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ORIGINAL ARTICLE

Entry by brute force: An unusual behaviour displayed by *Scaurus uncinus* (Coleoptera: Tenebrionidae), to access nests of *Messor barbarus* (Hymenoptera: Formicidae) in Spain

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Abstract. We describe a new and unusual myrmecophilous behaviour displayed by the darkling beetle, *Scaurus uncinus* (Forster, 1771) (Coleoptera: Tenebrionidae), accessing nests of the harvester ant, *Messor barbarus* (Linnaeus, 1767) (Hymenoptera: Formicidae), in southeast Spain. The beetles enter the interior of the ant nests using a relatively primitive strategy based only on brute force, which we name "brute force entries". To understand the significance of this behaviour, we have studied the interactions of these two species in the field and in the laboratory during six years. Our observations confirm that specimens of *S. uncinus* expend a considerable effort to enter the ant nests, despite the ants fiercely attacking them, to the extent that the beetles lose legs and antennae through the process. We describe and discuss this behaviour, and identify hypotheses about its development and significance.

INTRODUCTION

The close relationship between ants and other arthropods, known as myrmecophily, is an evolutionary strategy which has developed many times, involving members of many insect orders, as well as spiders, myriapods and crustaceans (Wasmann, 1894; Cushing, 2012). Among them, beetles (Coleoptera) are the most successful group in exploiting the resources offered by ants (Hölldobler & Kwapich, 2022: 336). Thousands of beetle species, belonging to at least 35 families, have been recorded as having some level of adaptation to exploit ants (Parker 2016); however, details on how those ant-beetle associations work are poorly or not known for about half that number (Mynhardt, 2013).

Kistner (1982) mentioned that myrmecophily has evolved independently in several lineages of darkling beetles (Tenebrionidae), although the number of species is relatively small in comparison to the large size of this family and their great diversity of feeding habits. Kistner (1982) and Matthews et al. (2010) gave numerous examples of darkling beetles attracted by food debris accumulated around the entries to ant nests. However, species that enter ant nests are few, one example being the European tenebrionid *Oochrotus unicolor* Lucas, 1852, which Parmentier et al. (2020) showed to be a case of obligate myrmecophily.

In arid and semiarid habitats, where darkling beetles are abundant, some of their food is obtained from seed-eating ants: in the Americas from species of *Pogonomyrmex* Mayr, 1868 (MacKay, 1983; Johnson, 2001), and in Eurasia and Africa from species of *Messor* Forel, 1890 (Sánchez-Piñero & Gómez, 1995; Parmentier et al., 2020). However, most darkling beetles do not have special traits for a myrmecophilous way of life, being mostly large and without morphological adaptations. Therefore, their exploitation of ants is limited to occasionally feeding on refuse piles left by ants near nest entrances, as an addition to their detritivorous diet.

Among facultative myrmecophiles, Slobodchikoff (1979) reported three species of *Eleodes* Eschscholtz, 1829 (Tenebrionidae) feeding on refuse piles left by harvester ants of the genera *Pogonomyrmex* and *Novomessor* Emery, 1915 in Arizona. Also, Slobodchikoff (1979) described how the beetles respond to ant attacks by taking defensive postures and by emitting secretions which temporarily paralyzed the ants. McIntyre (1999) confirmed that species of *Eleodes* and a species of *Gonasida* Casey, 1912 (Tenebrionidae) fed on refuse piles left by *Pogonomyrmex occidentalis* (Cresson, 1865). However, McIntyre (1999) did not observe any interaction between the beetles and the ants, concluding that the beetles were using the nest sites for oviposition and thermoregulation. Although these authors do not report any beetle entering ant nests, MacKay (1983) found seven species of six genera of darkling beetles associated with species of *Pogonomyrmex* in California where, besides the many beetles found outside the nests, some specimens of *Conibius parallelus* LeConte, 1851 and of one species of *Blapstinus* Dejean, 1821 were collected inside the nests. However, MacKay (1983) did not discuss further the possible significance of finding some beetles inside the nests. Therefore, the current hypothesis is that darkling beetles entering ant nests is sporadic, and that only a few partially adapted species are using the interior of ant nests as a regular niche for their development (Kistner, 1982; Matthews et al., 2010). Kistner (1982) suggested that species of the genera *Cossiphus* Olivier, 1791 and *Stenosis* Herbst, 1799 are facultative myrmecophilous in Europe.

Few species of darkling beetles are known with some level of adaptation to exploit resources inside ant nests. In Idaho, Hendricks & Hendricks (1999) observed a significant number of specimens of *Araeoschizus airmeti* Tanner, 1845 being carried by *Pogonomyrmex salinus* Olsen, 1934 around and inside their nests. We suggest that the small size and myrmecoid shape of *A. airmeti*, plus a possible chemical mimicry, may contribute to a peaceful association, as if the beetles were regarded as seeds. In southern Europe, two small darkling beetles have been recorded associated with *Messor barbarus* (Linnaeus, 1767): a species of *Dichillus* Jacquelin du Val, 1861 (Kistner, 1982), and *Oochrotus unicolor* (see Parmentier et al., 2020).

Some reports from Spain and Italy suggest a relationship between species of *Messor* and species of *Scaurus* Fabricius, 1775; members of this genus are large tenebrionids mainly living in arid habitats, with no morphological adaptations for a myrmecophilous way of life, and with poorly known biology (Ferrer et al., 2014). Most reports cite *Scaurus uncinus* (Foster, 1771), a species widely distributed over the Iberian Peninsula and northern Africa. Sánchez-Piñero & Gómez (1995) found 23 arthropod taxa, including 10 tenebrionids, associated with nests of *Messor bouvieri* Bondroit, 1918 in the vicinity of Granada, Spain; among them, they recorded *S. uncinus* (as *S. punctatus* Fabricius, 1798, see Labrique (2004: 335) for synonymy). Sánchez-Piñero & Gómez (1995) concluded that the beetles were attracted to feed on the refuse piles left by the ants, without mentioning any interaction between them, simply that the beetles were tolerated by the ants. In northeastern Spain, Blasco-Zumeta (1998) observed *S. uncinus* (as *S. punctatus*) attacked by ants, suggesting that it happened because the beetles moved too close to the refuse piles around the ant nest. In Italy, Aliquò & Leo (1999) recorded three species of *Scaurus* from Sicily, including *S. striatus*, associated with unidentified ants. Ferrer et al. (2014) regarded *S. uncinus* (as *S. punctatus*) and *Scaurus striatus* Fabricius, 1792 as myrmecophilous based on personal observations made in Madrid and Roma respectively, commenting that “A matter of future investigation is to explain the presence of *Scaurus* in anthills being tolerated by ants.”. Pichaco-García & Ramos-Sánchez-Mateos (2016) recorded specimens of *S. uncinus* in La Mancha, Spain, under stones near ant nests and inside the nests, feeding on debris or food the ants provided. However, they neither identified the ant, nor commented on the possible significance of such a large beetle entering the ant nest.

For several years, we have been studying the ecological associations between *Messor barbarus* and other arthropods in the region of Murcia, Spain (e.g., Delgado et al., 2020; Delgado & Palma, 2023). We found that the association between *Scaurus uncinus* and *M. barbarus* is not only real but, also, is more complex than implied by previous records. Ferrer et al. (2014) commented that this association is worthy of further investigation. Our preliminary observations in 2017 and 2018 showed that during the months of greatest activity of *M. barbarus*, aggressive interactions occurred between these ants and specimens of *S. uncinus*. Further detailed observations, made in the field and in the laboratory from 2019 to 2022, showed that those interactions could not be regarded as incidental. The beetles actively approach ant nests and, in many instances, they enter the nests, despite suffering aggressive assaults by the ants to prevent the incursion.

In contrast with commensals or symbionts that are tolerated by ants, *S. uncinus* provokes a violent reaction from the ants. If, according to Scharf et al. (2011), attack strategies correlate with potential threat, we can assume that *S. uncinus* is a serious enemy of *M. barbarus* colonies.

For this paper, we investigated this unusual behavioural interaction further, with the aim to describe it, as well as to answer these questions:

1. How specific is the interaction between *Scaurus uncinus* and *Messor barbarus*?
2. Are both sexes of *S. uncinus* equally involved?
3. Is there any temporal correlation between this behaviour and the life cycle of *M. barbarus*?
4. Which myrmecophilous strategies can be identified?
5. Considering mutilations and death: what is the benefit of the incursions for *S. uncinus*?

It should be noted that our answers to these questions are from both field and laboratory observations. Therefore, the behavioural interaction observed in the field outside the nest, including the ants’ defence and the successful penetration of beetles in the nests, are the only “natural” elements of this interaction. Observations of ant-beetle interactions inside the nest made in the laboratory are liable to be biased by methodological constraints. Therefore, the reader should consider our answers not as conclusive, but preliminary and worthy of further investigation.

MATERIALS AND METHODS

Study area

Field observations were made on the margins of the Rambla de las Monjas (38°05′03.9″N–1°09′23.3″W), a temporary stream running through the suburban area of Molina de Segura (Murcia, southeastern Spain). The area includes a corridor of vegetation highly modified by humans, with low growing weedy species, combined with the shrub *Retama sphaerocarpa* (L.) Boiss, the tree *Pinus halepensis* Mill., and exotic acacia trees. The soil is mostly marl rich in gypsum which, together with intense water

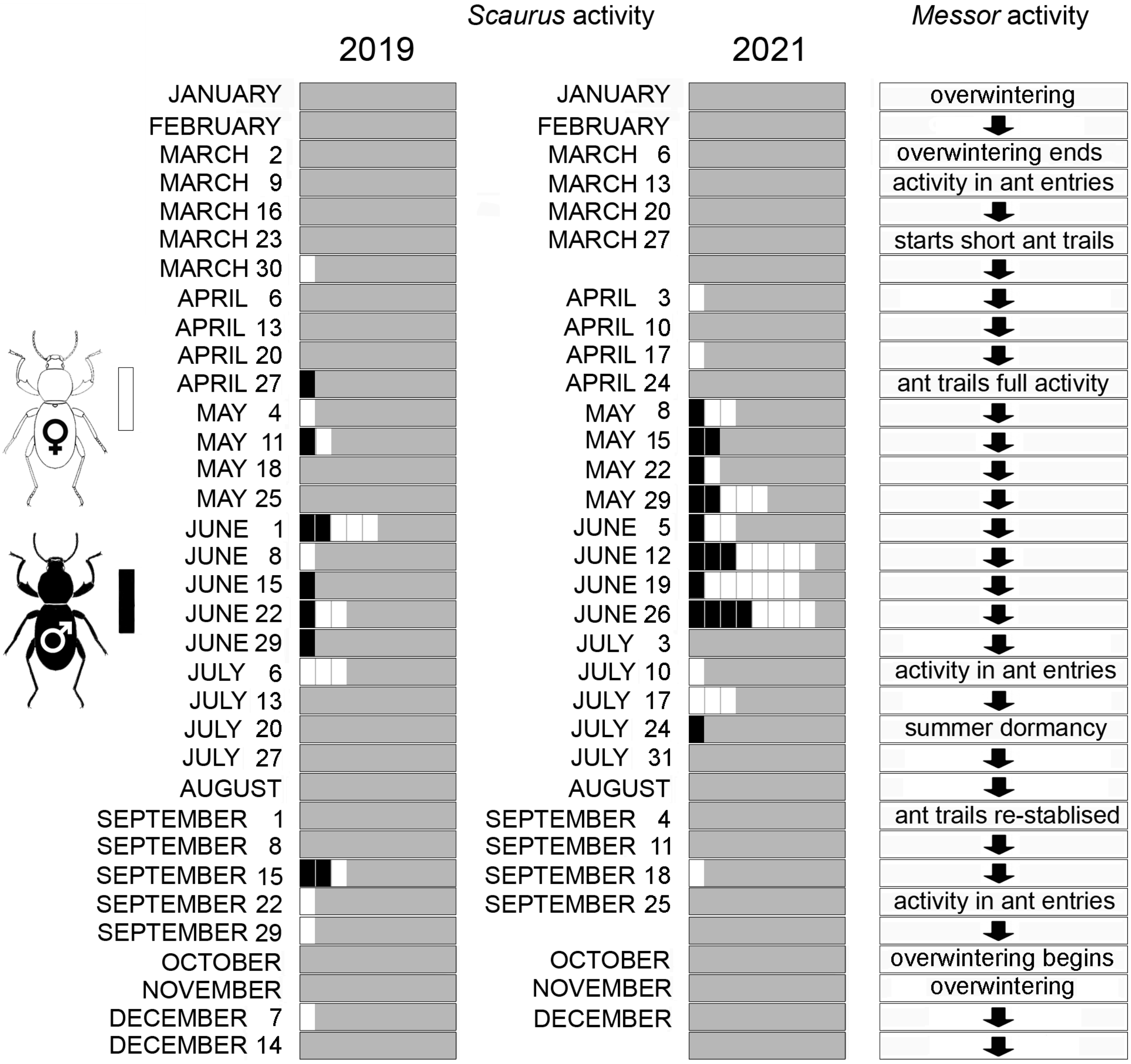

Fig. 1. Weekly records of *Scaurus uncinus* activity from January to December during 2019 and 2021.

evaporation, makes it very hard and difficult to excavate. Also, the ground is devoid of large stones, a feature that reduces the availability of suitable shelter for the beetles.

Fieldwork

To measure the abundance and observe the activity of *Scaurus uncinus* in the vicinity of nests of *Messor barbarus* over one-year cycle, direct observations were made at different intervals during the years 2019–2022. From January to December 2019 and from January to December 2021, visits to the study area were made weekly (Fig. 1), while from January to November 2022, visits were made daily (Fig. 2).

In both periods, data were taken along a straight transect about 500 m long, oriented approximately NW–SW. Observations made along the transect lasted one hour before sunset, except during April to July, the period of highest insect activity, when observations were made from 20.15 to 21.45 h. The number of specimens of *S. uncinus* was recorded along the entire transect. Ant nest entrances were also recorded, which were found separated by a minimum distance of 30 cm to a maximum of 34 m. Since the number of ant colonies was not relevant to this study, it was not necessary to determine if nest entrances located close to each other belonged to one colony or two. A total of 32 ant nest entrances were found in 2019, 27 in 2021, and 29 in 2022.

Behavioural methodology

We designed a process of data-collection based on irregular observations made in 2017 and 2018. To answer our questions about the behavioural interaction of these two insect species, we needed to make both field and manipulative observations. Hence, we collected field data during 2019–2021 and conducted manipulative and field observations during 2021 and 2022.

To describe the interactions between ants and beetles in the field, we made over 200 observations each lasting 15 to 20 min, from 2017 to 2021. We applied Altmann's (1974) "focal animal sampling" method to 26 detailed observations, each of 20 to 25 min, by selecting and studying one beetle, disregarding others nearby. Our observations began when an individual beetle was located at 50 cm or less from: (1) an ant nest entrance, (2) a refuse pile, or (3) a trunk trail. The observation lasted until the beetle entered the ant nest, or found refuge under litter, staying for more than 10 min before leaving the area close to the nest. The total ob-

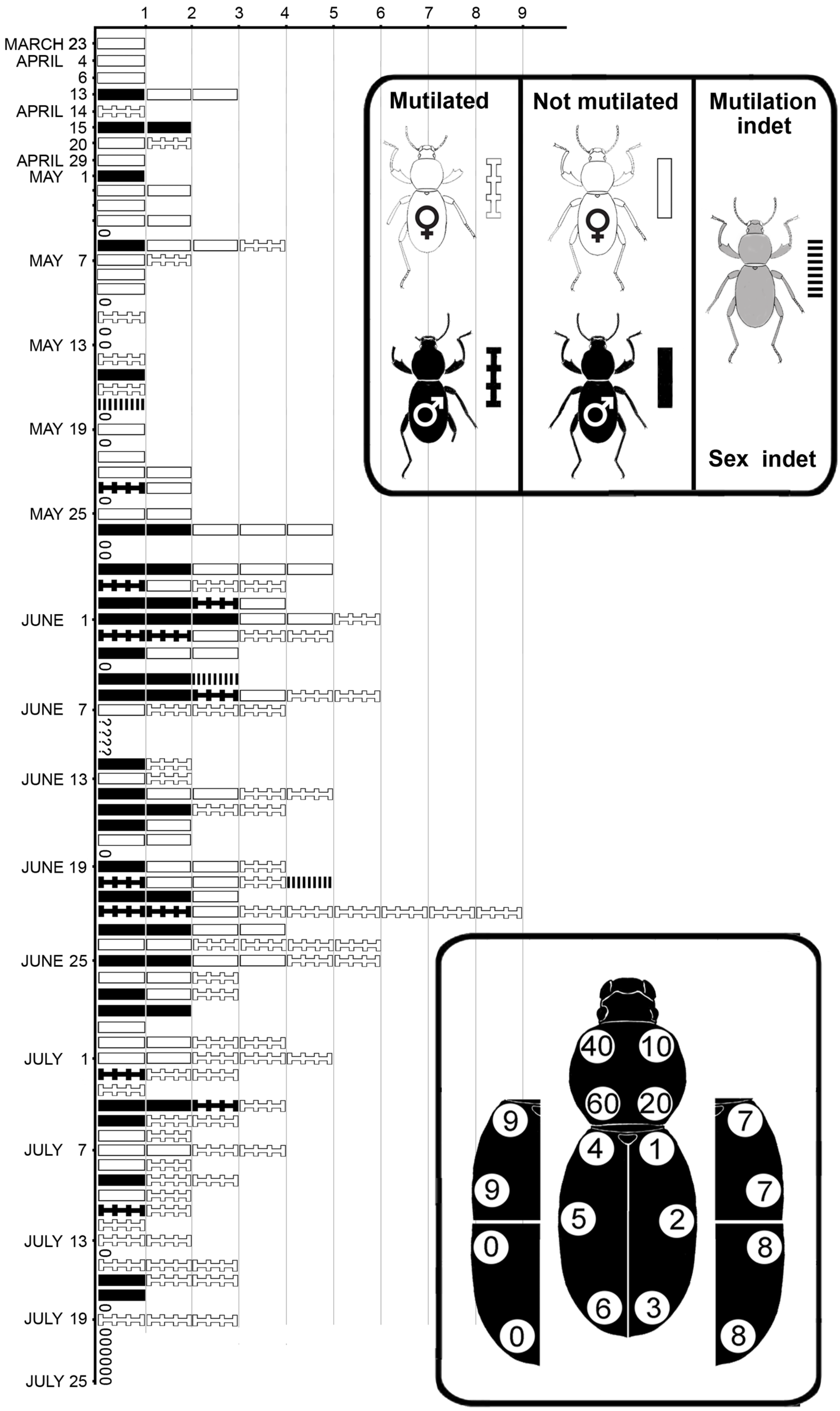

Fig. 2. Daily records of *Scaurus uncinus* activity from March to July 2022. Top insert: key to data on beetle mutilations. Bottom insert: individual beetle numbering system.

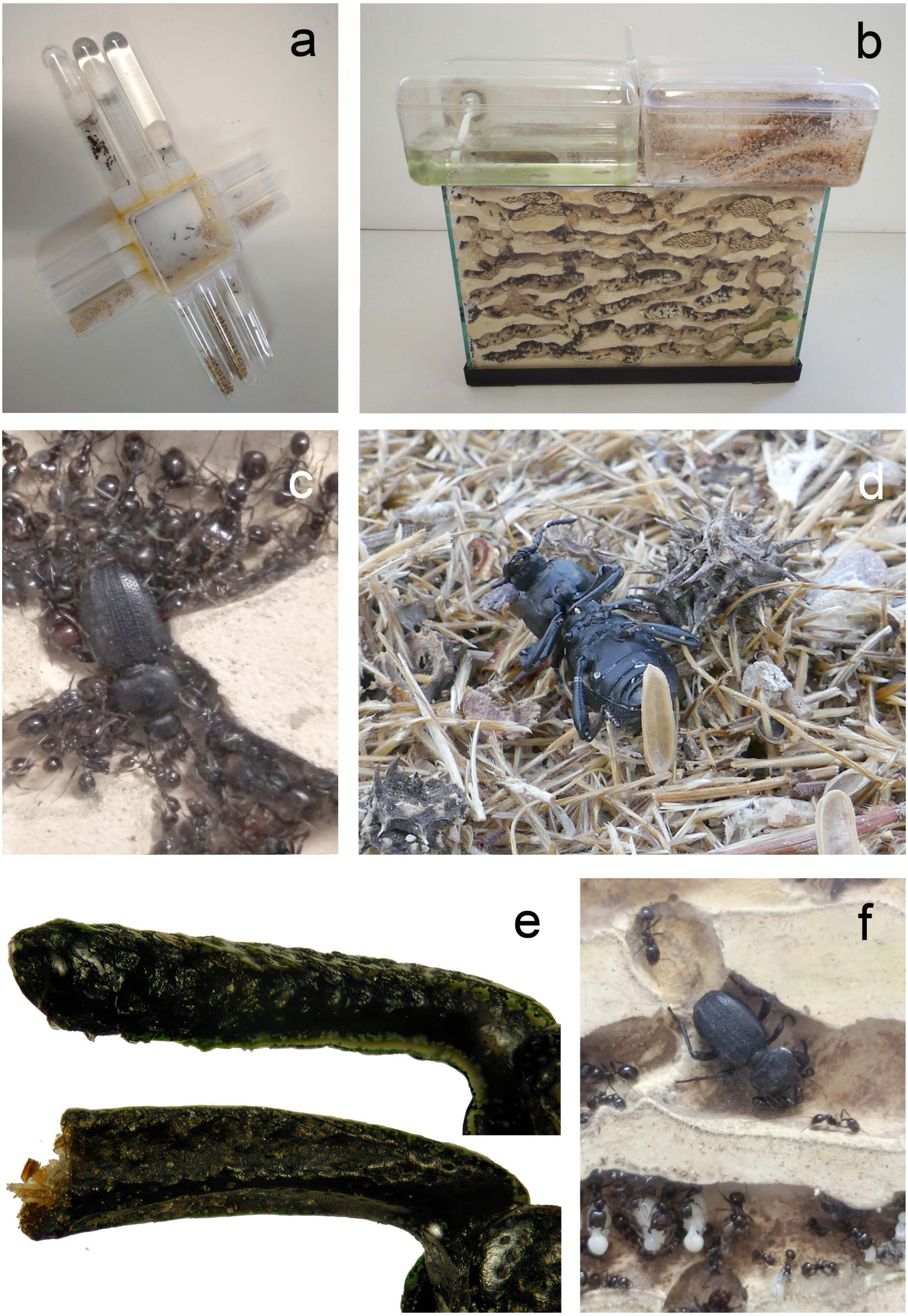

Fig. 3. a – test tube ant nest; b – complex ant nest; c – entry inside ant nest; d – dead beetle in a refuse pile; e – upper: mutilated leg with its end healed; lower: recently mutilated leg; f – beetle inside ant nest ignored by ants.

Fig. 4. a – beetle in approximation posture; b – interaction with ants; c – beetle in state of thanatosis; d – defensive strategy in refuse pile; e – defensive posture with extended forelegs; f – defensive posture with extended rear legs.

servation time was recorded with a chronometer and, usually, the behaviour of one beetle was observed each field day. The sex of each beetle was determined from its external morphology, without handling it. During the 2021 season, we manipulated some ants and beetles to confront each other.

During 2022, all beetles found within a circle of 50 cm radius from an ant nest entrance, were numbered by marking them with a white TexPen, highly resistant to abrasion (Bates & Sadler, 2004). The numbering was made according to a pattern shown on the lower right corner of Fig. 2, and calculated as in the following examples: number 1 had one spot on the anterior end of the right elytron; number 7 had two spots, one on the anterior and another on the middle points of the right elytron; number 11 had one mark on 1 plus a mark 10 on the anterior right end of the prothorax; number 35 had two marks on the right side of the prothorax adding 30, plus one mark 5 on the middle point of the left elytron; and so on. Thus, up to 140 specimens of each sex could be identified by different numbers.

Numbering beetles individually was necessary to ascertain any dispersal or loyalty to a particular ant nest, and to avoid duplicating data regarding beetle mutilations. Each day, newly found beetles were counted and marked, taking note of the codes of all previously marked specimens seen. Also, the level of appendage mutilations and their sex were recorded. Five beetles were not marked because they were seen just at the time when they were entering an ant nest (Fig. 2). Sex was determined by large body size and presence of a strong spine on the fore-femur in males, and smaller body and much reduced spine in females (see Figs 1–2 in López-Pérez, 2010). Once marked and recorded, each beetle was deposited around the ant nest from which it had been collected. Eventually, all the marked specimens entered the ant nest nearby (except one male on 19 June 2022).

Maintenance of ant nests in the laboratory

Considering that it was necessary to observe the behaviour of the beetles and ants inside the nests, we established several ant colonies in the laboratory. In September 2018, 28 newly fertilized, potential queens of *Messor barbarus* were collected in the field during the autumn swarms and they were placed in separate test tubes to develop founding colonies. Twenty-two of them formed colonies of around 100 workers which, after one year, were transferred to larger glass nests (Fig. 3a). Other two colonies, collected three years earlier with the same protocol, were placed inside glass boxes (25 cm long × 14 cm wide × 16.5 cm high) with internal galleries made of plaster of Paris (Fig. 3b) and developed between 2000 and 3000 workers. The ants were fed with seeds of canary grass (*Phalaris canariensis* L.). Ten of the small colonies were used to produce immature stages to be used in beetle feeding experiments. The remaining test tube nests and one box nest were used to observe the behaviour of beetles and ants in captivity.

Collection and rearing of beetles

To prevent altering the beetle population under observation, as described above, the specimens used in laboratory experiments were collected in another location, about 1200 m from the study area. To obtain data on oviposition and survival of the beetles in captivity, we collected 18 specimens, three males and three females on three separate occasions: early April 2019 and 2021, and mid-May 2022. Male-female pairs were placed in plastic boxes (14 cm long × 6.5 cm wide × 7 cm high) previously lined with filter paper, with a tube with water blocked with cotton wool, and three pieces of dry dog food, which were successfully eaten by the beetles. Paper, water and food were renewed as needed. Ambient temperature was kept at 18 to and 35 degrees Celsius, and the photoperiod was determined by natural light, through the windows of the laboratory. Eggs were laid from June to August but had to be removed from the boxes, otherwise, the adults would eat them. Larvae hatched and were used to test predation by the ants. All adult beetles were kept in captivity until their death.

Interactions between beetles and ants in the laboratory

To observe interactions between adult *S. uncinus* and *M. barbarus* in captivity, 8 beetles (2 males and 6 females) were collected in June–July 2021 and 2022 and introduced one at the time in the ant nest shown in Fig. 3b.

Detecting chemical cues

With the aim of establishing if *S. uncinus* used chemical cues to detect the presence of ants, in particular *M. barbarus*, we performed the experiment proposed by Dinter et al. (2002), with additional improvements given by Ramírez et al. (2000).

A circle of filter paper, divided with pencil lines into 16 numbered sectors, was placed in a 20.5 cm diameter glass Petri dish (Fig. 9a). Six *M. barbarus* workers were crushed on the wider end of sector 1 and their remains removed, while six workers of a different ant species, one at a time, were crushed in the opposing sector, number 9. The other ant species were: *Aphaenogaster iberica* Emery, 1908, *Camponotus sylvaticus* (Olivier, 1792), *Cataglyphis iberica* (Emery, 1906), *Pheidole pallidula* (Nylander, 1849) and *Tapinoma nigerrimum* Nylander, 1856. Other sectors of the paper were left clean as controls. All ants were collected a few hours before performing each experiment. A specimen of *S. uncinus*, collected a few days earlier and fed in captivity, was then placed in the centre of the dish. The time the beetle was motionless on each sector was recorded, but not the time it was moving between sectors. To eliminate any bias due to the position of the Petri dish or the illumination, the dish was turned anticlockwise by 90° every 7 min. Each trial lasted for 30 min. The dish was thoroughly cleaned with methanol, leaving it open until the following night. A new filter paper was used in each trial.

Thirty replications of this experiment were conducted during June 2022, one each day, inside a laboratory under dim light between 22.00 and 23.00 h. A different specimen of *S. uncinus* was used for each of the 30 trials, but 5 of them used as controls did not include ants, only beetles, four females and one male. Among the 25 trials with ants, females of *S. uncinus* were used in 15 of them, and males in the remaining 10. In the 15 trials with female beetles, the five species of ants mentioned above were used, three replicas for each species. In the 10 trials with male beetles, *Tapinoma nigerrimum* was used in two trials, *Camponotus sylvaticus* in three and *Pheidole pallidula* in five. Once the trials were finished, the beetles were returned to the field.

Tests to determine the food eaten by *Scaurus uncinus*

In June 2021, five males and five females of *S. uncinus* were collected in the field and placed in separate plastic boxes (14 cm long × 6.5 cm wide × 7 cm high), including adequate hydration, under 15L : 9D, and ambient temperatures between 26–31 degrees Celsius. Each day, each beetle received one of the following potential foods, in random order: three live *M. barbarus* workers; three freshly dead *M. barbarus* workers; three freshly dead *Aphaenogaster iberica* workers; three eggs, three live larvae and three live pupae of *M. barbarus*; one freshly dead bee (*Apis mellifera* Linnaeus, 1758) taken from a *M. barbarus* trunk trail. Before changing the potential food at 9.30 h, consumption of the previous food was noted, and the box was cleaned.

In June 2022, another set of five male-female pairs of *S. uncinus* were collected and treated as described above, but the potential foods offered were seeds. First, three seed capsules of the legumes *Hippocrepis ciliata* Willd. and *Medicago minima* (L.) L., and then three of the cruciferous *Eruca vesicaria* (L.) Cav. and *Sisymbrium irio* L. were tested. These species were chosen because they are seeds harvested by *M. barbarus* in the study area. Also, five seeds of the geranium *Erodium malacoides* (L.) L'Hér. and of the grasses *Piptatherium miliaceum* (L.) Cosson and *Hordeum murinun* L. were offered to the beetles. In all cases, care was taken to make sure that all the seeds were viable and not just empty capsules. All 20 beetles used in both years were returned to the field.

The manipulation of beetles and the food offered to them was done with forceps and wearing gloves, especially when it was not suitable to use forceps.

Statistical analyses

All statistical analyses were performed with SPSS 28.0 (SPSS Inc., Chicago). Male:female ratio was compared using a Pearson's chi-squared test (χ^2-test) without Yates correction. The χ^2-test was also used to analyse mutilations suffered by the beetles. In the experiments to detect chemical cues, the times that males and females spent in each sector were subjected to the Shapiro-

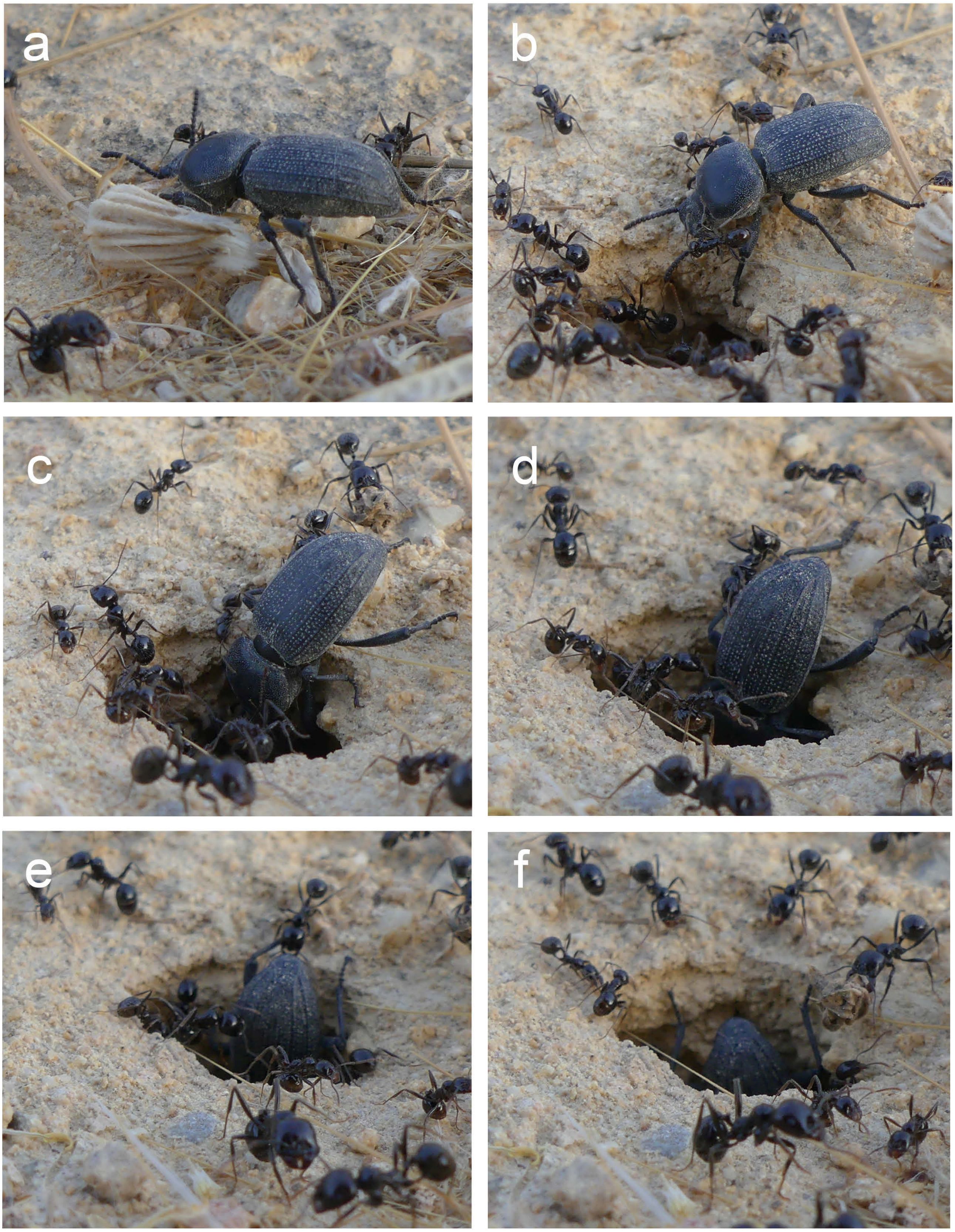

Fig. 5. Sequence of entry with minor ant resistance.

Wilk test of normality, and to the Levene's test of homogeneity of variances. Considering that data for males satisfied the parametric assumption (Levene's test = 0.063), their responses to the scent were tested using a one-way ANOVA with a 95% confidence. However, data for females were not normally distributed and Levene's test did not show variance homogeneity ($P < 0.001$). Therefore, a statistical analysis was performed using the non-parametric Kruskal-Wallis test, which was significant in one case. Hence, a Games-Howell post hoc test was performed to determine which sector differed from each of the other sectors.

RESULTS

Species present in the study area

Our observations made from 2017 to 2023 showed that the two species that interact in the study area were the darkling beetle *Scaurus uncinus* and the harvester ant *Messor barbarus*. Although *Scaurus rugulosus* Solier, 1838 and *Messor bouvieri* Bondroit, 1918 were also present, they were not observed in any of the interactions discussed in this paper.

Also in the study area, there were nests of the following ant species, in decreasing order of abundance: *Tapinoma nigerrimum*, *Pheidole pallidula*, *Cataglyphis iberica, Aphaenogaster iberica*, *Camponotus sylvaticus* and *Camponotus foreli* Emery, 1881. However, no specimen of *S. uncinus* was seen entering a nest of those ant species, with one exception, where a female beetle attempted to enter a narrow nest of *P. pallidula*, without succeeding. To see the reaction of these six ant species in the presence of *S. uncinus*, we placed live beetles at the entrance of their nests, but there was no interaction; as the beetles moved away the ants did not attack them, just showed minor signs of alarm. We repeated the trials with dead beetles but, again, there was no aggressive response; the species of *Pheidole*, *Tapinoma* and *Aphaenogaster* surrounded the dead beetles and tried to take them to their nests. The reaction of *Messor* was also without alarm, but the ants moved the dead beetles away from the nest, just as any other piece of refuse. Three other species of darkling beetles were found in the study area: *Pimelia baetica* Solier, 1836, *Tentyria laevis* Solier, 1835 and *Zophosis punctata* Brullé, 1832. We observed minor reactions by *Messor barbarus* when these beetles were in the vicinity of their nests, but there were very few mutilated specimens.

Sequence of an entry

In both, male and female beetles, the usual behaviour was to walk towards the ant nest and, as they approached the entrance, to carry on despite being attacked by several ants. Although the sequence of events varied slightly in different entries, it can be described in three steps: (i) initial approximation, (ii) contact with worker ants using brute force, (iii) entering the nest.

(i) Initial approximation

The beetle moves slowly in the vicinity of the ant nest and enters a circle of 50 cm radius, centred in the entrance of the ant nest, with its legs well extended, and the antennae pointing vertically (Fig. 4a). It walks short distances lasting 12.7 ± 6.2 s (mean ± SD, n = 16), alternated with stops lasting 11.5 ± 7.2 s (mean ± SD, n = 23). When it stops, it cleans its antenna with the mouth parts and foretibiae. Continuing with this walking sequence, the beetle points with precision towards refuse piles, where it begins its contact with ants. In other cases, the beetle detects an active or abandoned ant trail, and follows it. If active, the interaction with ants begins, but if abandoned, the beetle nears the nest entrance delaying its contact with ants. The beetle may take either direction when it begins walking on the ant trail but, if it is going away from the ant entrance, it soon reverses its direction towards the nest. We have not been able to determine how the beetle becomes aware of the wrong direction to change it so rapidly.

(ii) Contact with worker ants using brute force

When in contact with ants, the beetle changes its pace, walks faster with its legs folded and body closer to the ground. However, as ants, particularly majors, attack the beetle attaching themselves to its body, its speed reduces considerably. The duration of this phase is variable, lasting up to several minutes, when the beetle tries to get rid of the ants while attempting to reach the nest entrance, dragging up to 20 attackers attached to its body (Fig. 4b, Video S1). In some cases, due to the power of the ants, the beetle's access is not successful, walking away from the ant nest and gradually dislodging the ants while abandoning the area; however, the beetle often returns a few minutes later to try another entry. Alternatively, if the attacking ants are few, the beetle increases its speed, changes direction, and dislodges the ants to enter the nest successfully (Video S2). This interaction can last up to 20 min, but it is usually shorter (average 6.9 ± 5.05 min, n = 12).

We observed at least four different defensive postures used by the beetles when attacked by ants: (1) it raises its head by extending its forelegs (Fig. 4e); (2) it hides its head in sand and vegetable debris, extending its rear legs (Fig. 4f); (3) it finds refuge inside a refuse pile, burying itself a few centimetres deep and staying immobile for variable periods, up to 18 min (Fig. 4d, Video S3); then, it resurfaces to abandon the area or start a new incursion; (4) it freezes upside down for a few minutes (we measured one period of over 8 min); during this state of thanatosis, the ants gradually stop their attack (Fig 4c, Video S4); eventually, the beetle resumes its activity and walks away or initiates another entry. This strategy is common among myrmecophilous insects, which has been named as "tonic immobility" by Hölldobler & Kwapich (2022: 243), and recorded by Ferrer et al. (2014) among tenebrionid beetles.

We believe that these defensive postures, especially (1) and (2), are geared to protect the antennae, which are the appendages least affected by the ants (see below under *Beetle mutilations*). Also, while the beetle takes those postures, it distends its abdomen and releases phenolic compounds, which can be clearly smelled (see below under "Beetle chemical defences").

(iii) Entering the nest

Notwithstanding the defensive attacks by the ants, beetles succeeded in entering the nest in over 65% of incursions. Considering the 26 entries observed in detail (see above in Methods), a successful entry occurred in 17 (65.4%) of them. This phase varied in duration in response to different levels of ant resistance. On some occasions, a beetle entered rapidly, in what we call "clean entries", taking an average of 11.9 ± 2.1 s (n = 7) (Fig. 5a–f, Video S5). However, when the ant opposition was strong, "violent en-

Fig. 6. Sequence of entry with major ant resistance.

tries" lasted an average of 33.6 ± 10.7 s (n = 8) until the beetle entered the nest (Figs 6a–f, Video S6). Clean entries were more frequent at the end of the season, while violent entries prevailed during May and June (see below).

On some occasions, ants succeeded in preventing a beetle from entering the nest just at the entrance (Video S7), and even when it had penetrated almost completely. Successful beetle penetrations are assisted by narrow nest entrances, which force the ants to dislodge from the beetle (Video S5).

Beetle behaviour inside the ant nest

Most of our field observations were beetles entering ant nests. However, we witnessed beetles exiting nests on four occasions, but in three of them we could not ascertain the time each beetle had spent inside, i.e., it had been expelled immediately after entering, or it had spent longer time. The fourth exiting beetle was a female, marked as F58, which had entered the nest 20 min earlier.

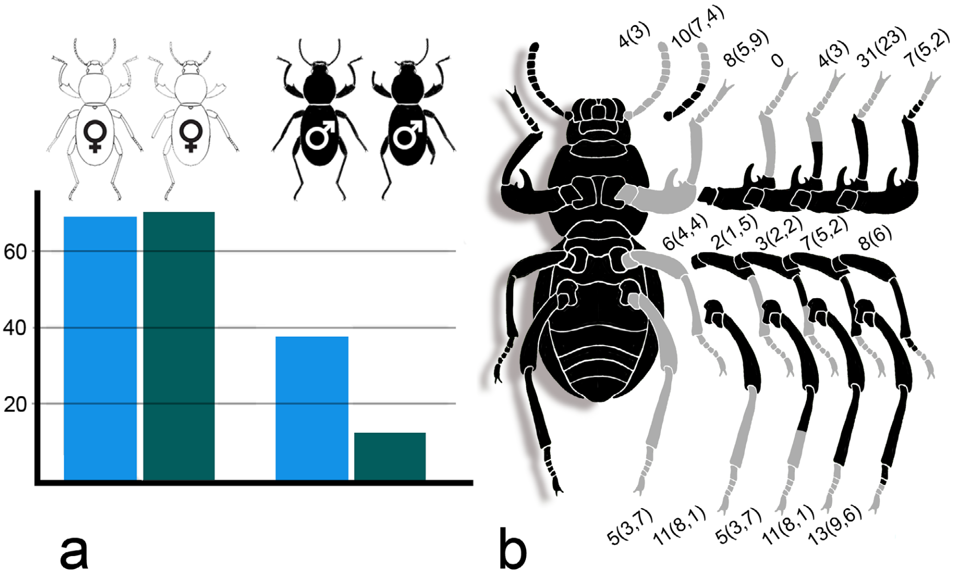

Fig. 7. a – numbers of mutilated beetles (green columns) and not mutilated (blue columns) for both sexes; b – numbers, (percentages) and types of mutilations recorded on 186 beetles (appendages removed in grey).

As it was not possible to observe beetle behaviour inside ant nests in the field, we used ant colonies in the laboratory. During June and July 2021 and 2022, we introduced a total of eight beetles, six females and two males, into different colonies. This procedure has the risk of altering the natural behaviour of both species (Scharf et al., 2011), especially the ants, which became very aggressive to the point that all eight beetles were eventually killed and eaten, in periods of two to six days.

Upon entering the nest, beetles walk slowly through the galleries, but the ants promptly react by attacking them. As the galleries are relatively narrow, beetles are mainly bitten on their anterior and posterior ends, protected on their middle body by the gallery walls (Fig. 3c). The colony becomes highly excited, especially in areas surrounding the beetle. Many ant nurses move immature stages to other chambers and, surprisingly, on some occasions, even the queen was observed aggressively attacking the intruders with its strong mandibles. It should be borne in mind that an attack by a queen in an artificial nest may be an artifact, and not an indication of natural behaviour because, in the field, the queen chamber may be located far deeper within the nest.

Notwithstanding the aggressiveness of the ant attacks, we noted that they are intermittent. During brief periods, the ants abandon the beetles, which wander inside the nest unharmed (Fig. 3f), but soon the ants resume the attacks. In the absence of attack, we observed two female beetles feeding on immature ants, but we did not see them feeding on seeds. Most of our observations showed that the beetles entered and exited the nests several times within a few hours, although in some instances they were inside more than two days without exiting. However, we do not know their behaviour during nighttime. Considering the physical limitations of our artificial nests, we observed that some beetles became stuck in narrow galleries, increasing the effectiveness of the ants to kill them. Dinter et al. (2002) – studying larval behaviour of several species of the beetle genus *Anthia* Weber, 1801 (Carabidae; cited as *Thermophilum*), which attack ant nests in north Africa – observed that they dug small cavities to hide and protect themselves from the ants. We are not aware of such behaviour in the case of *S. uncinus*.

Annual interaction between beetles and ants

In 2019 and 2021, beetles began to interact with the ants at the end of March, gradually increasing their activity to reach a peak in May–June, with a decrease in July, and no activity during August, but some in September. One specimen was recorded as late as December (Fig. 1). As expected, greater numbers of interacting beetles coincided with the period of maximum ant activity, when the nests were well supplied with food (Delgado et al., 2020) and there were great numbers of immature ants.

The results of our detailed daily field observations from January to the end of November 2022 are shown in Fig. 2. The first beetle was found at the end of March, and their numbers increased slightly from April to the end of May, when they began to appear in greater numbers, reaching a maximum around mid-June. Then, numbers decreased towards the end of July, and completely vanished during August. Only occasionally, beetles were observed from September to November. Fig. 2 also shows the sex and physical condition (mutilations) of each beetle recorded during 2022, and the pattern used to mark and identify each individual (see above in Methods).

In summary, in 2022, a total of 186 beetles were marked and their entry behaviour recorded, being 137 (73.6%) females and 49 (26.4%) males, with a sex-ratio of about 3 females to one male ($\chi^2 = 41.634$, $df = 1$, $P < 0.001$). Most of the mutilated males were concentrated in June–July. Besides recording mutilations, marking was a useful tool to record recaptures. Five beetles were recaptured during 2022: three females (F58, F101 and F128) and two males

(M10 and M39). Female F58 was recaptured while exiting an ant nest, only 20 min after being marked before penetration; female F101 was marked on 30 June and recaptured on 4 July; female F128 was marked on 12 July and recaptured on 13 July. Male M9 was marked on 29 May and recaptured on 31 May. The other male, M38, was unusual in that it was marked on 25 May and recaptured three times: on 27 and 28 June, as well as on 7 July, always around the same ant nest entrance and at about the same time, 20.45 h. Although the number of recaptures was insufficient to reach a firm conclusion, all of them were made within the same circle around the ant nest entrance, where the beetles had been previously marked.

Although the ground had few rocks, ants build chambers under them to store food remains or to use them as nurseries during winter. In addition to the beetles that enter ant nests through brute force, we recorded a few beetles inside those chambers under rocks. In July 2019, we found one female inside an empty chamber, and in October 2019, we found one female and three males inside two large chambers full of food remains.

During the three seasons studying the interaction between *S. uncinus* and *Messor*, we observed dead beetles outside the nests, deposited in the ant refuse piles. Beetle carcasses appeared to be intact or slightly damaged (Fig. 3d), and were mostly found during June–July, but also in September, when the ants clean their nests after their August aestivation. In 2019, we found six dead females, one male, and some unsexed remains; in 2021, there were two dead females and one male, and in 2022 we recorded 13 dead females, one male, and one abdomen.

Beetle mutilations

Although some cases have been described, mutilations suffered by myrmecophilous species appear to be rare. Masner & García (2002: 68) reported two Nearctic species of Diapriinae (Hymenoptera) collected from nests of the ant genus *Solenopsis*, having their wings occasionally clipped off by the ants. Zarca et al. (2022) found that individuals of the facultative myrmecophilous isopod *Porcelio scaber* Latreille, 1804 living close to red wood ants suffered more damage to their antennae than those not interacting with ants. Mutilations have also been described for *Attaphila paucisetosa* Bohn & Klass, 2021, a cockroach associated with *Atta cephalotes* (Linnaeus, 1758) (Ospina-Jara et al., 2022).

However, our preliminary observations in 2019 and 2021 showed an unusual number of beetles of both sexes with appendages (legs and antennae) removed by the defending ants (Fig. 7a). However, they were still alive and active enough to proceed with successful entries. During 2022, we made detailed records of mutilated beetles, which are shown in Fig. 2. Among the 186 specimens studied, 81 (43.5%) were mutilated, at least in one appendage, but several had more than one. Mutilated females were more abundant than males, with 69 (50.4%) from a total of 137 specimens, but only 12 (24.5%) of 49 males were mutilated (Fig. 7b). A chi-square test of association was conducted to determine if there was a relationship between beetle sex and mutilations. The test showed that there was sufficient evidence to suggest that females are more likely to be mutilated than males ($\chi^2 = 10.278$, $df = 1$, $P = 0.001$).

Among the 81 mutilated beetles, a total of 135 points of mutilation were recorded. Among the 69 mutilated females, 37 (53.6%) had one mutilation, and 32 (46.4%) more than one. Among males, 10 (83.3%) had one mutilation, and 2 (16.7%) more than one. Most of the beetles with multiple mutilations had 2 or 3 broken appendages, but we found two females with more: one with 5 and another with 7 mutilations. Despite having so many damaged appendages, both females were able to access an ant nest successfully.

Numbers and percentages of mutilations for each appendage are shown in Fig. 7. The antennae were mutilated to a much lesser degree than the legs, suffering only 10.7% of total mutilations. Legs were mutilated from the femur to the tarsi, the coxae remained in all specimens. Damage to palps, and the presence or absence of the trochanter were not recorded. The forelegs were most often damaged, with 50 (41.3%) mutilations; the mid-legs had 26 (21.5%) and the rear legs 45 (37.2%).

On 9 June 2019, we collected two beetles (one female and one male) mutilated by the ants, which we kept in captivity to record their survival. The female was missing the right anterior tarsus, the left middle tibia-tarsus and both rear tibia-tarsi, but lived over two years, until 23 September 2021. The male lacked the right antenna, and both anterior tarsi, and died on 4 December 2021, i.e., two and a half years later. These results imply that mutilated beetles may also survive under natural conditions for considerable time. We recorded one beetle with a mutilated leg, but with its end healed (Fig. 3e), indicating that the mutilation had occurred much earlier.

Food eaten by beetles in captivity

In laboratory feeding trials, beetles ate almost exclusively animal matter. Except for a few capsules of *Sisymbrium irio* L., they did not feed on seeds of any other species of plants consumed by *Messor* ants (Azcárate et al., 2005) (Fig. 8). Both male and female beetles fed primarily on immature stages of *Messor* ants, and to a lesser degree on

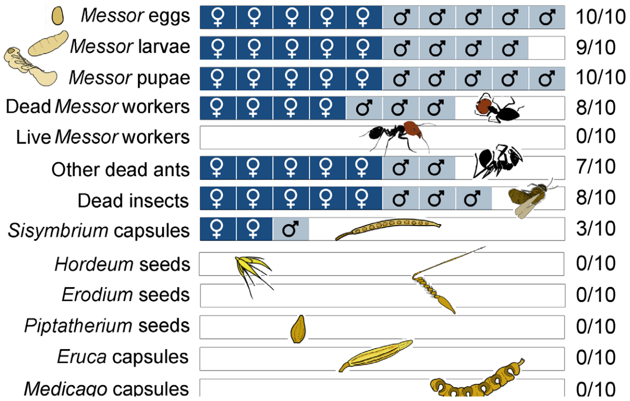

Fig. 8. Results of feeding beetles in captivity: variety of food offered and number of specimens which fed on each item. Each square represents one beetle.

dead adults, but not on live ants. Dead specimens of other ant species and other insects were also regularly consumed. We did not find differences between the feeding habits of males and females.

Detecting chemical cues

As expected, beetles did not show preference for any sector in the five control trials, i.e., without ant scent, they just moved around the 16 sectors of the Petri dish at random. When *Messor* ant scent was applied to sector 1, and scent from other ant species to sector 9, the behaviour of males and females differed significantly (Fig. 9a). Males walked randomly on the dish, not particularly attracted to sector 1 or sector 9, and staying on these sectors during a time range of 3 to 325 s, as for all the other 14 sectors. Our statistical analysis of the male data showed non-significant values (ANOVA test, $F = 0.627$, $df = 15$, $P = 0.848$). Therefore, we failed to reject the null hypothesis and conclude that there is no difference in the time spent by males on each sector.

Unlike males, the results of female trials were significantly positive. All 15 females used in the trials stopped on sector 1, during an average of 379 ± 282 s (mean ± S.D.), equivalent to 42.5% of the average times spent on all sectors of the Petri dish (Fig. 9a). When females picked up the *Messor* scent in sector 1, they stopped, touched the paper with their antennae and maxillary palps, and remained there without moving for up to 967 s, this being the longest recorded period.

Considering sector 9, where the scent of other ant genera was impregnated (see above in "Material and methods"), beetle females showed less interest than in sector 1, staying an average of 110 ± 178 s; (mean ± S.D.; n = 15). Our non-parametrical statistical test of the female data showed significant differences (Kruskal-Wallis test, $H = 59.194$, $df = 15$, $P < 0.0001$). A subsequent post hoc Games-Howell test confirmed that sector 1 differs significantly from all others, except from sectors 9 ($P = 0.193$) and 2 ($P = 0.051$), as summarised in Fig. 9b. The sector 9, impregnated with the scent of ants other than *Messor*, aroused some beetle interest, and sectors 2 and 16, as well as 8, also showed slightly higher values than the reminder because of their proximity to sectors 1 and 9, respectively.

Beetle oviposition in captivity

Females introduced into ant nests in the laboratory laid eggs in at least three of them. However, these eggs were eaten by the ants within three days. In another trial, one first-instar beetle larva was placed inside each of four ant nests, but they were eaten by the ants as soon as they were detected. These results were unexpected, especially the fate of the eggs, which were laid by females that were tolerated by the ants. Female beetles collected from the field in May 2022, kept at 33°C and under a photoperiod of 14L : 10D, laid eggs from mid-June to the end of July, but no more oviposition was observed before they were released into the field in September. Other females kept in captivity for periods of over two years only laid eggs in the season when they were collected, but not on the following two reproductive seasons, despite having frequent copulations.

DISCUSSION

1. How specific is the interaction between *Scaurus uncinus* and *Messor barbarus*?

Considering that our work was limited to a partial area of the total geographic distribution of *Scaurus uncinus*, we are not able to state that its myrmecophilous relationship with *Messor barbarus*, as described here, will be found over the entire distribution of the beetle where it overlaps with that of the ant. However, we believe that entering ant nests "using brute force" by a beetle is an unusual relationship, not comparable to any of the beetle-ant associations described in the literature.

A few cases of myrmecophilous interactions which use a degree of force to enter the ant nest have been described. For example, Verhoeff (1892) (cited by Hölldobler & Kwapich 2022: 128) found that females of the genus *Microdon* Meigen, 1803 (Diptera: Syrphidae) entered ant nests to oviposit, assisted by their large size and robust shape, but without any morphological adaptation. However, the flies do not make any contact with the ants, which try fiercely to prevent the fly from entering the nest. Other examples are species of *Liphyra* Westwood, 1864 (Lepidoptera: Lycaenidae), with mature caterpillars which enter ant nests to feed on their eggs and larvae, protected by a hard coriaceous cuticle, impervious to ant attacks (Hölldobler & Kwapich 2022: 204). These examples have similarities with *Scaurus* strategies but differ in that the insect entering the ant nest either does not make contact with the ants (*Microdon* spp.) or is protected from being damaged (*Liphyra* spp.). Although *S. uncinus* is devoid of any morphological, chemical, or behavioural adaptation, it does not avoid contact with ants, and suffers damage to its appendages. We are unaware of any myrmecophilous species of Coleoptera using brute force to enter ant nests, while being mutilated by the ants. The interaction between *S. uncinus* and *M. barbarus* represents a different type of beetle-ant relationship which we regard as facultative.

2. Are both sexes of *S. uncinus* equally involved?

Although both sexes are involved in entering ant nests, there was a clear bias towards females (ratio F : M 2.9 : 1). A similar sex ratio (3.3 : 1) was reported by Cartagena & Galante (2005) in an ecological study of three species of *Scaurus* (including *S. uncinus*, cited as *S. punctatus*; see Labrique (2004)) in the Iberian Peninsula. However, there was no mention of any interaction with ants in that study. Therefore, the sex ratio in the general population appears to be like that of the beetles accessing ant nests.

Considering that beetles of both sexes perform entries, we believe that their aim is to find food, especially for the females to produce eggs. If males were only searching for females to copulate, they presumably would avoid the risk of unnecessary mutilations. Knowledge of the life cycle of *S. uncinus* is needed to ascertain if females accessing ant

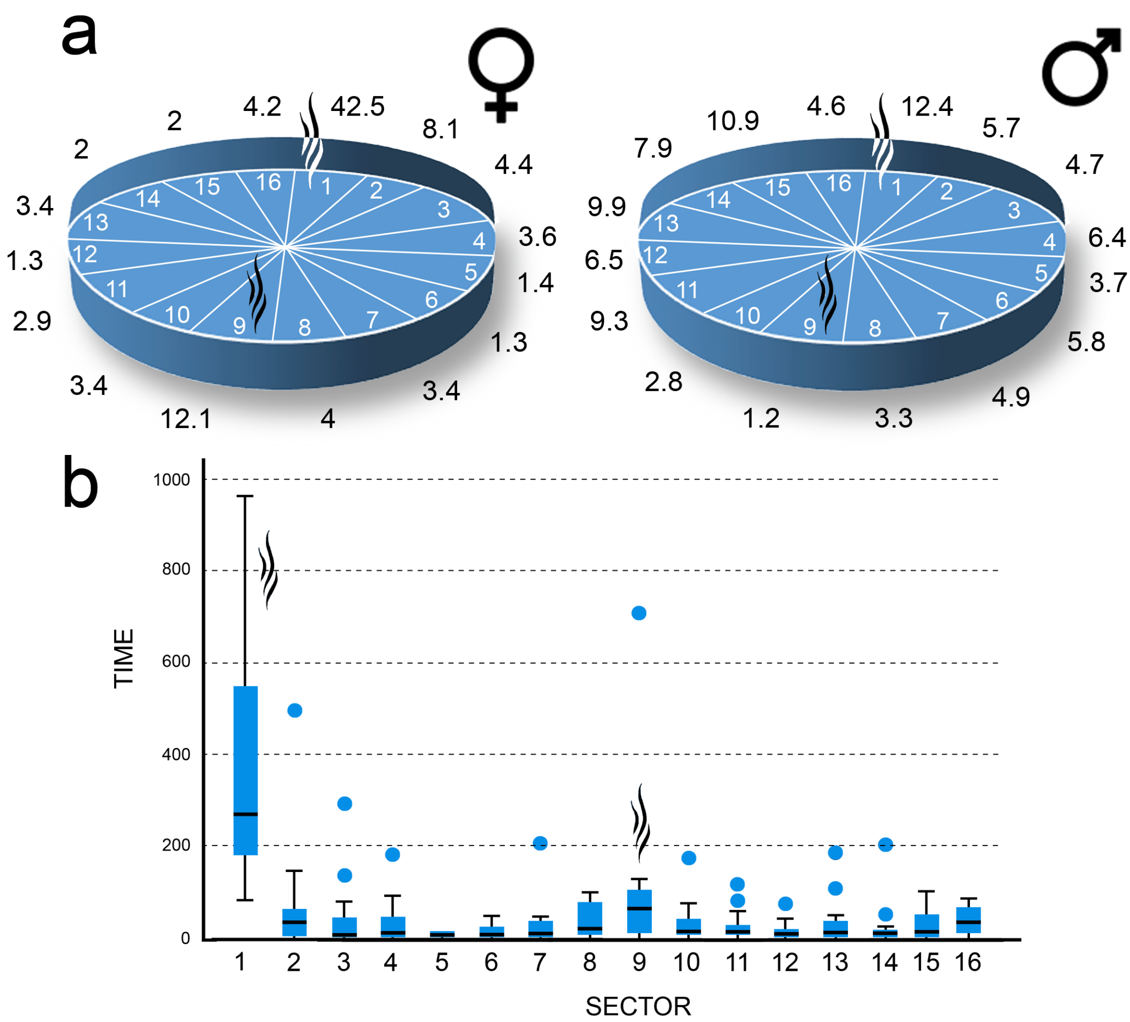

Fig. 9. a – percentages of time spent by male and female beetles on the 16 sectors of the Petri dish used for testing their attraction to ant scents. Sector 1 with *Messor barbarus* scent, sector 9 with other ant genera (see text for details); b – boxplot illustrating exploration time (in s) spent per sectors by female beetles. The black band inside the blue boxes represents the median, and the lower and upper box limits represent, respectively, the first and third quartiles. The whiskers indicate ±1,5 interquartile range limits; circles represent outliers (Kruskal-Wallis test followed by Games-Howell post hoc test; $p < 0.001$).

nests gain an advantage in their reproduction over those which do not enter ant nests.

3. Is there any temporal correlation between the behaviour of *S. uncinus* and the life cycle of *M. barbarus*?

Using pitfall traps, Giménez-Casalduero & Esteve-Selma (1995) and Cartagena & Galante (2005) reported data on the ecology of three species of *Scaurus* living in southeast Spain (including *S. uncinus*, cited as *S. punctatus*). Although our research was based on the activity of beetles around nests of ants, our results regarding temporal distribution and activity coincide with the results in the aforementioned publications. However, Cartagena & Galante (2005) found that August and September were the months of maximum activity, when only females were recorded, thus regarding these months as the time of oviposition. Our data showed that female beetles laid eggs from mid-June to the end of July, which coincides with both their entries to nests and with the maximum activity in the ant colony (Fig. 1), when there is more stored food (El-Boukhrissi et al., 2023) and numbers of preimaginal stages are at their peak (pers. observ., 2022).

4. Which myrmecophilous strategies can be identified?

(i) Location of ant trail and nest

Citing works by Akre & Rettenmeyer (1966, 1968), Hölldobler & Kwapich (2022: 260) reported myrmecophilous rove beetles of the genus *Tetradonia* Wasmann, 1894 regularly running along newly vacated trails of raiding columns of army ants, stealing their food or preying on the host ants, by following ant scent trails without other cues provided by the hosts.

There are three signals that *Scaurus uncinus* may use to locate ant nests: olfactory, visual, and auditory. As we have shown with laboratory tests, female beetles were clearly attracted to the scent of *Messor barbarus*, but males not so clearly (Fig. 9). However, from our field observations, we deduce that males also use scent signals to locate ant trails and nests. We have observed that both sexes follow foraging trunk trails for some metres towards the entrance of the ant nest, even on trails where there were no ants.

Considering auditory signals, although it is known that some species of *Messor* produce sound (Grasso et al., 2000), including *M. barbarus* (see Hernández et al., 2002), we have not been able to test if *S. uncinus* use auditory signals as a guide to the ant nest.

(ii) Beetle chemical defences

According to Tschinkel (1975), all tenebrionids have glands which secrete alkylated benzoquinones as a defensive mechanism against predators. During our manipulations of *S. uncinus* in the field and in the laboratory, we confirmed that they release chemicals, both by our smelling their scent and by the brown spots left on our hands. That emission of chemicals is more intense in May, at the beginning of the season, when entries to ant nests are more frequent. During some interactions, the scent was so strong that it could be perceived from up to two metres. This would suggest that *S. uncinus* uses chemicals as a defensive mechanism against the ants; however, unlike the report by Slobodchikoff (1979) where the beetle's secretions paralysed the ants, we did not observe any attacking ant negatively affected by such secretions.

(ii) Chemical camouflage

Any intruder attempting to survive inside an ant nest would benefit from being chemically insignificant or imitating the characteristic scent of the ants, either by producing identical chemicals – an exceptional feature among insects – or by acquiring them from the ants by contact (Hölldobler & Kwapich, 2022: 115). However, even in cases where the intruder is chemically protected, it can still be detected by the host, as shown by Parmentier et al. (2022) in ant-silverfish interactions. Vander-Meer & Wojcik (1982) reported the first known case of a scarab beetle with acquired chemical camouflage from its ant host. We ignore that *S. uncinus* can acquire such camouflage but, in a few instances, we observed that they entered ant nests without being attacked, implying a certain degree of invisibility. These instances happened in July, at the end of the season, suggesting that the beetles may have acquired some ant scent, if their visits or entries to nests were repeated to the same ant colony. Although our recapture data were not conclusive, all of them were made around the same ant nest entrance where the beetles had been previously marked. However, the evidence from the number of dead and mutilated beetles at the end of the season suggests that the level of chemical camouflage, if any, is negligible.

5. Considering mutilations and death: What is the benefit of entering ant nests for *Scaurus uncinus*?

Because we have not been able to observe *S. uncinus* interacting with *M. barbarus* inside nests in the field, it is challenging to attempt to describe which benefit/s the beetles could be gaining from so costly interaction, and which selective pressures may have operated to produce such interaction. In our opinion, the beetles obtain a net gain but we cannot be sure what that gain is. Based on the available evidence, we hypothesise that the beetles are searching for (1) refuge from desiccation and/or (2) food, either by brood predation or by kleptoparasitism.

There are various hypotheses attempting to explain behavioural traits which are adaptive, non-adaptive, or maladaptive (e.g. Emlen et al., 1991). If brute force entries evolved to obtain shelter and food, it would imply that is an adaptive trait, despite its costs. One possible evolutionary pathway may have begun with beetles sheltering in ant nests to avoid desiccation and, over time, this behavior expanded to include predatory and kleptoparasitic feeding on immature ant stages as a secondary adaptation. Considering that dead ants and seeds are available outside nests, these resources are unlikely to be cause for beetles to enter nests at such a high cost.

The hypothesis that brute force entries are non-adaptive would imply that the cost suffered by the beetles is not deleterious, which is a possible outcome as they appear to withstand mutilations without lethal results. To confirm or discard this hypothesis, it is necessary to have data from populations of *S. uncinus* not interacting with *Messor* ants, which is not available at present.

A third hypothesis, that brute force entries are maladaptive, where costs are higher than benefits, would imply negative effects and even extinction by natural selection. In this case, the attraction of *S. uncinus* to *Messor* nests could also be explained through the ecological trap concept (Robertson & Hutto, 2006). Examples of ecological traps are city lights and processed timber left within a forest. Under this concept, the beetles are lured into a poor-quality habitat which appears to be advantageous but, in fact, is not. However, ecological traps are difficult to demonstrate in nature, and we do not believe this concept applies in the *Scaurus*–*Messor* relationship. Admittedly, the habitat occupied by *S. uncinus* is degraded, but this is not a novel feature, because the current arid mediterranean environment goes back to the Miocene and the interaction between darkling beetles and ants could also be that old (Mas-Peinado et al., 2018).

If the brute force entries practised by *S. uncinus* are non-adaptive or maladaptive, we can also invoke the concept of "evolutionary trap" (Schlaepfer et al., 2002). Thus, the current behaviour could have been adaptive in the past, or for its ancestor, but it has been maintained by "evolutionary inertia", with an uncertain effect for the future of the species. Gould (2002: 1270) reminds us that the specialisation of one species may compromise the adaptation of its descendants and, conversely, "… the genuine junk of today can be exapted for the triumphs of tomorrow." (Gould, 2002: 1274).

CONCLUSION

Although our results are preliminary, considering the apparent uniqueness of the relationship between *S. uncinus* and *M. barbarus*, we believe that reporting them is justified. We still have many questions that we hope to be able to answer with further field observations and tests. Are the beetles obtaining any benefits inside the ant nests? For example, barcoding of beetle gut contents could show if they are in fact feeding on ant immatures. Does this unusual relationship exist in some areas or in all areas where both species occur? Can *S. uncinus* survive in the absence of *M. barbarus* nests? Do other species of *Scaurus* behave as *S. uncinus*, with *M. barbarus* or other ant species? No doubt, this is a potentially interesting model worthy of further research

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Online supplementary files:
S1 (http://www.eje.cz/2024/034/S01.mp4).Video S1. Successful defence by *Messor barbarus* against an entry by *Scaurus uncinus.*
S2 (http://www.eje.cz/2024/034/S02.mp4). Video S2. *Scaurus uncinus* entering a nest of *Messor barbarus* with little ant resistance.
S3 (http://www.eje.cz/2024/034/S03.mp4). Video S3. *Scaurus uncinus* finding refuge in a refuse pile.
S4 (http://www.eje.cz/2024/034/S04.mp4). Video S4. *Scaurus uncinus* defence by state of thanatosis.
S5 (http://www.eje.cz/2024/034/S05.mp4). Video S5. *Scaurus uncinus* entering ant nest without ant attack.
S6 (http://www.eje.cz/2024/034/S06.mp4). Video S6. Successful entry by *Scaurus uncinus*, despite strong ant defence.
S7 (http://www.eje.cz/2024/034/S07.mp4). Video S7. Unsuccessful entry by *Scaurus uncinus*.