



Ants as prey for the endemic and endangered Spanish tiger beetle *Cephalota dulcinea* (Coleoptera: Carabidae)

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(Accepté le 29 juin 2020)

Summary. Among the insects inhabiting endorheic, temporary and highly saline small lakes of central Spain during dry periods, tiger beetles (Coleoptera: Carabidae: Cicindelinae) form particularly rich assemblages including unique endemic species. *Cephalota dulcinea* López, De la Rosa & Baena, 2006 is an endemic, regionally protected species that occurs only in saline marshes in Castilla-La Mancha (Central Spain). Here, we report that *C. dulcinea* suffers potential risks associated with counter-attacks by ants (Hymenoptera: Formicidae), while using them as prey at one of these marshes. Through mark-recapture methods, we estimated the population size of *C. dulcinea* at the study marsh as of 1352 individuals, with a sex ratio slightly biased towards males. Evident signs of ant defensive attack by the seed-harvesting ant *Messor barbarus* (Forel, 1905) were detected in 14% of marked individuals, sometimes with cut ant heads still grasped with their mandibles to the beetle body parts. Ant injuries have been more frequently recorded at the end of adult *C. dulcinea* yearly activity, and in similar proportions in males and females, perhaps because the similar body mass of the two sexes makes the output of interactions similar. Because antennae and tarsi were particularly involved in such injuries, consequences on both chemosensory and locomotion abilities may be expected. Future studies may discover if ants are effectively a costly prey for this endangered tiger beetle.

Résumé. Les fourmis comme proies de *Cephalota dulcinea* (Coleoptera: Carabidae), Cicindèle espagnole endémique et menacée d'extinction. Parmi les insectes qui habitent les petits lacs endoréiques, temporaires et très salins du centre de l'Espagne pendant les périodes sèches, les Cicindèles (Coleoptera: Carabidae: Cicindelinae) forment des assemblages particulièrement riches comprenant des espèces endémiques uniques. *Cephalota dulcinea* López, De la Rosa & Baena, 2006 est une espèce endémique protégée régionalement qui ne se trouve que dans les marais salins de Castilla-La Mancha (centre de l'Espagne). Nous rapportons ici que *C. dulcinea* souffre des risques potentiels associés aux contre-attaques par les Fourmis (Hymenoptera: Formicidae), tout en les utilisant comme proies dans l'un de ces marais. Grâce à des méthodes de marquage-recapture, nous avons estimé la taille de la population de *C. dulcinea* dans le marais étudié à au moins 1352 individus, avec un sex-ratio légèrement biaisé en faveur des mâles. Des signes évidents d'attaque défensive par la fourmi moissonneuse *Messor barbarus* (Forel, 1905) ont été détectés chez 14% des individus marqués, parfois avec des têtes de fourmis coupées encore attachées par leurs mandibules aux parties du corps du coléoptère. Des blessures dues aux fourmis ont été plus fréquemment enregistrées à la fin de l'activité annuelle de *C. dulcinea* chez l'adulte, et dans des proportions similaires chez les mâles et les femelles, peut-être parce que la masse corporelle similaire des deux sexes rend ces interactions similaires. Les antennes et les tarses étant particulièrement impliqués dans de telles blessures, on peut s'attendre à des conséquences sur les capacités chimio-sensorielles et de locomotion. Des études futures pourraient permettre de découvrir si les fourmis sont effectivement une proie coûteuse pour ce coléoptère menacé.

Keywords: Cicindelinae; Formicidae; predation; prey defense; Castilla-La Mancha

One of the main problems to face insect conservation is the lack of biological data for the species of interest. Basic information concerning the biology, distribution, phenology and abundance of many endangered and threatened insects is still poorly known, making targeted conservation efforts difficult at best (Bossart & Carlton 2002; Samways 2007). This is especially relevant for species

inhabiting particularly restricted habitats or subject to unusual climates, generally not occupied by megafauna and therefore lacking information on habitat availability or quality (Abellán et al. 2005).

Tiger beetles (Coleoptera: Carabidae: Cicindelinae) present high habitat specificity and, at local scale, patterns of their species richness are generally not correlated with

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those of other taxa (Pearson & Cassola 1992). In a recent monitoring study of tiger beetles in steppe areas of the Iberian Peninsula (Rodríguez-Flores et al. 2016), a hot spot of species richness has been found in an area of high concentration of salt-marshes (Castilla-La Mancha, Central Spain). This area harbors the largest concentration of species of tiger beetles in a single degree latitude/longitude square in Europe (Rodríguez-Flores et al. 2016). Among the nine species occurring in the area, it is of particular interest the endangered local endemic *Cephalota dulcinea* López, De la Rosa & Baena, 2006.

C. dulcinea appears in a small fraction of the saline-lake and salt-marsh system of Castilla-La Mancha. Originally, the species was described from four saline lakes (López et al. 2006), and now it is known from 19 lakes and salt marshes in La Mancha (Rodríguez-Flores et al. 2016). The species seems to show preferences for soft, non-compact, saline substrates with typical halophytic vegetation; it has been hypothesized that their populations could respond to a metapopulation system, dispersing from marsh to marsh, through paths and trails across cultivated unfavorable habitats. Despite its interest in conservation biology, *C. dulcinea* is still a largely unknown species from ecological and behavioral points of view.

Along with other factors influencing conservation of geographically restricted endangered species such as habitat fragmentation, use of pesticides, introduction of alien species, and human disturbance, inter-specific interactions with native species are important. For example, adults and larvae of tiger beetles are known to prey often on ants (Hymenoptera: Formicidae) (Pearson & Vogler 2001; Pearson et al. 2006), a group of insects with excellent defensive capacities due to their strong mandibles and a sting (Feldhaar 2011). Ant colonies are generally abundant in terrestrial habitats shared by tiger beetles, and interactions between the former and the latter involve reciprocal predation (Laroche 1974; Rogers 1974; Wilson 1978; Pearson & Vogler 2001; Pearson et al. 2006). In particular, while tiger beetles are attacking ants, ants may defend themselves by biting certain appendages of the predators, leaving injuries such as amputation of tarsi and antennae (Valenti & Gaimari 2000). Even when ants finally are devoured, tiger beetles may present such injuries as well as, sometimes, the head of the ant grasping an appendage (Kippenhan 1990; Valenti & Gaimari 2000; Pearson & Vogler 2001).

However, it is unclear if at least some of these injuries are the result of ant attacks on tiger beetles rather than being produced by ant defensive behavior to tiger beetle predation. Ant predatory attacks on tiger beetles are sparsely reported in the literature, but this may also be due at least partially by the fact that ant predation was basically estimated by the injuries on the beetles, which could be hiding the real number of devoured prey (Laroche 1972; Rogers 1974). Independently from which of these

two situations (ant predation on beetles or vice versa) is the main case of leaving injuries on tiger beetles, this phenomenon, together with main threats such as inadequate agricultural activities, may also affect tiger beetle populations if occurring at high frequencies.

Here, we studied the predator-prey relationships between *C. dulcinea* and ants. In particular, (1) we estimated the population size of *C. dulcinea* in order to suggest how many adult tiger beetles could be potentially affected by the interactions with ants; (2) assessed the trophic group of biting ants (based on ant remains) to help decipher whether attacks are offensive (predatory ants) or defensive (harvester ants); and (3) calculated the sex-ratio of injured individuals and tested if males and females of *C. dulcinea* differ in body mass, since ant-produced injuries may depend on sex-biased size variation. For example, ants may be targeting larger prey to optimize their hunting trips (Cerdá & Dejean 2011). Alternatively, smaller (and thus likely weaker) beetles may be more vulnerable to be counter-attacked by their prey, that might have more chances to escape (a not uncommon phenomenon, e.g. Rewicz & Jaskuła 2018). Furthermore, if the identified ants are not beetle predators, we also hypothesized that (4) ant-produced injuries may be more common on the beetle appendages closer to the head (i.e. the antennae), since tiger beetles hunt their prey quickly and grasp them with their mandibles (Pearson & Vogler 2001). At last, we evaluated if the frequency of injuries varies across the day and among days, which may give insights on hunting activity patterns.

Material and methods

Study area

The study was carried on a small lake and its adjacent marshes (Laguna de Tirez, Villacañas, Toledo) (Figures 1–5) which belong to an ancient endorheic system consisting in more than 25 wetlands, included in the region traditionally known as “La Mancha Húmeda” (Rodríguez-Flores et al. 2016, 2017) (Figures 1 and 2). La Mancha Húmeda is located among portions of Ciudad Real, Cuenca, and Toledo provinces in the central area of the Iberian Peninsula, and it is included as protected space in the Natura 2000 network. This area is located at 600 m of altitude, with a substrate formed mostly of evaporite deposits (gypsum and salt) from a large endorheic lake system that covered the Iberian plateau during almost all Tertiary period. The “Laguna de Tirez” (Figure 3) is a shallow, small and salty lake. During the dry season, a solid salt crust covers the surface of the basin; during the rainy season this salt is dissolved and incorporated to the small lake, which becomes saline. The lake’s peripheral vegetation consists in halophytic and steppe vegetation; most of it protected by European Union Habitat Directive (Rodríguez-Flores et al. 2017).

Sampling methods for the population status estimate of *Cephalota dulcinea*

Sampling was performed during the activity period of the species in 2015, after first monitoring the area in 2014 to assess the



Figure 1-7. 1, Location of the La Mancha salt lake system in central Spain (rectangle); 2, location of the study site (triangle) in the La Mancha salt lake system; 3, the study site (Laguna de Tirez); 4, the area where the data on abundance (mark-recapture) were collected (delimited by black line); 5, a picture of the sampled area; 6, a marked individual of *Cephalota dulcinea*; 7, *Cephalota dulcinea* in courtship.

occurrence of the species. The methodology to detect the species in the wetlands is explained in detail in Rodríguez-Flores et al. (2016). The area of study, in the northern shore of the protected Laguna de Tirez (Figures 4 and 5), was selected for having a high density of specimens during 2014 (Rodríguez-Flores et al. 2016), so it could be treated as a healthy subpopulation, and for its relative isolation from other subpopulations of the species in the area of Laguna de Tirez. The isolation is almost complete towards the north (dirt road flanked by cultivated land), to the west (inadequate habitat for this species), and to the south (salt crust), but possibly incomplete towards the east. The eastern side is flanked by crops, but its inadequacy as *C. dulcinea* habitat depends on the level of flooding after spring–summer storms and the type of soil structure resulting in the flooded area. *Cephalota dulcinea* was the only tiger beetle present in the selected area at the time of sampling, although other species occurred in nearby areas (Rodríguez-Flores et al. 2016).

Population size estimates of *C. dulcinea* were carried out in the study area by implementing a mark–recapture method. The mark–recapture experiments consisted in three sessions of consecutive captures that were carried out during a week with a time lapse of two days in between each sampling (10.VI.2015, 12.VI.2015, and 14.VI.2015), plus an additional sampling made six days later (20.VI.2015). The three first sampling sessions (i.e. those used to estimate the population size, see below) were exhaustive: the area was not abandoned until all the visible individuals were marked, or until no active specimens were observed, which entailed 9 hours per day. The specimens were easily captured with an entomological net, since escape behavior in *C. dulcinea* is limited to short fast running and low flight.

Once captured, the specimens were marked with paint stains (nail polish) in the middle or terminal portion of the elytra and released *in situ*. The marking scheme consisted of a thick dot of orange lacquer coating in the right elytra during the first sampling (Figure 6), green lacquer coating in the left elytra during the second sampling, orange lacquer coating in the left elytra in the third, and “unmarked” in the fourth sampling. The marked specimens were easy to identify without needing to be recaptured.

After the marking and release of the specimens, some of them were followed for a few moments to check their activity. These specimens behaved normally and were observed in the usual areas of activity. Hours later, specimens were observed hunting or involved in courtship or copula (Figure 7) in mixed pairs (marked/unmarked) or involving two marked individuals. Therefore, it was considered that the markings did not negatively affect the survival of the specimens. The fourth exhaustive sampling was carried out to determine population decline (or migration). The flooded area to the east was monitored twice a day to determine if it was used as escape area for disturbed *C. dulcinea*.

During the mark–recapture experiments, sex and damaged appendages or elytra were recorded. We also annotated the number of specimens that were found associated with beheaded ants holding onto their antennae or legs, and pictures of both free-walking ants and ant grasped heads were checked by ant specialists (see acknowledgements) for species identification. During the week, and coinciding with the observations previously made, a clear reduction in the number of captured specimens was detected, indicating the proximity of the end of the activity period of the species in the area of study.

On 11.V.2017 we came back to the area in order to obtain the body mass of males and females of *C. dulcinea*. A total of 11 females and 26 males were netted and weighed with a field electronic balance to the nearest 0.1 mg. Only intact, not injured

individuals were weighed. We did not weigh injured individuals during the mark–recapture study to limit as much as possible their manipulation and because we aimed to obtain representative size data, i.e. not from injured individuals.

Statistical analysis

To estimate population size (N) from the mark–recapture data, we used the Schnabel Index (Schnabel 1938), which is a more accurate extension of the commonly employed Lincoln–Petersen method (based on only two sampling periods), and it is used for closed populations (i.e. in which the effect of births, deaths, and movements are negligible) (Silver 2008). Salt marshes in Castilla-La Mancha can be considered as closed systems for poorly flying insect species, such as tiger beetles, and the interval between sampling (two days) was constant and short enough to assume that the number of emergences and deaths were similar (Krebs 1999). Other assumptions underlying the use of this method, i.e. all individuals are equally likely to be captured in each sample, capture and marking do not affect catch-ability, each sample is random, and marks are not lost between sampling occasions, are very likely to be met in our study. This estimation method was previously used to study other tiger beetle populations (e.g. Eusebi et al. 1989).

The population size was calculated as follows:

$$N = \frac{\sum_{i=1}^m M_i C_i}{\sum_{i=1}^m R_i}$$

where M_i = the total number of previously marked animals at time i , C_i = the number caught at time i , and R_i = the number of marked animals caught at time i .

Student's t -test was used to verify differences in body mass between males and females, while the χ^2 test (with Yeat's correction) was used to verify if population sex-ratio differs from 1:1, and to verify differences in the number of males and females damaged by ants, in the number of damages by ants in different body parts (antennae or tarsi), and in the number of damages by ants recorded in the morning and in the afternoon.

Results

We estimated the population size of *Cephalota dulcinea* at the study marsh as of 1352 individuals, with a sex ratio slightly biased towards males (1:1.3, $\chi^2 = 6.12$, $p < 0.05$, $n = 618$ marked individuals).

We found evident signs of ant attack in 86 marked individuals of *C. dulcinea* (14%, $n = 618$), sometimes with cut ant heads still grasped with their mandibles to the beetle body parts (six individuals) (Figures 8 and 9). The ant species was identified as *Messor barbarus* (Forel, 1905), which nested copiously in the study area (Figure 10).

Damages inflicted by ants were recorded in similar proportions on antennae (43%) and tarsi (36%) ($\chi^2 = 0.18$, ns, $n = 86$), while a lower number of individuals showed damage on both antennae and tarsi (20.9%, $n = 86$).

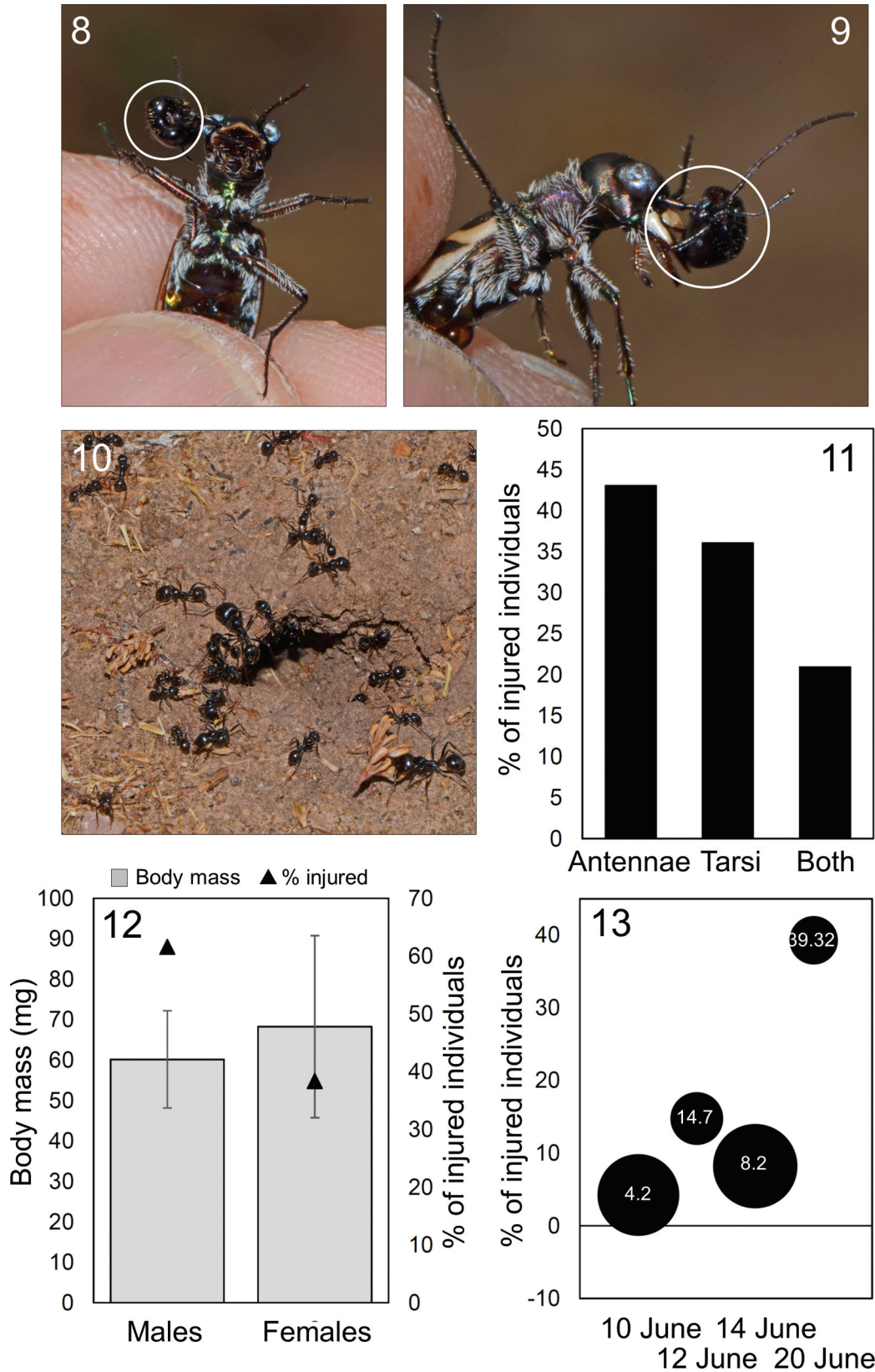


Figure 8-13. 8-9, Individuals of *Cephalota dulcinea* with ant (*Messor barbarus*) heads grasping the antennae (in the circles); 10, *Messor barbarus* individuals at their nest; 11, % frequency of individuals recorded with injuries on antennae and tarsi; 12, mean body mass (\pm standard error) of males and females (bars) and % frequency of male and female injured individuals (triangles); 13, % frequency of injured individuals (values in the circles) recorded across the study period (size of circles proportional to the total number of sampled individuals).

(Figure 11). Males and females of *C. dulcinea* did not differ in body mass (60.2 ± 12.0 mg vs. 68.3 ± 22.5 mg, respectively) (Student's *t*-test: $t = 1.42$, $df = 36$, $p = 0.16$), and similar proportions of injured males and females were observed (8.5% and 5.3%, respectively, $n = 618$) ($\chi^2 = 2.09$, ns) (Figure 12). Ant injuries have been recorded with similar frequencies in the afternoon (15.00–20.00: 61.6%, $n = 86$) and in the morning (11.00–14.59: 31.4%, $n = 86$) ($\chi^2 = 2.09$, ns). This was true even if males and females were considered separately ($\chi^2 = 0.6$ and $\chi^2 = 1.5$, ns, respectively). On the other hand, ant injuries seemed to increase in frequency with date (Figure 13).

Discussion

Tiger beetle fauna of La Mancha salt marshes is rich in species and a previous report showed that from May to June *Cephalota dulcinea* is quite abundant in its areas of occupation (Rodríguez-Flores et al. 2016).

Our calculations indicate a healthy and relatively abundant population for *C. dulcinea* at the study area, particularly if we consider that this species inhabits restricted, almost isolated salted lake areas. Both higher and lower population sizes were reported for other tiger beetle species inhabiting similar environments. For example, a population of *Cephalota circumdata leonschaeferi* (Cassola, 1970) occurring near a saline lake in Tuscany was found to have an estimated population size of around 700 individuals at its peak, then decreasing in correspondence with increasing ground temperature (Eusebi et al. 1989). The size of a population of *Cicindela patruela* Dejean, 1825 at Pinery Provincial Park (Canada) was (very roughly) estimated at only 400–1000 individuals including both larvae and adults (COSEWIC 2009). On the other hand, *Habroscelimorpha dorsalis dorsalis* (Say, 1817) may reach over 5000 individuals at a single colonized site along the Chesapeake Bay shoreline in Virginia, USA (Knisley et al. 2016). Differences in these values may change accordingly to the extent of movements across large areas, since *C. dulcinea*, *C. circumdata leonschaeferi* and *C. patruela*, which have relatively small populations, were not seen to disperse at large distances (Eusebi et al. 1989; Willis 2001; Rodríguez-Flores et al. 2016). In general, besides some movements within habitat patches, tiger beetles do not readily disperse to other patches even when these are relatively close (Pearson & Vogler 2001).

Previous information on the attack of ants on ant-hunting tiger beetles report occurrence of injuries/ant heads, sometimes with the identification of the injured body parts (Table 1). In particular, an inspection of the literature revealed a total of 29 recorded cases of ant heads on the appendages of tiger beetles, involving a total of 25 tiger beetle species/subspecies (plus one

undetermined one) and ant species from at least 7 genera (*Formica* Linnaeus, 1758, *Pogonomyrmex* Mayr, 1868, *Camponotus* Mayr, 1861, *Lasius* Fabricius, 1804, *Dorymyrmex* Mayr, 1866, *Polyergus* Latreille, 1804 and *Solenopsis* Westwood, 1840) (Table 1). About half of ant–tiger beetle interactions were recorded to date for taxa of *Cicindela* (16 cases and 14 species/subspecies) and all previous information regarded North American species. *Messor* was never recorded previously in such interactions. Because we observed in several occasions individuals of *C. dulcinea* while devouring these ants, and since *Messor* species are seed-predators rather than insect predators (Azcárate & Manzano 2011; Plowes et al. 2013), we can conclude that the found injuries on tiger beetles are not a product of failed predatory attempts by the ants but of defensive counter-attacks to *C. dulcinea* predation. Frequency of injured individuals (14%) suggest that this ant is an important component of the *C. dulcinea* diet, which also includes other small hymenopterans and small dipterans (Rodríguez-Flores et al. 2016).

As in our study, observations retrieved from the available literature show antennae and tarsi to be the body parts most commonly grasped by ant heads, with one additional case of ant head attached to maxillary palpi (*Cicindelidia marginipennis* LeConte, 1851) (Valenti & Gaimari 2000). Partially in accordance with our hypothesis, antennae may be heavily involved in the interactions because tiger beetles grasp their prey with their mandibles (Pearson & Vogler 2001). Because antennae and tarsi were frequently injured, consequences on both chemosensory and locomotion abilities may be expected. From one side, total antennal length is known to make a significant contribution to tracking ability in insects (Lockey & Willis 2015), and experimentally shortened antennae, i. e. cutting off the antennal segment richest in sensilla (e.g. Polidori et al. 2012), drastically reduce behavioral responses to odors (Ali et al. 2016). From the other side, leg amputation is known to interfere with normal movements in insects (Wilson 1966) and tarsi are also rich in sensilla and thus used not only in locomotion but also in tasting (Loy et al. 2016).

Ant injuries have been recorded with similar frequencies across the day, but they seemed to increase in frequency with date. These trends may be due to the daily and seasonal activity of ants at the study site, which however remains to be evaluated. Both *Messor* ants and *Cephalota* tiger beetles are known to have a daily activity heavily affected by soil temperature, strongly decreasing at temperatures $>35^\circ\text{C}$ (Eusebi et al. 1989, Azcárate et al. 2007), so it is possible that predator and prey share a similar activity window during which the frequency of their interactions is roughly constant. On the other hand, ant colony phenology may explain variation of attacks by *C. dulcinea* during the year. For example, some *Messor*

Table 1. Available information in the literature on ant heads found attached on tiger beetle body parts. Tiger beetle species names found in the literature were updated following Pearson et al. (2015).

Tiger beetle species/subspecies	Ant genus/species	Country	Reference
<i>Brasiella viridisticta arizonensis</i> (Bates, 1884)	Undetermined	USA	Larochelle (1974)
<i>Cephalota dulcinea</i> López, De la Rosa & Baena, 2006	<i>Messor barbarus</i> (Forel, 1905)	Spain	This study
<i>Cicindela bellissima</i> Leng, 1902	<i>Formica pallidefulva nitideventris</i> Emery, 1893	USA	Valenti & Gaimari (2000)
<i>Cicindela formosa gibsoni</i> Brown, 1940	<i>Pogonomyrmex barbatus</i> (Smith, 1858)	USA	Valenti & Gaimari (2000)
<i>Cicindela formosa gibsoni</i> Brown, 1941	Undetermined	USA	Larochelle (1974)
<i>Cicindela formosa</i> Say, 1817	Undetermined	USA	Pearson et al. (2006)
<i>Cicindela limbalis</i> Klug, 1834	<i>Camponotus</i> sp.	USA	Valenti & Gaimari (2000)
<i>Cicindela pugetana</i> Casey, 1914	<i>Pogonomyrmex barbatus</i> (Smith, 1858)	USA	Valenti & Gaimari (2000)
<i>Cicindela pulchra</i> Say, 1823	<i>Pogonomyrmex barbatus</i> (Smith, 1858)	USA	Valenti & Gaimari (2000)
<i>Cicindela repanda</i> Dejean, 1825	Undetermined	USA	Larochelle (1974)
<i>Cicindela scutellaris lecontei</i> Haldeman, 1853	<i>Camponotus</i> sp.	USA	Valenti & Gaimari (2000)
<i>Cicindela scutellaris lecontei</i> Haldeman, 1854	Undetermined	USA	Larochelle (1974)
<i>Cicindela scutellaris</i> Say, 1823	<i>Pogonomyrmex barbatus</i> (Smith, 1858)	USA	Valenti & Gaimari (2000)
<i>Cicindela sexguttata</i> Fabricius, 1775	Undetermined	USA	Larochelle (1974)
<i>Cicindela</i> sp.	<i>Pogonomyrmex occidentalis</i> (Cresson, 1865)	USA	Willis (1967)
<i>Cicindela tranquebarica</i> Herbst, 1806	<i>Formica</i> sp.	USA	Valenti & Gaimari (2000)
<i>Cicindela tranquebarica kirbyi</i> LeConte, 1867	<i>Pogonomyrmex barbatus</i> (Smith, 1858)	USA	Valenti & Gaimari (2000)
<i>Cicindela willistoni</i> LeConte, 1879	Undetermined	USA	Larochelle (1974)
<i>Cicindelidia marginipennis</i> Dejean, 1831	<i>Formica</i> sp.	USA	Valenti & Gaimari (2000)
<i>Cicindelidia obsoleta</i> Say, 1823	<i>Pogonomyrmex rugosus</i> Emery, 1895	USA	Valenti & Gaimari (2000)
<i>Cicindelidia obsoleta santaclarae</i> Bates, 1890	Undetermined	USA	Larochelle (1974)
<i>Cicindelidia punctulata</i> Olivier, 1790	<i>Lasius</i> sp.	USA	Valenti & Gaimari (2000)
<i>Cicindelidia punctulata chihuahuae</i> Bates, 1890	Undetermined	USA	Valenti & Gaimari (2000)
<i>Ellipsoptera hamata lacerata</i> (Chaudoir, 1854)	<i>Polyergus</i> sp.	USA	MacRae (2019)
<i>Ellipsoptera hirtilabris</i> LeConte, 1875	<i>Dorymyrmex bureni/flavus</i>	USA	MacRae (2019)
<i>Ellipsoptera marutha</i> Dow, 1911	Undetermined	USA	Larochelle (1974)
<i>Eunota circumpicta johnsoni</i> Fitch, 1857	Undetermined	USA	Larochelle (1974)
<i>Eunota togata</i> (LaFerté-Sénectère, 1841)	<i>Polyergus</i> sp.	USA	Pearson & Vogler (2001)
<i>Parvindela celeripes</i> LeConte, 1846	Undetermined	USA	MacRae (2019)
<i>Tetracha virginica</i> (Linnaeus, 1766)	<i>Solenopsis molesta</i> (Say, 1836)	USA	Valenti & Gaimari (2000)

species studied in Spain increase their foraging activity during summer and reach their maximum in September–October (Cros et al. 1997). Thus, towards the end of adult *C. dulcinea* yearly activity (end of June), tiger beetle abundance is lower but *M. barbarus* abundance may be higher than in early June, perhaps resulting in an increased use of ants as prey and in consequence in an increased frequency of ant injuries.

Similar incidence of injuries in both sexes may be due to the fact that body mass did not differ between males and females. Thus, if we assume that smaller and, thus, likely weaker individuals, would suffer more injuries by counter-attacking ant prey, the similar body mass of the two sexes would make the output of interactions similar. Valenti & Gaimari (2000) also found tiger beetle males and females with similar frequencies of ant heads attached to

appendages. Despite the sex-ratio for the population being slightly biased toward males, we did not find differences in the frequency of ant injuries between *C. dulcinea* sexes, which suggests that males may be overall a little bit less susceptible to ant counter-attacks.

The generally narrow ecological requirements of tiger beetles (Pearson 1988) make them good taxa to monitor environmental changes (Pearson & Cassola 1992), as well as making these insects particularly vulnerable to local population extinctions (Schultz 1989; Knisley & Hill 1992). Besides human-driven disturbing factors such as habitat modifications, the negative impact of high-frequency predation, as well as the consequences of hunting particularly strong prey such as ants, may contribute to shaping population size. In our study, 14% of all marked individuals were injured by ants, which makes a minimum of roughly 6% of the total estimated population during one single reproductive season, which is not a negligible value. We thus suggest that improving our knowledge on these biotic interactions will help to determine tiger beetle status and to make predictions on their future (Cordero-Rivera & Zhang 2018), particularly in localized populations of species with an overall restricted distribution.

Acknowledgements

We thank Alberto Sánchez-Vialas and Pablo Pichaco, for their support in the field during some of the sampling sessions, and Jose Manuel Serrano and Diego López (Universidad Complutense de Madrid) for the identification of ants; Amanda del Río and Eduardo de Miguel (Global Nature) for their support; the Consejería de Medio Ambiente de Castilla-La Mancha for providing us with permits for the study of tiger beetles in La Mancha protected areas. This study was possible thanks to the facilities provided by the Museo Nacional de Ciencias Naturales (MNCN-CSIC).

Funding

This study was funded by the Fundación Global Nature through the European Life European Community Project Humedales de La Mancha (LIFE10 NAT/ES/000563) (Spanish Ministry of Agriculture, Food and Environment) and to a post-doctoral contract (SECTI) by the Universidad de Castilla-La Mancha to CP.

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