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A preliminary investigation of rabbit carcass decomposition and attracted ants (Hymenoptera: Formicidae) on the seaward coastal beach of Al-Jubail City, Saudi Arabia

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The current study was carried out in the seaward coastal beach environment of Al-Jubail City, Saudi Arabia, to analyze the rabbit carcass decomposition process, the succession pattern of associated ants, and their potential utility in forensic investigation. Experiments were conducted over a 4-season course (from autumn 2018 to summer 2019). A total of 9 species belonging to the 2 subfamilies, Myrmicinae and Formicinae, were recorded. The myrmicine species were *Crematogaster aegyptiaca* Mayr, 1862; *Messor ebeninus* Santschi, 1927; *Messor foreli* Santschi, 1923; and *Monomorium abeillei* Andre, 1881. The formicine species were *Camponotus xerxes* Forel, 1904; *Cataglyphis albicans* (Roger, 1859); *Cataglyphis hologerseni* Collingwood & Agosti, 1996; *Cataglyphis viaticoides* (André, 1881); and *Nylanderia jaegerskioeldi* (Mayr, 1904). *M. abeillei* was the only species recorded in all 4 seasons, while *M. abeillei* and *C. albicans* were the dominant species in summer and *C. aegyptiaca* and *C. albicans* in spring. Diversity was lowest in the autumn, with only 4 species recorded. The *COI* gene sequences of 5 species have been successfully deposited in the GenBank database for the first time. In total, 4 carcass decomposition stages were observed, with the longest duration in winter (13 days), the shortest in summer (11 days), and in between for both autumn and spring. Most ant species were present during both decay and dry stages, while *M. abeillei*, *C. aegyptiaca*, *M. ebeninus*, and *C. albicans* were observed in all decomposition stages. These data may indicate that ants on this coastal beach showed seasonal and geographical succession patterns that could be taken into consideration in forensic investigations.

Key words: ants, rabbit carcass, seasonal, coastal, Al-Jubail

Introduction

Forensic entomology explores necrophagous insects that colonize carcasses, forming distinct communities dependent on local soil and climatic conditions (Anderson 2010). In-depth investigations of such insects are useful for criminal investigations, and forensic entomology has tremendously evolved with several recent studies (Al-Qahtni et al. 2019, 2020, Byrd and Tomberlin 2019, Mashaly et al. 2019b, 2019a, 2020b, 2020a). In legal situations, understanding the ecology, taxonomy, physiology, and carcass decomposition process is essential. Decomposition is a critical part of the nutrition cycle that redistributes organic nutrients and minerals into the food chain (Weathers et al. 2012). Many interacting organisms, including insects, are involved in this intricate process (Byrd and Tomberlin 2019).

Because necrophagous insects are the first animals attracted to carcasses (Tantawi et al. 1996), once understood, their community succession patterns can be used to develop forensically important evidence. The larvae of many dipteran and coleopteran insects feed on

decomposing flesh and represent the majority of carcass-colonizing insects (Greenberg 1991, Byrd and Tomberlin 2019). Thus, they can be used in developing forensically important evidence, such as: (i) proving the location and time of committed murders and estimating the post-mortem interval (PMI) (Tarone and Sanford 2017, Bajerlein et al. 2018, Al-Qahtni et al. 2019, 2020, Al-Khalifa et al. 2020), (ii) ascertaining if a carcass was moved from the original crime scene (Cruz 2016, Byrd and Tomberlin 2019), and (iii) creating a link between suspects and the crime scene (Amendt et al. 2011).

Ants (Hymenoptera: Formicidae) are one of the most diverse and successful insect groups on the planet (Hölldobler and Wilson 1990, Sleigh 2003), constituting one of the biggest families (Formicidae) of aculeate Hymenoptera, the third largest order of insects after Coleoptera and Diptera (Taylor 2023). However, their role in the forensic context is still understudied, with relatively few studies published worldwide (Eubanks et al. 2019). Although coleopteran and dipteran insects are the most common carrion insects (Payne 1965, Greenberg 1991), ants can also play an active role in the decomposition process and, thus, have an impact on carcass ecology (Eubanks et al. 2019). In this context, ants visit carcasses to prey on the other carrion-colonizing insects, to feed on the carrion's tissues, or both (Smith 1986, Mashaly et al. 2018). Moreover, disruption of the carcass by ants can attract other types of omnivorous or predatory insects to carcasses (Gunn 2019).

Ants can delay the colonization of carcass feeders or exclude other insects from the carcass, thereby slowing down the decomposition process (Wells and Greenberg 1994, Campobasso et al. 2009, Lindgren et al. 2011). From the point of view of forensic investigation, this may significantly affect succession-based estimations of the PMI. Therefore, ant–carcass interaction and competition with other carcass colonizers are critical features that should be thoroughly investigated. The current study was carried out to update, document, and assist in building a larger database on rabbit carcass decomposition rate and the associated ant taxa. The impact of meteorological factors in the Saudi coastal city of Al-Jubail on carcass decomposition and carcass-attracted ant species was investigated over the course of 4 seasons, from the autumn 2018 to the summer 2019.

Material and Methods

Site of Study

This study was carried out in Al-Jubail, a port city located in the eastern region of the Arabian Gulf coast (26.9598° N, 49.5687° E) (Fig. 1, Google.com 2021). According to the Saudi Meteorological Station, the climate of Al-Jubail is "desert" during the year, with an average annual temperature of 25.2°C (Climate-Data.org 2018). The experiments were carried out at the seaward coastal beach environment (27°11'14.7"N, 49°31'24.4"E), 10 m away from the water, throughout the 4 seasons from the 17th of November (Autumn, 2018) to the 11th of July (Summer, 2019). This beach is sandy with no vegetation, and the nearest human dwelling is approximately 3 km away.

Meteorological Parameters

The meteorological parameters of the experimental site were monitored in each of the 4 seasons during the study period. The atmospheric temperature and humidity were measured daily at midday using digital thermometer and hygrometer devices (Elitch, China) according to the instruction manuals. The temperature and speed of the wind were measured using a Skywatch Wind Meter device (Skywatch®, Switzerland) by holding it against the wind for 1 min, according to the instruction manual.

Experimental Animals and Design

Rabbits, *Oryctolagus cuniculus* (Linnaeus, 1758), were used as an experimental model according to Mapara et al. (2012) and following several other studies (Azwandi et al. 2013, Silahuddin et al. 2015, Mashaly et al. 2018, Al-Qahtni et al. 2021, Khalil et al. 2023). A total of 12 adult male rabbits (≈ 2.5 Kg each) were used. In each of the 4 seasons, 3 individuals ($n = 3$) were euthanized with CO_2 prior to each experiment according to Conlee et al. (2005). Upon confirmed death, carcasses were immediately moved to the experimental site and placed in specially designed iron cages (50 × 40 × 25 cm each) to protect them from predators and scavengers (Mashaly 2016, Fig. 4). Cages were lined with stainless steel wire mesh

Fig. 1. The location of Al-Jubail City on Saudi Arabia's eastern coast (Google.com 2021), in where the seaward coastal site of the study is located.

(1 × 1 cm) to provide access by insects, while preventing access by predators. To secure each cage to the ground, about one-third of the cage was buried in the soil, and one carcass was put inside a cage in direct contact with the sand. Each cage was covered with a movable tightly fitting wire mesh lid (with 30 mm openings) to allow access for collecting insects. Carcasses were spaced equidistance 10 m apart to provide an isolated resource of carcass-colonizing insects (Lewis and Benbow 2011, Khalil et al. 2023). Carcasses were observed daily from the time of initial exposure until complete skeletonization. All experiments were carried out in accordance with the Research Ethical Committee at King Saud University (approval code: KSU-22-82).

Carcass Decomposition

Carcasses were examined (for 10 min each) from day 0, the day the rabbits were sacrificed, until complete dryness, according to Parmenter and MacMahon (2009). Four previously categorized stages of decomposition; fresh, bloated, decay, and dry (Fig. 4) were observed (Gennard 2007, Abouzied 2014, Mashaly 2016). The duration, in days, of each stage was recorded. The temperature (°C) of each carcass was measured daily at midday through the anus for 1 min using a Lascar EL-USB-2 digital thermometer (Omron, China) according to the instruction manual. The humidity and temperature (°C) of the soil in the experimental area were also monitored and recorded daily using the same device. The sensor was inserted 5 cm deep into the soil for 1 min until a fixed reading was recorded.

Ants Collection

During the first 3 days after death, each carcass was examined hourly for 10 min, from 9 AM to 4 PM, then examined once a day at 9 AM from day 4 onward until completely dried, according to Mashaly et al. (2018). Ants were collected from and underneath each carcass using soft forceps and a spatula (3 cm in width and 10 cm in length), according to Singh et al. (2020). Ants running within an area of 1 m around each carcass were also collected. Pitfall traps were also used to maintain monitoring beyond the time of collection (Majer 1997) and to reduce the disturbance of carcasses and their inhabitant insects for later sampling. To reduce detrimental impacts on the next collection, selective sampling was performed without significant depletion of ants and other carcass-inhabitant insects, according to Michaud and Moreau (2013). Collected ants were immediately preserved in 70% ethanol and stored at 4°C for subsequent identification.

Ants Identification

Morphological identification.

Collected ants were preserved in ethanol and mounted on triangle cards, labeled, and morphologically identified to species rank by M. Sharaf using the relevant keys (Hölldobler and Wilson 1990, Bolton 1994, Collingwood and Agosti 1996, Fig. 5). Voucher specimens of each species are preserved at the King Saud University Museum of Arthropods, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia.

Molecular *i*dentification.

In order to accurately characterize the collected ants, to avoid any misidentification, and to overcome challenges associated with cryptic species, a molecular barcoding of the collected specimens has been performed, as detailed below.

DNA *e*xtraction.

Random ant individuals (one from each species) were processed for molecular identification. DNA was extracted from a complete individual of each ant using the QIAamp DNA Mini Kit (50) (Qiagen, Germany) according to the manufacturer's instructions. Upon extraction and purification, DNA was quantitated using the NanoDrop 8000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The concentration and purity of the DNA were determined through 260/280 nm absorbance measurements.

PCR *a*mplification.

Extracted DNA was processed for amplification of the partial sequence of the cytochrome C oxidase I (COI) mitochondrial gene using a combination of 3 primers (Table 1). Polymerase chain reaction (PCR) was performed in a T100 Thermocycler apparatus (Biorad, Canada) as initial denaturation for 5 min at 95°C followed by 35 cycles at 94°C for 20 s, annealing for 20 s at 48–52°C, extended for 1 min at 72°C, and a final extension step of 5 min at 72°C. A negative control PCR tube, free of DNA, was included in each run to check for any false positive amplification. To confirm amplification, samples from the resulting amplified DNA fragments were run on 1.0% agarose gel, then visualized under ultraviolet light (UV) in a BDA compact-gel imager (Biometra, Germany) (Fig. 6a). The remaining PCR products were then purified using the ExoSAP-ITTM (USB, Afymetrix) prior to sequencing.

DNA sequencing and phylogenetic analysis.

The PCR products for each sample were sequenced using Big Dye terminator cycle sequencing (Macrogen, Seoul, South Korea) with the same primers as used for PCR amplification. The resulting chromatograms were edited using BioEdit and used to query related species in the GenBank database using BLAST (Altschul et al. 1997) with a similarity cut-off of 99.5% to identify the ants based on sequence homology. DNA sequences were aligned with ClustalX 2.1.0.12 (Larkin et al. 2007) applying the default parameters. Bayesian inference (BI) and maximum likelihood (ML) methods were used to create a phylogenetic tree. For the BI analyses, 4 chains (*n*chains = 4) of the Markov chain Monte Carlo algorithm were analyzed using MrBayes version 3.2.6 software (Ronquist et al. 2012). Two separate runs of the study were performed over a period of 10 million generations, with sampling occurring every 100 generations and 25% of the first trees being removed using a burn-in technique. Posterior probability was expressed as nodal support. ML analyses were performed with the help of MEGA software version 11 by applying a GTR (general-time-reversible) model with a uniform gamma distribution of all characters and invariant sites (GTR + G + I) (Kumar et al. 2012). A topology was chosen based on its high log probability value. Positions with gaps and missing

Table 1. List of primers used for the amplification and sequencing of the *COI* partial gene for ants

Primers	Forward 5'→3'	Reverse 5'→3'	Reference
LCO1490/HC02198	GGTCAACAAATCATAAAGATATTGG	TAAACTTCAGGGTGACCAAAAAATCA	(Vrijenhoek 1994)
dgLCO/dgHCO	GGTCAACAAATCATAAAGAYATYGG	TAAACTTCAGGGTGACCAAARAAYCA	(Meyer 2003)
LepF1/LepR1	ATTCAACCAATCATAAAGATATTGG	TAAACTTCTGGATGTCCAAAAAATCA	(Hebert et al. 2004)

data were removed from all positions (complete deletion option). Bootstrap support was calculated using 1,000 replicates.

Statistical Analysis

All statistical analyses were carried out using MINITAB software (MINITAB, State College, PA, v. 18.1, 2018, UK). Basic statistical analyses were performed first for calculating means and standard errors (SE). Prior to any further analysis, data were processed for normality using the Anderson–Darling normality test according to Morrison (2002). The meteorological data, as well as the overall counts of ants members of the order Hymenoptera, were normally distributed and, thus, analyzed using the Students' *t*-test. However, data of families and species counts were not normally distributed and, hence, were first analyzed by the nonparametric Kruskal–Wallis to test for differences between seasons, and then the multiple comparisons for the ant succession patterns between seasons were carried out using the Mann–Whitney *U*-test. All experiments were performed using 3 different rabbit carcasses ($n = 3$), and results are presented as means ± standard errors (SE). Finally, some particular ant species were reported as singletons or doubletons, where only 1 or 2 individual ants, respectively, were collected, according to Novotný and Basset (2000) and Mashaly et al. (2020a).

Results

Meteorological Parameters

It was important to monitor the meteorological parameters of the experimental site throughout each experimental period in each season. As shown in Fig. 2, the temperature of the ambient weather, wind, and soil varied according to the season. Students' *t*-test showed that the mean values of these parameters were similar in autumn and summer ($n = 13$ and 11, respectively; $P > 0.05$) and had the lowest value in winter ($n = 23$; $P \leq 0.05$). Although the soil humidity was significantly lower than the atmospheric humidity ($P \leq 0.05$), it was constant throughout the 4 seasons, which is characteristic of this coastal city. Finally, wind speed fluctuated and showed the lowest speed in spring compared to the other seasons ($P \leq 0.05$). Because the experimental duration varies among seasons, the presented meteorological values are the means of 13, 23, 13, and 11 measurements ($n = 13$, 23, 13, and 11) in autumn, winter, spring, and summer, respectively. Finally, the mean carcass temperature was similar both in autumn and summer and both in winter and spring ($P > 0.05$; $n = 3$). However, it was significantly lower in winter and spring compared to autumn and summer ($P \leq 0.05$; $n = 3$).

Seasonal Effects on Decomposition Stages

Fresh, bloating, decaying, and dry were the 4 decomposition stages assigned to the experimental rabbit carcasses. The fresh stage (Fig. 3a) showed the same features as the prior killing, with no bad odors, and lasted for 1 day in all seasons (Fig. 4). The bloating stage began with abdominal swelling with slight putrefying odor (Fig. 3b) and lasted for 2 days in autumn, 1 day in both spring and summer, with the longest duration recorded in winter (Fig. 4). The decay stage with declining swelling, body fluids leaked out, and a strong putrefying odor of decay (Fig. 3c) and lasted for 7 days in both autumn and summer, and 8 days in spring. However, the longest duration (13 days) was recorded in winter (Fig. 4). The dry stage showed hardened remains, as all the soft tissue and flesh had been consumed and slightly putrefying odor (Fig. 3d), and lasted for 3 days in autumn, spring, and summer, and 4 days in winter (Fig. 4). The entire carcass desiccation process lasted for 13, 23, 13, and 11 days in autumn, winter, spring, and summer, respectively. Overall, it was noticeable that the shortest duration was recorded in the summer, while the longest was recorded in the winter.

Ants Identification

Morphological identification.

Nine ant species belonging to 2 sub-families (Myrmicinae and Formicinae) were identified (Fig. 5, Table 2). Myrmicinae were represented by 4 species: *Monomorium abeillei* Andre, 1881, *Crematogaster aegyptiaca* Mayr, 1862, *Messor foreli* Santschi, 1923, and *Messor ebeninus* Santschi, 1927. These constituted 46.97%, 12.24%, 1.89%, and 0.93% of the collected ants, respectively (Fig. 5, Table 2). Formicinae was represented by 5 species: *Cataglyphis albicans* (Roger 1859), *Cataglyphis hologerseni* Collingwood & Agosti, 1996, *Cataglyphis viaticoides* (André, 1881), *Nylanderia jaegerskioeldi* (Mayr, 1904), and *Camponotus xerxes* Forel, 1904. These constituted 22.77%, 8.54%, 4.27%, 1.42%, and 0.93% of

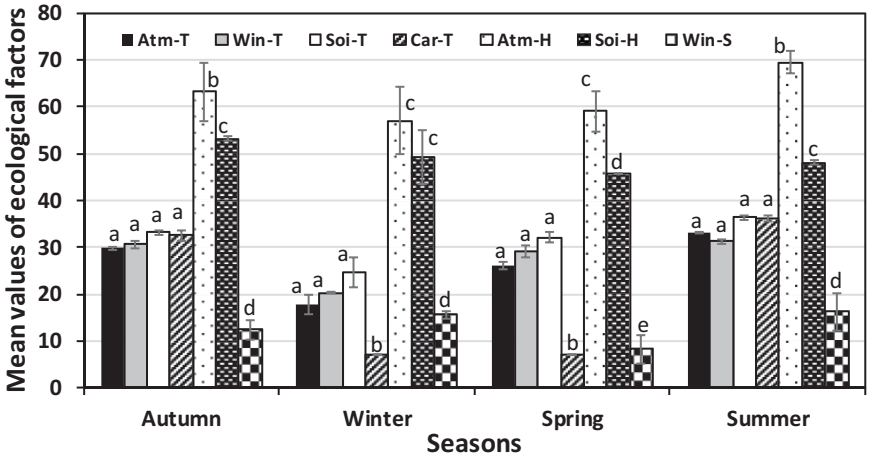

Fig. 2. The meteorological factors in each of the 4 seasons at the study site over the experimental period in each season. Error bars represent the mean values of 13, 23, 13, and 11 measurements ($n = 13$, 23, 13, and 11) in autumn, winter, spring, and summer, respectively. Mean values within each season with different letters on top of the histograms (a, b, c, and d) indicate significant differences ($P \leq 0.05$), however, similar letters indicate no significant difference ($P > 0.05$). Atm-T: atmospheric temperature, Win-T: wind temperature, Soi-T: soil temperature, Car-T: carcass temperature, Atm-H: atmospheric humidity, Soi-H: soil humidity, Win-S: wind speed.

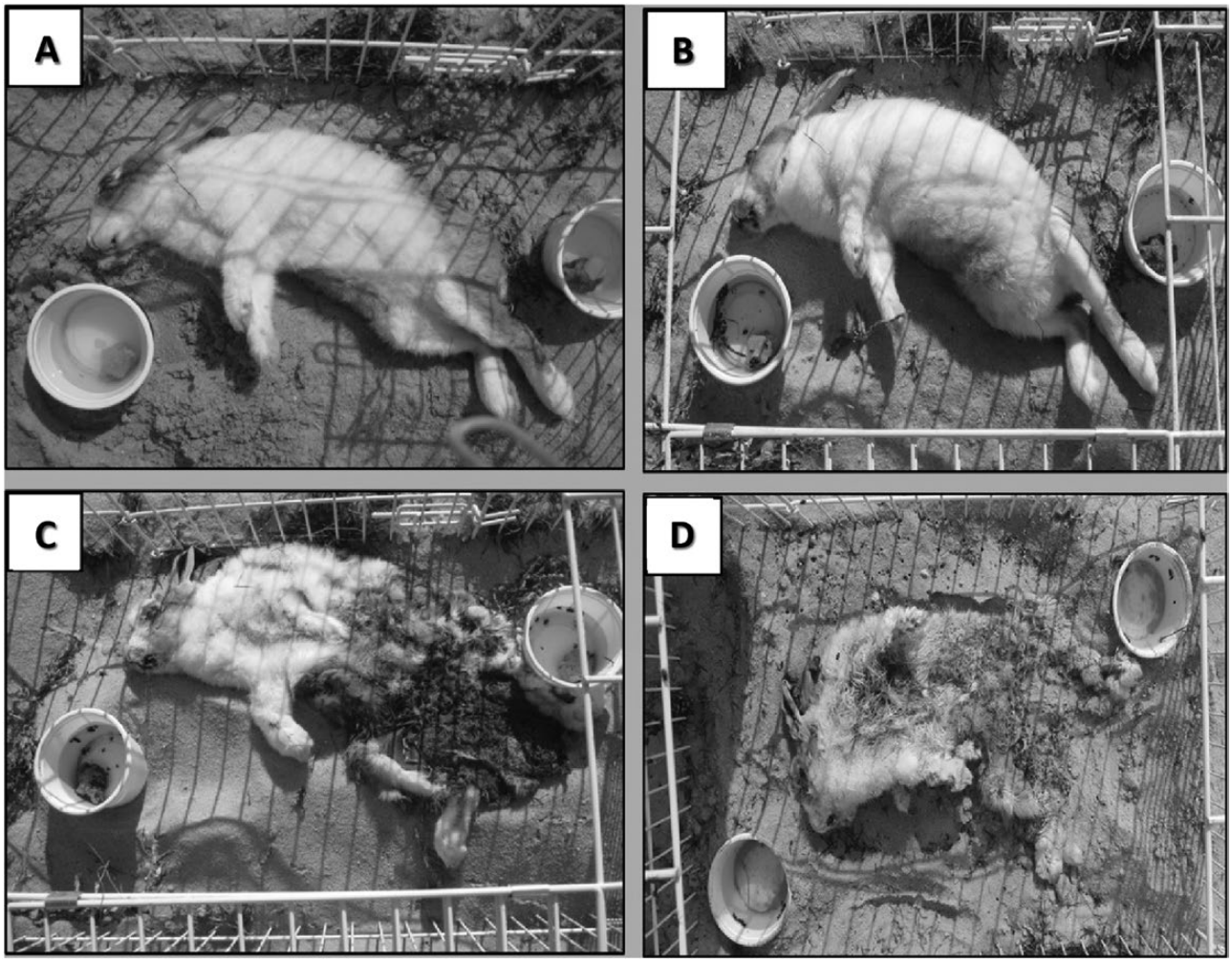

Fig. 3. Representative images of the 4 decomposition stages of experimental rabbit carcasses inside the metal cage. a) Fresh, b) bloating, c) decaying, and d) dry.

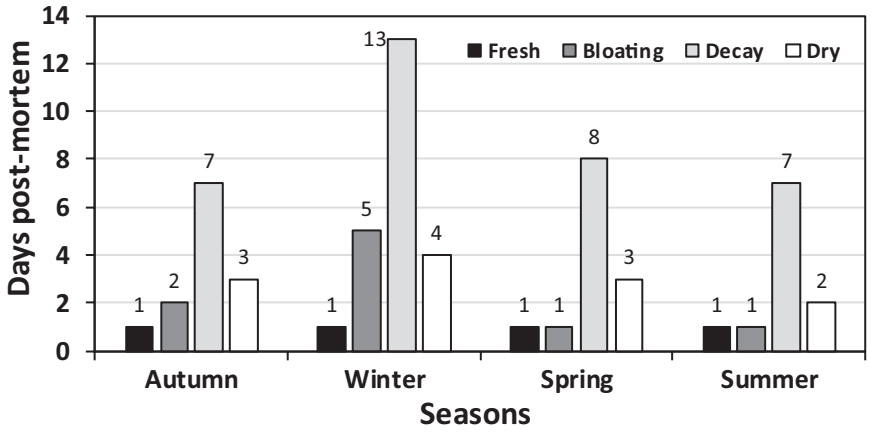

Fig. 4. Durations (in days) of the main features of the decomposition stages of experimental rabbit carcasses in each of the 4 seasons over the experimental period.

the collected ants, respectively, throughout the 4 seasons (Fig. 5, Table 2).

Molecular identification.

Amplification and sequencing were performed using samples from each of the 9 morphologically identified ant species. Seven species successfully yielded amplicons of the predicted size of the *COI* gene (~620 bp). The amplified target bands (~620 bp) were detected by agarose gel electrophoresis for all tested ants before being sequenced (Fig. 6a). Initial results of the BLAST analysis of the nucleotide sequence showed that these sequenced samples were all highly homologous to the Formicidae genome (96–99% homology in all cases). Of

Fig. 5. Auto-montage profile views of the 9 recorded ant species. a) *Camponotus xerxes*, CASENT0264128 (Will Ericson); b) *Cataglyphis albicans*, CASENT0906295 (Estella Ortega); c) *Cataglyphis hologerseni*, CASENT0912233 (Zach Lieberman); d) *Cataglyphis viaticoides*, CASENT0906298 (Estella Ortega); e) *Crematogaster aegyptiaca*, CASENT0906375 (Estella Ortega); f) *Messor ebeninus*, CASENT0249861 (Ryan Perry); g) *Messor foreli*, CASENT0249826 (Ryan Perry); h) *Monomorium abeillei*, CASENT0913864 (Zach Lieberman); and i) *Nylanderia jaegerskioeldi*, CASENT0922276 (Michele Esposito). (AntWeb).

Table 2. Abundance of ant species attracted to experimental rabbit carcasses throughout the experimental period

Subfamilies	Species	Accession number of COI gene[a]	Mean no. ± SE (n = 3)
Myrmicinae	*M. abeillei*	ON248452	33.0 ± 1.53
	C. aegyptiaca	ON255476	8.6 ± 0.66
	M. ebeninus	ON248453	0.66 ± 0.33
	M. foreli	ON248454	1.33 ± 0.33
Formicinae	*C. albicans*	ON738572	16 ± 2.08
	C. viaticoides	ON248451	3.0 ± 0.33
	C. hologerseni	NA	6.0 ± 0.57
	C. xerxes	NA	0.66 ± 0.33
	N. jaegerskioeldi	ON248455	1 ± 0.00
Mean total	9		70.25 ± 5.83

[a]The accession numbers of our recorded ant species on the NCBI. SE: Standard error of means of 3 replicates (n = 3) for each species. NA: not available.

the 7 species, only 2 were represented in GenBank (*C. albicans* and *C. viaticoides*). The sequences of the other 5 species are deposited in the GenBank database for the first time (Table 2). In order to investigate the genetic affinity of the sequenced specimens from the study area, we have performed a robust phylogenetic analysis including related species worldwide. The phylogenetic analysis obtained from BI and ML methods yielded trees with similar topology, with some differences in nodal supports (Fig. 6b). For each sequenced species, available congeneric sequences in the NCBI database were included (https://www.ncbi.nlm.nih.gov/nucleotide/). The newly sequenced specimens were attributed to 5 clusters. As shown in Fig. 6b, clade I included *C. aegyptiaca*, while clade II contains 2 *Messor* species: *M. foreli* and *M. ebeninus*. In clade III we found *Monomorium* spp., *M. abeillei*. Clade IV includes *C. albicans*, *C. viatocoides* with other *Cataglyphis* species, and finally, *N. jaegerskioeldi*, which was positioned in clade VI with congeneric *Nylanderia* species.

Abundance of Ants Attracted to Carcasses

Seasonal abundance.

A total of 212 ants were recorded in this study from November 2018 to June 2019. In autumn, the experiment ran for 13 days, during which 37 (17.5%) ants were recorded in 2 peaks (Fig. 7a). The first peak was on days 6 and 7 as singletons, and the second peak was on days 12 and 13, showing the highest percentage of attracted ants. Both peaks coincided with the highest atmospheric and soil humidity, respectively, and low wind speed. No ants were recorded on the remaining days. In winter, the experiment ran for 23 days, during which 20 (9.4%) ants were recorded at many peaks. Three peaks showed the highest number of attracted ants on days 5, 11, and 18. While peaks on days 3, 7, 8, 10, 13, 15, and 16 showed singletons of species (1 ant each) (Fig. 7b).

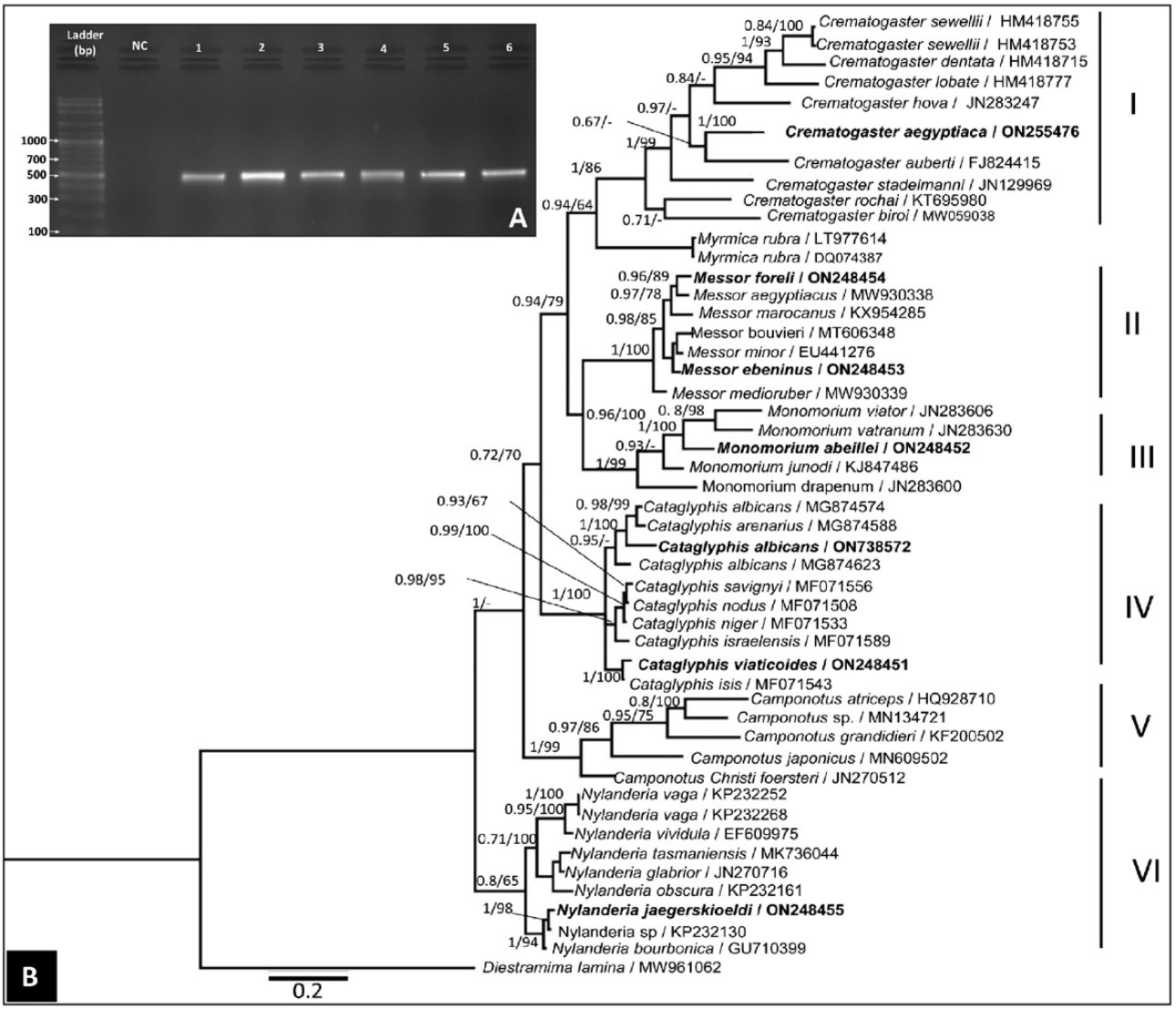

Fig. 6. a) An example of 1.0% agarose gels shows 6 different PCR products of the COI for the negative control (NC) and 6 ant species (1–6). b) Phylogenetic relationships tree based on the BI analysis of the partial COI gene showing the position of newly sequenced ants' species.The nodal support is given as Bayesian posterior probabilities (BI)/and maximum likelihood (ML) bootstrap values. Dashes (-) indicate a non-supported node or a value less than 60%.The new sequence identified in this study is in bold. GenBank accession numbers for sequences are given after the species. *Diestramima lamina* was used as an outgroup.

These peaks coincided with the high atmospheric and soil humidity, respectively, and the low wind speed. No ants were recorded on the remaining days. In the spring, the experiment ran for 13 days, during which 48 (22.6%) ants were recorded; most of them were shown in 3 peaks on days 3, 8, 9, and 13 (Fig. 7c). Singletons of attracted ant species were noted on days 1, 2, and 6, since only one ant was recorded. These peaks coincided with the high atmospheric and soil humidity, respectively, and the low wind speed. In the summer, the experiment ran for 11 days, during which 107 (50.5%) ants were recorded in 3 peaks on days 1, 4, and 9 (Fig. 7d). The first peak showed the highest number of species compared to the other 2 peaks. Doubletons and singletons of attracted ant species were noted only on days 10 and 11, respectively. This may indicate that the highest number of attracted ants to carcasses was recorded in the summer, followed by the spring, then the autumn, while the fewest number was recorded in the winter.

Differential abundance.

According to the Mann–Whitney *U*-test, the differential abundance, in terms of species abundance, is shown in Fig. 8. The *M. abeillei* was the dominant species and was recorded in all seasons. It was significantly more abundant in summer (accounted for 25.62 ± 2.31%) compared to the other seasons and the other species ($P \leq 0.05$, $n = 3$), but the lowest percentage abundance was recorded in winter and spring (accounted for 2.36 ± 0.33% and 4.74 ± 0.66%, respectively). The *C. albicans* showed the second significant highest percentage abundance in summer (accounted for 16.59 ± 0.66%) compared to the other seasons ($P \leq 0.05$, $n = 3$) and as a doubletons in autumn but was completely absent in winter. The *C. aegyptiaca* showed the third significantly high percentage abundance in spring (accounted for 9.96 ± 1.0%) compared to the other seasons, and as a doubletons in winter, but was completely absent in autumn. Five species were absent in autumn (*C. aegyptiaca*, *M. foreli, N. jaegerskioeldi, C. hologerseni*, and *C. xerxes*), 3 were absent in winter (*M. ebeninus*, *N. jaegerskioeldi*, and *C. albicans*), 4 were absent in spring (*M. ebeninus, M. foreli*, *C. hologerseni,* and *C. xerxes*), and 3 species were absent in summer (*M. ebeninus, N. jaegerskioeldi,* and *C. xerxes*). *M. ebeninus*, *N. jaegerskioeldi*, and *C. xerxes* were only recorded in autumn, spring, and winter, respectively. The highest

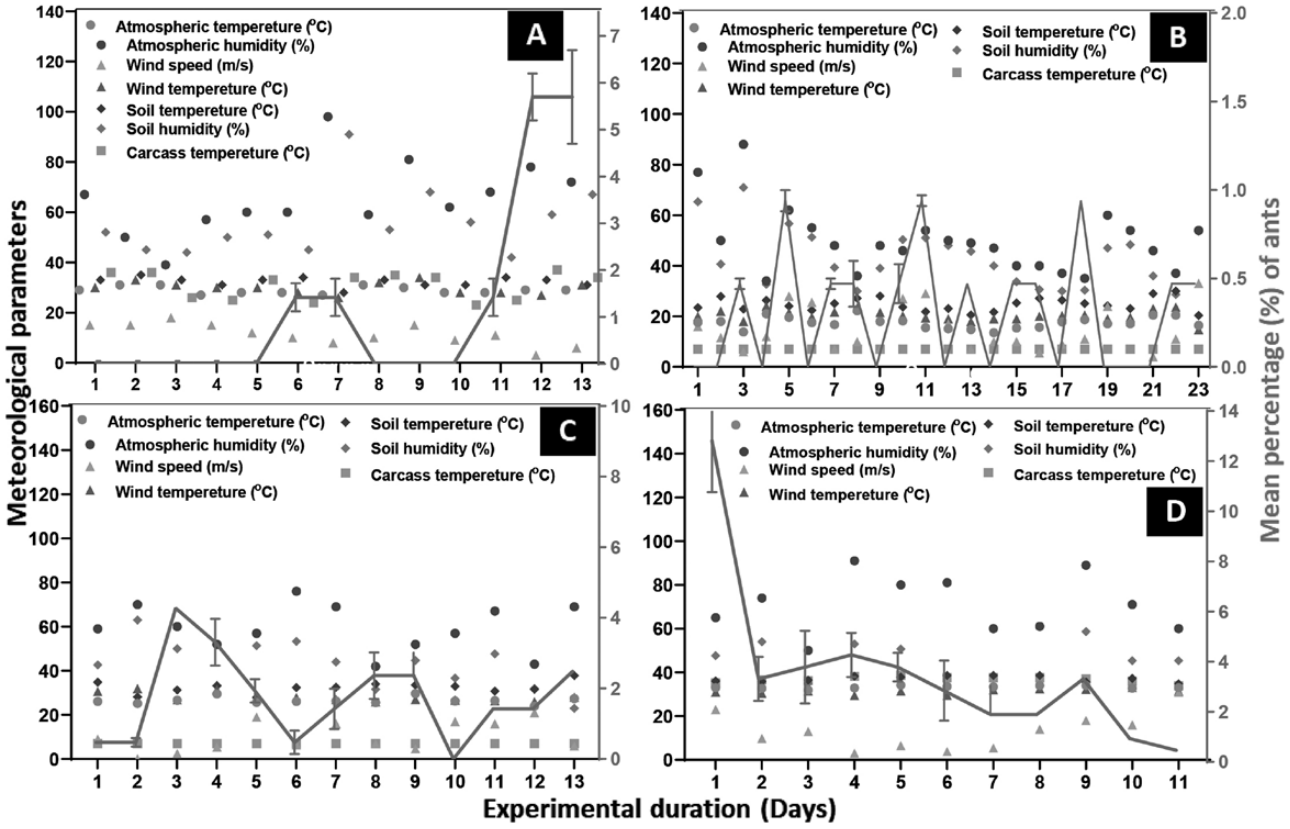

Fig. 7. The effect of meteorological factors on the abundance percentage of ants attracted to rabbit carcasses during autumn a), winter b), spring c), and summer d) in the study site throughout the experimental periods (days).

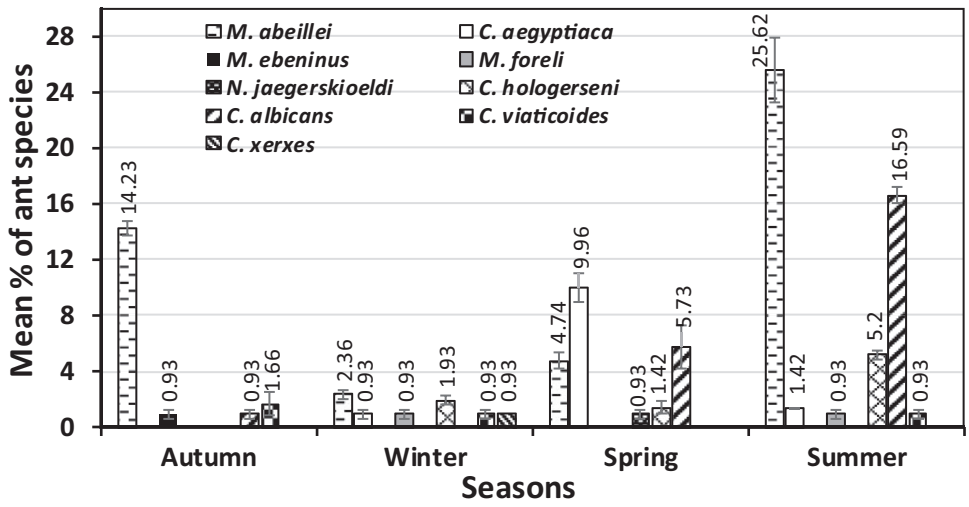

Fig. 8. The abundance percentage of ant species attracted to rabbit carcasses during the 4 seasons in the study site.

abundance of species was recorded in the summer season (50.73%), followed by, spring and autumn seasons (accounted for 22.76% and 18.47%, respectively). Winter had the lowest abundance of recorded species (8.01%).

Differential succession to carcasses.

The differential succession of ant species to the different decomposition stages of rabbit carcasses is summarized in Table 3. The fresh stage showed no attracted ants in all seasons except in summer since the myrmicine species *M. abeillei*, *C. aegyptiaca*, and *M. foreli* and the formicine species *C. viaticoides* and *C. hologerseni* were recorded. In the bloating stage, the myrmicine species *M. abeillei* and *C. aegyptiaca* were recorded in winter, while the formicine species *C. viaticoides*, *C. hologerseni*, and *C. xerxes* were recorded in summer. In the decay stage, the myrmicine species *M. abeillei* was recorded in all seasons, while *C. aegyptiaca* was absent only in autumn. However, the formicine species *C. albicans* and *N. jaegerskioeldi* were recorded in spring and winter, respectively, while *C. viaticoides*, *C. hologerseni*, and *C. xerxes* were

Table 3. Recorded ant species at different decomposition stages of rabbit carcasses during the 4 seasons

Subfamilies	Ant species	Decomposition stages	Seasons			
			Autumn	Winter	Spring	Summer
Myrmicinae	*M. abeillei*	Fresh				+
		Bloating		+		
		Decay	+	+		+
		Dry	+	+		+
	C. aegyptiaca	Fresh				+
		Bloating		+		
		Decay		+	+	+
		Dry			+	
	M. ebeninus	Fresh				
		Bloating				
		Decay				
		Dry	+			
	M. foreli	Fresh		+		+
		Bloating				
		Decay				
		Dry				
Formicinae	*C. albicans*	Fresh				
		Bloating				
		Decay			+	
		Dry				
	C. viaticoides	Fresh				+
		Bloating		+		+
		Decay		+		+
		Dry		+		
	C. hologerseni	Fresh				+
		Bloating				+
		Decay	+		+	+
		Dry				
	C. xerxes	Fresh				
		Bloating				+
		Decay	+	+		+
		Dry				
	N. jaegerskioeldi	Fresh				
		Bloating				
		Decay		+		
		Dry				

+: indicates recorded ants. Gray squares indicate no records.

recorded in all seasons except autumn, winter, and spring, respectively. The dry stage showed attracted myrmicine species *M. abeillei* in all seasons except the spring, and *M. ebeninus* and *C. aegyptiaca* in only the autumn and spring, respectively. However, no formicine species were recorded in this stage in all seasons. These data may indicate that the myrmicine species *M. abeillei* and *C. aegyptiaca*, and the formicine species *C. viaticoides* and *C. hologerseni* were the predominant carcass visitors throughout the experimental period. While the myrmicine species *M. ebeninus* and *M. foreli* and the formicine species *N. jaegerskioeldi* were the least attracted species to carcasses. Moreover, species richness was higher during the summer in most of the decomposition stages, followed by winter, spring, and finally autumn. In addition, individual assessments of all species found that the blooding and dry stages were the least attractive, which may indicate a relationship between specific ant species and decomposition stages.

Discussion

There are several studies investigating the ant fauna in the Arabian Peninsula in general (Sharaf et al. 2014b, 2014a, 2015, 2017, 2018) and Saudi Arabia in particular (Collingwood 1985, Collingwood and Agosti 1996, Al-Khalifa et al. 2010, 2015, Sharaf et al. 2018). However, carcass-attracted ants were investigated in only 2 studies in 2 locations: Al-Ahsaa Oasis in the Eastern region (Shaalan et al. 2017) and Riyadh City (Mashaly et al. 2018, 2020c, Al-Khalifa et al. 2021) of the Kingdom of Saudi Arabia (KSA). The current study is the first to investigate carcass-attracted ant species in a third location, the seaward coastal beach environment of Al-Jubail, an eastern city of the Arabian Gulf coast in KSA. In this beach environment, 212 ants, comprising 9 species belonging to the 2 subfamilies Myrmicinae (4 species) and Formicinae (5 species), were collected. This number of collected ants is small compared to cultivated areas, which is not unusual. Based on several previous studies (Boomsma and Vries 1980, Bonte et al. 2003, Feagin et al. 2005, Chen et al. 2014, 2015, Andrade-Silva et al. 2015, Fonseca et al. 2015, Leong et al. 2019, Schultheiss et al. 2022), this small number could be attributed to several factors: (i) the lack of resources, which limits ants' population size; (ii) the harsh ecological conditions such as the intrusion of the salty water, high salinity of the sandy-soil of the beach, as well as the exposure of ants to strong winds (AL-Barrak 1997, Akram et al. 2009, Wafa'a 2010, Galaledin and H El-Raey 2013), which are unfavorable for their activities and, hence, impact

their populations; and (iii) the higher predation pressure from other insects and animals, which make ants a direct target for predation and, hence, decreases their populations. The cultivated areas, on the other hand, have a higher ecological diversity and wider varieties of animals and plants, which provide a wide range of habitats and resources to larger and more stable ant populations compared to coastal areas (Ghazanfar and Fisher 1998).

Ants have larger populations than most other insects (Hölldobler and Wilson 1990) and most of them prefer proteinaceous foods compared to other types of foods (Mashaly et al. 2013). This could be one of the reasons why ants are attracted to animal cadavers for feeding and are thus categorized as omnivorous insects (Tabor et al. 2005). Moreover, ants can also feed on the carcass-colonizing insects and, thus, affect the carcass decomposition process (Eubanks et al. 2019). During our study, we observed ants actively feeding on eggs, larvae, and newly emerged flies. This observation was also reported by Chen et al. (2014) and Mashaly et al. (2018). Although the role of ants in the forensic context has been investigated for decades (Luederwaldt 1926), it is still poorly studied and understood (Eubanks et al. 2019). In KSA, only 2 studies have been conducted on ant species attracted to rabbit carcasses in Al-Ahsaa Oasis, in the Eastern Region (Shaalan et al. 2017), and the capital, Riyadh (Mashaly et al. 2018). The current study constitutes a further step forward toward building a larger database on carcass-associated ant species in the seaward coastal beach environment of Al-Jubail City.

Data from the literature show that ants can significantly affect forensic investigation in various ways: (i) by directly affecting the decomposition of the carcass by feeding on it (necrophagy); (ii) by indirectly affecting the decomposition of the carcass by predating on other carcass-colonizing insects; (iii) by reducing access for other necrophagous insects; and (iv) by causing fatal misleading in forensic analyses because the lesions or wounds they cause on the carcass can be misinterpreted as antemortem injuries or burns (de Carvalho Moretti et al. 2011, Lindgren et al. 2011, Byard and Heath 2014, Byrd and Tomberlin 2019, Eubanks et al. 2019). Additional evidence was provided by a considerable number of previous studies, for instance (Wells and Greenberg 1994, Anderson and Vanlaerhoven 1996, Anderson and McShea 2001, Campobasso et al. 2009). Consequently, this highlights the necessity for more in-depth studies of carcass-attracted ant communities in a variety of habitats and environments. During our study period, we observed ants attracted to carcasses within the first hour of exposure, particularly to the mouths and eyes, and were feeding on the dipteran immature stages and newly emerged flies, as reported by Chen et al. (2014) and Mashaly et al. (2018).

As with previous research, for instance (Schroeder et al. 2003, Abouzied 2014, Shaalan et al. 2017), the current study reported seasonal variation in meteorological factors that significantly affected ant attraction to carcasses. In addition, the seasonal availability of insects and the decomposition process were found to be influenced by the biogeoclimatic zone (Mabika et al. 2014, Byrd and Tomberlin 2019). Moreover, it has been reported that ants have the potential as seasonal, geographic, and agroecosystem conditions indicators (Peck et al. 1998, Neto-Silva et al. 2018). The combined data from these studies may indicate that the duration of the stages of carcass decomposition and attracted ants could be considered geographical and seasonal indicators and should be taken into consideration during forensic investigations while estimating the PMI. On the other hand, the lower carcass temperatures recorded in this study compared with those of the atmospheric and soil temperatures could be attributed to the postmortem changes and deterioration in biochemical parameters (Micozzi 1986, Goff and Win 1997). Furthermore, it has been reported that the soil temperature is usually higher than the ambient temperature (Zhan et al. 2019) and thus higher than the carcass temperature.

Identification of carcass-associated insects is a powerful tool that significantly strengthens forensic evidence but can pose major challenges to forensic scientists (Tang et al. 2012, Byrd and Tomberlin 2019). Advances in DNA barcoding provide an accurate means of insect identification (Zhou et al. 2009, Byrd and Tomberlin 2019). The ants in the present study were identified using traditional morphological methods and confirmed *via* amplification of a partial sequence of the *COI* gene (Kumar et al. 2012, Ronquist et al. 2012). Compared with previously published studies in the KSA (Shaalan et al. 2017, Mashaly et al. 2018), 4 myrmicine and 1 formicine species were reported to be associated with rabbit carcasses for the first time in Al-Joubail and, thus, could be unique to this particular geographical coastal city. In addition, *M. abeillei* was the only species recorded in all seasons, while *C. xerxes* was recorded only in the winter. The other species were reported differently in the rest of the seasons. These findings may indicate that ants in this coastal city did not show a specific pattern of succession. Rather, they have the potential to be seasonal and/or geographic markers.

Four decomposition stages were reported in this study, as reported in previous studies in different habitats (Bucheli et al. 2009, Al-Mesbah et al. 2012, Chen et al. 2014, Richards et al. 2015, Mashaly and Al-Mekhlafi 2016), but the recorded duration of each stage was different. These differences could be attributed to the coastal meteorological factors of Al-Jubail City. Supporting results have been provided by previous studies, which reported several factors affecting the decomposition process (Dillon 1997, Anderson 2010, Al-Mesbah et al. 2012, Abouzied 2014). Data also showed carcass decomposition-stage-dependent species succession and abundance of attracted ants. The importance of these data is supported by a previous study that proved that ants usually delay colonization by some carcass-feeders and exclude others (Wells and Greenberg 1994, Campobasso et al. 2009, Byrd and Tomberlin 2019, Eubanks et al. 2019).

In conclusion, this study investigated for the first time the carcass-attracted ant species in the coastal environment of Al-Jubail City, KSA, over the course of a whole year. The study recorded 212 ants, including 9 species belonging to the subfamilies Myrmicinae (4 species) and Formicinae (5 species). The recorded species were molecularly identified, and the COI mitochondrial gene sequences of some of them have been successfully deposited in the GenBank database for the first time. The summer and spring attracted the highest number of ants, and *M. abeillei* and *C. albicans* were the dominant species in the summer, while *C. aegyptiaca* and *C. albicans* were the dominant species in the spring. Autumn was the poorest season for attracted ants since 5 species have not been recorded. *M. abeillei* was the most abundant and common species in all seasons and all decomposition stages. However, *C. xerxes* was the least abundant, as it was only recorded in the decay stage in the winter. These data may contribute to improving our understanding of the impact of seasonal variation on ant-carcass interaction, carcass colonization by other sarcosaprophagous insects, and, consequently, the impact on the succession-based forensic investigation, which could result in biased estimates of PMI if the effects of ants are not taken into consideration. Finally, these findings highlight the need for more regional in-depth investigations of carcass-attracted ant communities in other localities. Moreover, the impact of direct sunlight, shade, raid behavior, swarm-forming behavior, and the location of carcasses near ant nests on carcass colonization is still to be investigated.

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