Italy. We attribute the release of blood from the ant-inflicted lesions to the eroding action of the formic acid and other glandular chemicals released during the feeding process of *Crematogaster* cf. *liengmei* on the epidermal layer and engorged capillaries of the carcass skin [8, 21, 36].

Ant-inflicted skin lesions became attractive fly oviposition sites

On ~~d~~Day 3 of the mid-autumn trial (May 2021), we observed several egg masses in and around the ant-inflicted lesions between the forelimbs and upper region of the trunk of one of the carcasses (i.e., carcass 1) (Fig. 5a₂ ~~and~~ 5b). The lesions were fresh and moist, and this might plausibly be the reason why the Calliphorid flies were attracted to them. Similar observations have been reported by Meyer et al. [18] in Mississippi, USA. The direct oviposition of eggs in and around the ant-inflicted lesions might potentially alter the process of decomposition and may contribute to a reduction in the amount of feeding time in which the emerging larvae will have to go through to gain access to the internal tissues of the carcass.

Predatory activity: prevention of continuous fly landing and oviposition, larval removal, and adult fly predation

The large aggregation of *Crematogaster* cf. *liengmei* on one of the carcasses (i.e., carcass 2) as early as ~~d~~Day 4 in the early spring trial (Fig. 2a), prevented not only the landing, and continuous oviposition/larviposition by flies, but also, the formation of large maggot masses on the carcass. This observation is consistent with the findings of other similar studies [11, 16, 33]. In the mid- and late autumn trials (May 2021), several individuals of *Crematogaster* cf. *liengmei* were seen removing some eggs away from the egg masses deposited between the forelimbs and on the trunk of the carcass (Fig. 5a, ~~and~~ 5b). Furthermore, some ants were seen underneath the carcass preying on a larva and an adult Calliphorid fly (data not shown). In the early winter trial (June 2021), one *Crematogaster* cf. *liengmei* individual was seen carrying a fly larva away from the carcass (Fig. 6a), while in the mid-winter trial (July 2021), several *Crematogaster* cf. *liengmei* individuals were seen preying on the adult of a Calliphorid fly (Fig. 6b). In the literature, the predatory behaviour of several ant species including members of the genus *Crematogaster* on the immature and adult stages of flies and other insects ~~have~~ has been reported [9, 11, 20, 37]. In this study, we did not attempt to quantify the impact of the predatory behaviour of *Crematogaster* cf. *liengmei* on the duration of decomposition. Overall, the predatory activities of ants could pose a problem in human forensic cases especially if the post-mortem intervals are to be estimated using the developmental data of the immature insect specimens (i.e., eggs and larvae) found in or around the corpse [9].

Present but exhibiting no observable necrophagous and/or predatory activity

Despite the presence of *Crematogaster* cf. *liengmei* in all the summer trials, mid- (October 2020) and late (November 2020) spring trials, early autumn (March 2021), and late winter trials (August 2020), they were not observed to feed on the flesh of the carcasses or prey on other carrion-associated insects present. Little is known about the biology, ecology, and foraging behavior of this ant species; thus, we cannot definitively explain differences in behavior within the different trials. Nevertheless, it is possible to make some assumptions based on the foraging behaviors of its congeners and other ant species. Firstly, most *Crematogaster* species nest arboreally with a few species nesting on the ground, with generalized and omnivorous feeding habits, hence, may explain their selectiveness in feeding and preying on vertebrate carcasses and other associated insects [38, 39]. Secondly, while we did not attempt to track the distance between the carcasses and the ants' nesting site(s), it is possible to associate the lack of the above-mentioned behaviors of *Crematogaster* cf. *liengmei* despite their presence, not only to the potentially lengthy distance between the food source (i.e., carcasses) and the ant nest(s), but also, to the delay in the transmission of information regarding food location and/or recruitment of foraging nest mates by the scouting ant individuals [38, 40]. Thirdly, most *Crematogaster* species are polydomous in nature with multiple nesting sites [38, 39]. Therefore, we may further associate the absence of necrophagy/predation on and around the carcasses to the scouting/foraging ants' nest nutritional status and size, physical caste, prior experience and age, and food quality [17, 40, 41].

Conclusion

We report for the first time in the Western Cape Province of South Africa, the aggressive necrophagous and predatory behavior of *Crematogaster* cf. *liengmei*. We documented several individuals of *Crematogaster* cf. *liengmei*

inflicting numerous skin lesions on the skin of the pig carcasses in different seasons. We also reported several individuals of *Crematogaster cf. liengmei* predated on the eggs, larvae, and adult stages of flies. In one of the trials, haemorrhage was observed as a consequence of the ant-inflicted lesions on the skin of the pig carcass. Also, we report the occurrence of numerous fly eggs in and around the ant-inflicted skin lesions. We suggest that crime scene investigators, forensic pathologists, and forensic entomologists should examine the presence or absence of ants in and around the region where the corpse was found. This will help in providing more information especially on the origin or source of wounds, or lesions that might be encountered on a decomposing human corpse. We acknowledge that there is a need for empirical studies on the biology, ecology, and foraging behaviour of *Crematogaster cf. liengmei* on food resources including vertebrate carcasses and carrion-associated insects. Notwithstanding, we suggest that further studies are needed specifically with increased number of carcass samples and sizes in different seasons and several locations to fully establish the effect of the presence of ants on decomposition and the possible extrapolation of the results to human forensic cases. In addition, future studies should investigate the impact of the presence or absence of ants (including *Crematogaster cf. liengmei*) not only on the duration of decomposition but also on the diversity and successional patterns of insects on vertebrate carrion. This may reveal interesting trends as to whether the presence or absence of ants influences the duration/pattern of decomposition and succession of insects on vertebrate carrion.

Supplementary information

Acknowledgements

We thank the Authorities of the South African National Parks and Table Mountain National Park for hosting the research project within their premises. We thank Mr. John Morris (Farm Manager: Mariendahl Experimental Farm, Stellenbosch University) for providing the neonate pig carcasses used for this research. We thank Dr. Caswell Munyai of the University of KwaZulu-Natal, South Africa, for the identification of the collected ant specimens. We are grateful to the South African National Research Foundation and University of Cape Town for providing Adeyemi Daniel Adetimehin with a Grantholder Student-Linked Bursary and International Students' Scholarship respectively for his academic programme. The financial assistance of the National Research Foundation towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the National Research Foundation.

~~CRediT authorship contribution statement~~ Author contribution

Adeyemi Daniel Adetimehin: conceptualization, methodology, writing - original draft, writing - review and editing, visualization, and investigation.
Marise Heyns: conceptualization, methodology, resources and funding acquisition, writing - review and editing, supervision, project administration.
Devin Alexander Finaughty: conceptualization, methodology, writing - review and editing, and supervision. Calvin Gerald Mole: writing - review and editing, supervision, and project administration.

Funding

This study was funded by the South African National Research Foundation (NRF) through a Research Grant awarded to Dr. Marise Heyns (grant number: CSUR116299).

Data availability

All data generated or analyzed in relation to this study are included in this published article and its supplementary information files.

~~Statements and~~ Declarations

Ethics approval

Ethical clearance for the use of stillborn pigs and the entire study was obtained from the University of Cape Town, Faculty of Health Sciences Animal Ethics Committee (FHS AEC Reference Number: 019_036).

~~Declaration of~~ Competing interests

~~The authors have no relevant financial and non-financial interests to disclose. This work is not currently under consideration or published in another journal~~ The authors declare no competing interests.

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Fig. 1 Close-up view of the neonate pig carcass within the steel cage

Fig. 2 a Numerous individuals of *Crematogaster cf. liengmei* aggregating in and around several parts of the carcass on dDay 4 during the early spring trial. **b** Reduced presence of *Crematogaster cf. liengmei* in the morning of dDay 5 after a brief rainfall. **c** Bite marks and fleshy lesions (red arrows) inflicted on the carcass due to the feeding activities of *Crematogaster cf. liengmei*. **d** Close-up view of the dry reddish-brown lesions (red arrows) inflicted on the skin of the carcass by *Crematogaster cf. liengmei* (dDay 9)

Fig. 3 a, b Several individuals of *Crematogaster cf. liengmei* aggregating in and around several regions of the carcasses on dDay 2 during the mid-autumn trial. **c, d** Moist and fleshy lesions (red arrows) inflicted on the carcasses due to the feeding activities of *Crematogaster cf. liengmei*

Fig. 4 a–c Individuals of *Crematogaster cf. liengmei* aggregating, feeding, and creating fleshy lesions between the hindlimbs and forelimbs of the carcass on dDays 4 and 6 respectively during the mid-winter trial. **d** Hemorrhage (red arrow) observed from one of the lesions between the forelimbs on dDay 7

Fig. 5 a, b Distant and close-up views of *Crematogaster cf. liengmei* preying on the eggs (red arrows) of Calliphoridae flies deposited in and around the fleshy ant-inflicted lesions

Fig. 6 a An individual *Crematogaster* cf. *liengmei* carrying a fly larva away from the carcass in the early winter trial. **b** Several individuals of *Crematogaster* cf. *liengmei* preying on the adult of a Calliphorid fly in the mid-winter trial