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4

5 **Pre-adaptive Shift of a Native Predator (Araneae, Zodariidae) to an Abundant Invasive Ant Species**  
6 **(Hymenoptera, Formicidae)**

7

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28 **Abstract**

29 Invasive species often displace native species and thus affect ecological processes in invaded habitats. If invasive  
30 species become abundant changes in prey availability may affect the specialist predators. The Argentine ant,  
31 *Linepithema humile* Mayr, is one of the most important invasive species on nearly all continents. Spiders of the genus  
32 *Zodarion* are specialised ant-eating predators native to the Mediterranean. These spiders may exploit invasive ant  
33 species. Indeed, we found in the field that one species, *Zodarion cesari* Pekár, was preying exclusively on these ants.  
34 Here we studied spatial and temporal abundance of this invasive ant and the native spider during four years in four  
35 citrus groves. Circadian activity of both spiders and ants, and capture efficiency and prey specificity of the predator  
36 were also evaluated.

37 The abundance of *Z. cesari* was strongly correlated to the *L. humile* abundance. The predatory activity of  
38 spiders varied during season with differences on the relative frequency of spiders capturing ants depending on the time  
39 of the year. In laboratory, *Z. cesari* displayed most efficient capture upon the native ant *Tapinoma nigerrimum*  
40 (Nylander) and the invasive ant *L. humile* in comparison with five other native ant species. These results demonstrate  
41 that the native spider *Z. cesari* is successfully exploiting the invasive ant species *L. humile* and is likely a locally  
42 monophagous predator. We consider that *Z. cesari* shifted away from native *T. nigerrimum*. Such shift was made  
43 possible due to the close relationship between these two ant species: both being phylogenetically related and of similar  
44 size.

45

46 **Key-words:**

47 *Linepithema humile*, specialist predator, *Zodarion*, prey specialisation, community ecology

48

## 49 **Introduction**

50 Trading of goods between different geographic regions is known to have implications on the ecology and evolution of  
51 native ecosystems (Perrings et al. 1992; Mooney and Cleland 2001; Wittenberg and Cock 2001; Strauss et al. 2006).  
52 Invasive ants are widely recognized as an important component of human-caused global environmental change, often  
53 resulting in a significant disruption of native communities and are, thus, considered some of the most ecologically  
54 damaging invasive species (Moller 1996; Holway et al. 2002). The Argentine ant, *Linepithema humile* Mayr  
55 (Formicidae, Dolichoderinae), is a highly invasive species that has successfully spread from its native range in South  
56 America across many zones of the globe, being introduced by humans to more than 30 countries worldwide, particularly  
57 in areas with Mediterranean climate (Suarez et al. 2001). In Southern Europe, two continental supercolonies have been  
58 identified, the Catalanian supercolony, and the main European supercolony spreading in Portugal, Corsica and Greece  
59 (Giraud et al. 2002; Blight et al. 2010).

60 One factor that facilitates the spread of the Argentine ants is an unusual social structure, unicoloniality, in  
61 which individuals mix freely among physically separated nests allowing them to reduce cost on territoriality and  
62 defence, and to increase the colony growth, foraging and recruitment activities (Holway et al. 2002; Pedersen et al.  
63 2006). In alien habitats, unicoloniality permits Argentine ants to displace native ant species, thus monopolising the  
64 invaded environments (Human and Gordon 1996; Holway 1998, 1999). Furthermore, these ants reduce natural  
65 resources to levels at which competition becomes a main factor (Hölldobler and Wilson 1990). As a consequence, a  
66 reduction of other arthropods, vertebrate and plant diversity is generated (Holway et al. 2002; Blancafort and Gomez  
67 2005; Lanch 2008).

68 Ants enter into an array of mutualistic interactions in agro-ecosystems, and as a result can be categorized as  
69 pests. In this way, the Argentine ant is regarded as a significant pest in many agricultural systems, such as vineyards and  
70 citrus groves (Vega and Rust 2001; Daane et al. 2008), because it protects honeydew-producing homopterans from their  
71 predators. As a consequence, an effectiveness reduction of this component of biological control is obtained (Bartlett  
72 1961; Moreno et al. 1987). The predatory role of invasive ants, albeit of significant benefit in other arthropod pest  
73 control (Wong et al. 1984; Urbaneja et al. 2006), may have negative effects on beneficial arthropods, such as spiders. It  
74 seems likely that certain spiders (e.g., web-building species) are more negatively affected by the presence of ants  
75 (Lenoir et al. 2003; Sanders and Platner 2007) than others (e.g., ground-dwelling species) (Halaj et al. 1997). However,  
76 it is difficult to establish generalizations about the relation between ant presence and its effect on spiders at a group  
77 level (e.g., Schuch et al. 2008). There is a lack of available data about the influence of invasive ants on spiders (Holway  
78 et al. 2002). Touyama et al. (2008) observed a higher density and frequency of the myrmecophagic jumping spider *Siler*

79 *cupreus* Simon in sites infested with Argentine ants than in ant-free sites urban/residential areas. However, nutritional  
80 values or preferences for invasive ants compared to native ants remain unexamined.

81 Ant invaders might also be foreseen as a source of food for indigenous generalist predators because they can  
82 reach very high densities (Maerz et al. 2005; King et al. 2006). For example, ant-lions, *Myrmeleon* spp. (Neuroptera,  
83 Myrmeleontidae), fared better in habitats where *L. humile* became the dominant ant species than in habitats where the  
84 invasive ant was not present (Glenn and Holway 2008). But for native prey-specialised predators, which are often  
85 strictly adapted to their exclusive prey (e.g., Pekár et al. 2008), shifts to novel prey might entail behavioural and  
86 physiological trade-offs. Some ant-eating spiders have adapted to their native prey to such an extent that exploitation of  
87 alternative ants has negative effects in terms of low survival and slower development (Pekár et al. 2008).

88 In the Mediterranean region, spiders of the genus *Zodarion* (Araneae, Zodariidae) are very abundant (Pekár  
89 and Lubin 2003) and known to catch no other prey but native ants (Pekár 2004; Pekár and Toft 2009). Species of this  
90 genus were found to target certain ant taxa (Pekár et al. 2005), but the specialisation is limited to a subfamily level  
91 (Pekár et al. 2008). Therefore, it is expected that shifts towards other ant species might be possible only among closely  
92 related species (for example, within the same subfamily). At present, the Argentine ants are potential prey to *Zodarion*  
93 spp. in citrus groves, where they were recently reported (Urbaneja et al. 2006). Yet, no evidence on the exploitation of  
94 this abundant invasive ant by this indigenous ant-eating predator has been recorded.

95 Here, we have studied to what extent *Zodarion cesari* Pekár, a new species described recently (Pekár et al.  
96 2011), is exploiting an invasive ant species, *L. humile*. First we investigated the association between the two species in  
97 time and space. For an obligatory exploitation of the prey it is essential that predator and prey have similar spatial and  
98 temporal activities. Absence of such a pattern would indicate facultative predation. Additionally, we investigated prey  
99 specificity and capture efficiency of *Z. cesari* for different ant species in order to reveal trade-offs in utilisation of *L.*  
100 *humile*. We hypothesize that if *Z. cesari* has preadaptations for the exploitation of *L. humile*, the capture efficacy should  
101 be similar to that for the native prey. Since *L. humile* individuals have similar body size and morphological traits to  
102 some of the native and closely related ant species, we expected to find the native prey among Dolichoderinae species.  
103 The importance of such effects will have implications for the control of this invasive ant species and for conservation  
104 programs enhancing indigenous ant-eating predators.

105

## 106 **Material and methods**

### 107 *Spatial co-occurrence of ants and spiders*

108 Citrus ground ant communities were studied in four commercial citrus groves located in the Valencia region (Spain):  
109 Bétera grove (UTM: X722106, Y4388610; 30 m asl), Náquera (UTM: X722427, Y4385216; 110 m asl), Olocau (UTM:

110 X706741, Y4400206; 330 m asl) and Picassent grove (UTM: X715482, Y4358584; 75 m asl). In Bétera grove, the ant  
111 community was dominated by the exotic ant *L. humile*. Twelve pitfall traps were regularly distributed diagonally across  
112 each grove with a distance between traps of 20 m approximately. Each trap consisted of a plastic jar (12.5 cm diameter  
113 and 12 cm depth), with a plastic funnel fitted to the upper edge of the jar. A plastic 150 ml container half filled with a  
114 3:1 mixture of water and ethanol, and 0.1% detergent, was placed inside the plastic cup. Sampling was performed from  
115 April 2005 to April 2006. Traps were changed every 14 days and all ant specimens collected were counted and  
116 identified to the species level. In each grove the number of *Z. cesari* specimens in pitfall traps was also recorded.

117 To establish the seasonal activity pattern of the Argentine ant and the spider *Z. cesari*, in the grove located in  
118 Bétera, the number of specimens belonging to both species captured per trap was recorded every 14 days using the same  
119 pitfall traps design described above from April 2004 to December 2007.

120

#### 121 *Circadian activity*

122 In Bétera, the circadian activity of the Argentine ants and the ant-eating spider, *Z. cesari*, was studied at three different  
123 dates during the season when ants and spiders were active. The first observation was performed on November 13, 2008,  
124 the second on May 29, 2009, and the third on October 8, 2009. Sunrise, sunset and temperature minimum and  
125 maximum for each date are given in Table 1. On each sampling day, the activity was observed for 24 hours and was  
126 measured every two hours as the number of ants and spiders found during 5 min in a 50 x 50 cm<sup>2</sup> square area that  
127 surrounded the opening of a *L. humile* nest, in six randomly selected locations of the grove. For the third observation,  
128 sampling hours were restricted from 3 hours before sunset to 2 hours before sunrise because the previous observations  
129 revealed nocturnal activity of spiders.

130

#### 131 *Natural prey*

132 Observations on the natural prey of *Z. cesari* were conducted in Bétera during night along with the circadian activity  
133 measurements and in the vicinity of three nests of the native ant species, *Cataglyphis gadeai* De Haro & Collingwood,  
134 *Messor barbarus* (Linnaeus) and *Pheidole pallidula* (Nylander), that were located along the border of the infested  
135 grove. We counted spiders either capturing or feeding on ants. Each individual spider was collected and the prey was  
136 identified.

137

#### 138 *Capture efficiency*

139 In order to investigate the degree of prey specialisation of *Z. cesari*, field observations were followed by laboratory  
140 investigations. The laboratory experiments were performed to reveal the capture efficiency of the spider to different ant

141 species. Subadult or adult specimens of *Z. cesari* were field collected in Bétera and individually placed in 1.5 ml  
142 polypropylene microtubes (Deltalab, S. L., Barcelona, Spain) and kept in starvation for 5 days in the laboratory. Water-  
143 soaked cotton wool was supplied as a source of water. Twenty-four hours prior to the experiment each spider was  
144 transferred to a Petri dish (60 mm diameter). The Petri dishes had a rough bottom to ease spider movements. The wall  
145 of the Petri dishes were Fluon (Polytetrafluoroethylene 60%, Sigma-Aldrich®, St. Louis, MO, USA) coated to avoid ant  
146 and spider escape. Each spider was tested only once by offering a single ant individual from any of the following ant  
147 species: *L. humile* (body length = 2.9 mm, n = 18), *Tapinoma erraticum* (Latreille) (3.3 mm, n = 10) and *Tapinoma*  
148 *nigerrimum* (Nylander) (3.9 mm, n = 10) (Dolichoderinae); *Formica rufibarbis* Fabricius (5.3 mm, n = 10) and *Lasius*  
149 *grandis* Forel (3.9 mm, n = 15) (Formicinae); *Messor barbarus* (Linnaeus) (6.9 mm, n = 14) and *Pheidole pallidula*  
150 (Nylander) (2.5 mm, n = 11) (Myrmicinae). These species are the most representative of the Western Mediterranean  
151 citrus ant communities (Vanaclocha et al. 2005; Cerdá et al. 2009). All ant individuals were field-collected 3-5 h prior  
152 to the experiment. The size of each ant species was estimated by measuring the distance from the front of the head to  
153 the end of the gaster. Latency to the first attack, number of attacks, and the paralysis latency were recorded for each  
154 trial. The latency to the first attack was estimated as the time from the first encounter (between the spider and the ant) to  
155 the first attack. The number of attacks was the frequency of attacks during the experimental time and showed the  
156 aggressiveness of the spider to each ant species, as the spider made multiple attacks. The paralysis latency was  
157 estimated as the time between the first attack and the complete immobilisation (i.e. when the ant could not rise on its  
158 legs). The tests lasted at most 120 min. All the experiments were performed after sunset and under red light. To confirm  
159 the nocturnal activity of *Z. cesari* a day trial, also under dark conditions, using *L. humile* as prey (n = 11) was  
160 performed. The number of attacks was compared between day and night trials.

161

#### 162 *Data analyses*

163 As there were several traps in each orchard arranged in a transect we expected that the samples might not be  
164 independent. Thus data on total annual abundance of ants and *Z. cesari* spiders from 12 pitfall traps were tested for  
165 spatial correlation using autocorrelation function. As no correlation was significant the traps were considered  
166 independent. Data on annual abundance of each ant and *Z. cesari* spider species were then subjected to a multivariate  
167 analysis within CANOCO (Ter Braak and Šmilauer 2002), Detrended Correspondence Analysis (DCA). Long gradient  
168 (4.9) suggested a unimodal response of species. Therefore, Canonical Correspondence Analysis (CCA) was used.  
169 Significance of study sites was assessed from Monte Carlo permutation test on the first axis with 499 simulations.

170 All other analyses were performed within R environment (R Development Core Team 2009). Spearman  
171 correlation was used to study the relationship between abundance or density of spiders and ants as the data were ordinal.

172 ANOVA was used to compare annual diversities between sites by means of the Shannon-Wiener index (Ludwig and  
173 Reynolds 1988). Otherwise Generalised Linear Models (GLM) with various error structures and links were used (Pekár  
174 and Brabec 2009). To compare annual abundances between sites and the number of attacks between ants, GLM with  
175 quasiPoisson and log link (GLM-qp) was used due to overdispersion. To compare latency to the first attack and  
176 paralysis latency among different ant subfamilies or species GLM with Gamma error structure and log link (GLM-g)  
177 was used.

178

## 179 **Results**

### 180 *Spatial co-occurrence of ants and spiders*

181 The total number of ants captured was not similar in all four study sites (GLM-qp,  $F_{3,44} = 15.9$ ,  $P < 0.0001$ ), with the  
182 highest abundance in Bétera and the lowest ant abundance occurring in Náquera, Olocou and Picassent (Table 2). The  
183 diversity of the ant community was significantly different among groves (ANOVA,  $F_{3,44} = 7.6$ ,  $P = 0.0003$ , Table 2),  
184 with the lowest diversity occurring at Bétera. The ant community composition differed significantly between Bétera and  
185 the rest of the groves (CCA,  $F = 54.3$ ,  $P = 0.002$ , Fig. 1). In Náquera, Olocou and Picassent four native ant species, *L.*  
186 *grandis*, *P. pallidula*, *M. barbarus* and *F. rufibarbis*, dominated the ant community, whereas Bétera only *L. humile* was  
187 eudominant with 93.4% of all specimens (Table 2).

188 The number of *Z. cesari* specimens captured per trap in the invaded grove (Bétera) was significantly higher  
189 than in all the non-invaded groves (Náquera, Olocou and Picassent) (GLM-qp,  $F_{3,44} = 72.5$ ,  $P < 0.001$ , Fig. 2a). CCA  
190 revealed that the number of *Z. cesari* spiders is related to the number of *L. humile* and *P. membranifera* ants. There was  
191 a strong positive correlation between *Z. cesari* and *L. humile* (Spearman correlation,  $\rho = 0.80$ ,  $S = 3637$ ,  $P < 0.0001$ ,  
192 Fig. 2b) and *P. membranifera* (Spearman correlation,  $\rho = 0.54$ ,  $S = 8429$ ,  $P < 0.0001$ ).

193

### 194 *Seasonal activity*

195 Altogether 30,692 *L. humile* specimens and 1,978 *Z. cesari* spiders were collected from April 2004 to December 2007.  
196 The seasonal abundance of *L. humile* and *Z. cesari* spiders was low between December and April and markedly  
197 increased between May and November (Fig. 3). In 2004 the peak of *L. humile* ants occurred in June and July (Fig. 3a);  
198 in 2005 it was in September and October (Fig. 3b); in 2006 it was in May and August (Fig. 3c); in 2007 it was in June  
199 and September (Fig. 3d).

200 The abundance of *Z. cesari* spiders followed a rather similar pattern as that of the ants, being low between  
201 December and April and markedly increased between May and November (Fig. 3). In all four years, there was a small  
202 peak in early summer and a large peak in autumn. The abundance of spiders was strongly correlated to the ant



203 abundance in 2004 (Spearman correlation,  $\rho = 0.69$ ,  $S = 720$ ,  $P = 0.0002$ ), 2005 (Spearman correlation,  $\rho = 0.67$ ,  $S =$   
204  $757$ ,  $P = 0.0003$ ) and in 2007 (Spearman correlation,  $\rho = 0.45$ ,  $S = 1285$ ,  $P = 0.03$ ), but not in 2006 (Spearman  
205 correlation,  $\rho = 0.16$ ,  $S = 2178$ ,  $P = 0.44$ ).

206

#### 207 *Circadian activity*

208 The circadian activity of *L. humile* varied during the season (Fig. 4). In early summer (May), ants were active from  
209 early evening (18.00) until morning (10.00) (Fig. 4a); in early autumn (October) from afternoon until midnight (22.00)  
210 (Fig. 4b); in November from morning (8.00) until evening (22.00) (Fig. 4c).

211 The spider *Z. cesari* was active only during darkness. There was a shift in its circadian activity pattern during  
212 the season. The peak always occurred 30 min after sunset. In May it was at 22.00 (Fig. 4a); in October at 20.00 (Fig.  
213 4b); in November at 18.00 (Fig. 4c). Activity of *Z. cesari* spiders was not correlated to the activity of *L. humile* ants in  
214 May (Spearman correlation,  $\rho = 0.15$ ,  $S = 243$ ,  $P = 0.65$ ), in October (Spearman correlation,  $\rho = -0.40$ ,  $S = 117$ ,  $P =$   
215  $0.33$ ) or in November (Spearman correlation,  $\rho = -0.13$ ,  $S = 322$ ,  $P = 0.70$ ).

216

#### 217 *Natural prey*

218 In the grove invaded with *L. humile* the spiders were found to catch only *L. humile* (100%,  $n = 241$ ) (Video S1). The  
219 predatory activity of *Z. cesari* spiders varied during season. In May the percentage of spiders observed capturing an ant  
220 was 6% ( $n = 70$ ); in October it was 26.5% ( $n = 147$ ); in November it was 4% ( $n = 24$ ). No *Z. cesari* was found in the  
221 proximity of any of the three native ant nests that were located along the border of the grove.

222

#### 223 *Capture efficiency*

224 In the laboratory, spiders had a 3-times higher capture activity on *L. humile* ants during nocturnal conditions than during  
225 diurnal ones (GLM-p,  $X^2_1 = 13.5$ ,  $P = 0.0002$ , Fig. 5a). In the nocturnal conditions the number of attacks was similar for  
226 all seven ant species (GLM-p,  $X^2_6 = 0.1$ ,  $P = 1$ , Fig. 5b). Latency to the first attack was significantly different among the  
227 three ant subfamilies tested (GLM-g,  $F_{2,85} = 12.2$ ,  $P < 0.0001$ , Fig. 6a, Video S2). It was significantly shorter for  
228 Dolichoderinae than for Myrmicinae or Formicinae ants (contrasts,  $P < 0.01$ ). Within Dolichoderinae, it was shortest for  
229 *T. nigerrimum*, followed by *L. humile* and *T. erraticum* (Fig. 6b). Paralysis latency was significantly different among  
230 the three ant subfamilies (GLM-g,  $F_{2,87} = 11.6$ ,  $P < 0.0001$ , Fig. 7a). It was significantly shorter for Dolichoderinae than  
231 for Myrmicinae or Formicinae ants (contrasts,  $P < 0.001$ ). Within Dolichoderinae, it was similar for *T. nigerrimum* and  
232 *L. humile*, but longer for *T. erraticum* (Fig. 7b). The paralysis latency was independent of ant size (GLM-g,  $F_{1,87} = 1.2$ ,  
233  $P = 0.28$ ).

234

235 **Discussion**

236 Invasive ants are notorious for directly displacing native ant species and posing serious threats to native biodiversity and  
237 ecosystems worldwide. The Argentine ant, *L. humile*, is one of the world's most damaging invasive insects (Suarez et  
238 al. 2001; Holway et al. 2002). By acting as either predator or fierce competitor, Argentine ants can displace native ants  
239 and other invertebrates (Holway 1999; Holway et al. 2002; Sanders et al. 2003). Previous studies on ant communities in  
240 the Valencia region found that two native ant species, a formicine, *L. grandis*, and a myrmicine, *P. pallidula*, are the  
241 most prevalent species (Vanaclocha et al. 2005; Cerdá et al. 2009; Pekas et al. 2010), and that once *L. humile* become  
242 established, the diversity of the ant community was reduced. Accordingly, our results showed that the ant community  
243 composition differed significantly between the invaded and non-invaded groves. In the non-invaded grove four native  
244 ant species, *L. grandis*, *P. pallidula*, *M. barbarus* and *F. rufibarbis*, dominated the ant community, whereas in the  
245 invaded one only *L. humile* was eudominant representing 93.4% of all specimens.

246 A change in the ant community was expected to affect the composition of the ant-eating predators. The  
247 abundance of *Zodarion* spiders was strongly and positively correlated with two ant species, *L. humile* and *P.*  
248 *membranifera*, but observations in the field confirmed only the exploitation of the first one. Moreover, in those groves  
249 where *L. humile* has not yet been established, the abundance of *Z. cesari* was significantly lower.

250 It is expected that specialised predators have finely adapted to their prey. This includes temporal and spatial  
251 co-occurrence and high hunting and physiological efficacy. As such, the recently described indigenous ant-eating  
252 spider, *Z. cesari*, preyed very successfully upon the prevalent Argentine ant, *L. humile*, under field conditions,  
253 suggesting that its acceptance of the new prey was quite rapid.

254 The seasonal activity of *L. humile* extends throughout the season like that of other ant species (e.g. López et al.  
255 1992), with one large peak from early summer to late autumn. Seasonal activity of *Z. cesari* overlaps with that of *L.*  
256 *humile*. In this case, it is bimodal, with one peak in early summer and the other in early autumn. The two peaks would  
257 correspond with two main discrete reproductive periods. Thus, there are at least two generations per year. Such a  
258 pattern is typical for other *Zodarion* species occurring in the Mediterranean (Pekár et al. 2005). Interestingly, *Z. cesari*  
259 is much smaller in size than the other *Zodarion* species with two generations, so it could even produce several  
260 generations as adults were found throughout the year. The observed correlation in seasonal activities between *Z. cesari*  
261 and *L. humile*, however, does not appear to be a result of adaptation of the predator to its prey.

262 The circadian activity of *L. humile* is rather broad, but the activity of *Z. cesari* is more restricted, in particular  
263 to the night. Such activity is advantageous due to the absence of visually oriented predators, and higher humidity in dry  
264 and hot areas (Foelix 1996). Actually, many other *Zodarion* species possess nocturnal foraging activity (e.g., Harkness

265 1976; Pekár and Král 2001). Besides the reasons mentioned above, *Zodarion* spiders avoid high activity-densities of  
266 ants, in particular if these are large, as the ants are a dangerous prey (Pekár 2005). *Zodarion cesari* was active  
267 throughout the night with a marked peak of hunting activity at dusk when the Argentine ant foraging activity declined.

268 *Zodarion cesari* was able to attack all seven ant species but with a different efficacy that was measured as the  
269 number of attacks, latency to attack and to paralysis. The spiders made a similar number of attacks for all the ant species  
270 indicating that this parameter cannot be used as an indicator of prey specificity, in contrast to previous results (Pekár  
271 2005; Pekár et al. 2005). Previous research on the capture efficiency of *Zodarion* spiders has revealed that the latency to  
272 the first attack is mainly influenced by the taxonomic status (similar within subfamily), the size of the ant species (the  
273 larger the longer), and their aggressive behaviour (the more aggressive the sooner) (Pekár 2004, 2005; Pekár et al.  
274 2005). In this study the longest latency to the first attack was obtained with the formicine ant, *F. rufibarbis*. This is a  
275 large ant that showed a very aggressive behaviour towards the spider. On the other hand, *Messor barbarus*, an ant even  
276 larger than *F. rufibarbis*, also showed a long latency to the first attack. However, this latency was shorter than in the  
277 case of *F. rufibarbis*, probably due to its slower movement and less aggressive behaviour. When similarly sized  
278 medium and small ants were used as prey, differences in the latency to the first attack are not easily attributable to any  
279 of these factors. It is remarkable that the Argentine ant had the second shortest latency to the first attack; indeed, this  
280 parameter was shorter than for *T. erraticum*, a native species of the same subfamily. Paralysis latency seemed not to be  
281 influenced by the size or aggressive behaviour of the prey, but by the taxonomic status. The venom was especially  
282 efficient for two species of dolichoderine ants, *T. nigerrimum* and *L. humile*. In general, myrmicine ants showed a  
283 higher resistance to the venom. After being attacked myrmicine ants possess a lower risk to *Z. cesari*, so the venom  
284 does not need to be as effective as in case of aggressive and fast moving ants.

285 Together with the native ant species *T. nigerrimum*, the invasive ant *L. humile* had the shortest latency to the  
286 first attack and paralysis latency of all the ants assayed. *Linepithema* is phylogenetically closely related to the genus  
287 *Tapinoma* (Shattuck 1992). *Linepithema humile* has a similar behaviour to *Tapinoma* ants, but it is slightly smaller. The  
288 two species probably share numerous traits in their biology, including circadian activity (Cerdá et al. 1998) and  
289 physiology (Buczowski and Bennett 2008). According to our preliminary observations in the field, *Z. cesari* was found  
290 in association with *T. nigerrimum* in sites where *L. humile* was absent. All these results show that *Z. cesari* is  
291 specialised on a subfamily level, on the dolichoderine ants. The fact that it is likely exploiting only one ant species  
292 during its entire life cycle suggests a strict monophagy. Monophagy is possibly more common in *Zodarion* spiders than  
293 expected, in particular if the ant prey is either small or strongly polymorphic (Pekár 2009).

294 What was the native prey of *Z. cesari* before *L. humile* became available? All evidence suggests that *Z. cesari*  
295 was hunting another dolichoderine ant, such as *T. nigerrimum*. Due to the biological similarities between the two ant

296 species as outlined above, we assume that the Argentine ants are successfully exploited due to the concurrent pre-  
297 adaptations of *Z. cesari* (Strauss et al. 2006). So the shift between these prey species likely did not require evolution of  
298 adaptive changes.

299 The invasion of *L. humile* has probably triggered a chain of evolutionary processes that affects all trophic  
300 levels at the study habitats and that has involved a reconfiguration of the native arthropod communities. Pre-adaptive  
301 processes affecting some of the potential predators of the invasive species, may also affect in the long term the role of  
302 the exotic species in the ecosystems. The consequence would be the loss of the invasive status of the exotic species.  
303 This could be the case of the feeding adaptation showed in this study of *Z. cesari* when the Argentine ant spread in its  
304 habitat.

305 Further studies are needed to reveal whether the high population density of ant-eating spiders is only due to the  
306 high density of the prey or to what extent the spiders also obtain a nutritional benefit from the new prey. They should  
307 also provide further evidence that behavioural traits are correlated with the physiological specificity (Pekár et al. 2008).  
308 At least such comparative studies would permit us to track the pre-adaptive traits that lead to the change of the feeding  
309 habits of this native specific predator when it preys solely upon this invasive ant.

310

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315

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420 **Figure Captions**

421 **Fig. 1.** Ordination diagram of the CCA showing the effect of four study sites (triangle) on the number of ants and  
422 *Zodarion cesari* specimens (star) captured by pitfall traps along first two axes. The first two eigenvalues were:  $\lambda_1 =$   
423  $0.72$ ,  $\lambda_2 = 0.15$ .

424

425 **Fig. 2.** A. Comparison of the mean number of *Zodarion cesari* spiders per trap collected by pitfall traps in four citrus  
426 groves, the first one being the invaded one. Whiskers are SE of the mean. B. Relationship between the number of  
427 *Zodarion cesari* spiders and *Linepithema humile* ants.

428

429 **Fig. 3.** Comparison of the activity-density (mean  $\pm$  SE per trap) of ants and spiders captured by pitfall traps during a  
430 season of three years: 2004 (A), 2005 (B), 2006 (C), and 2007 (D) in Bétera.

431

432 **Fig. 4.** Circadian activity (mean  $\pm$ SE) of ants and spiders in late spring (A), early autumn (B) and late autumn (C).

433

434 **Fig. 5.** Comparison of the number of attacks for diurnal and nocturnal experiments (A). Number of attacks on 7 ant  
435 species from 3 subfamilies that are found in western Mediterranean citrus orchards. Ant species: MB – *Messor*  
436 *barbarus*, PP – *Pheidole palidulla*, LH – *Linepithema humile*, TE – *Tapinoma erraticum*, TN – *Tapinoma nigerrimum*,  
437 FR – *Formica rufibarbis*, LG – *Lasius grandis*. Bars are means, whiskers are 95% confidence intervals of the mean.

438

439 **Fig. 6.** Comparison of the latency to the first attack for 3 subfamilies (A) and 7 ant species (B). Ant species: MB –  
440 *Messor barbarus*, PP – *Pheidole palidulla*, LH – *Linepithema humile*, TE – *Tapinoma erraticum*, TN – *Tapinoma*  
441 *nigerrimum*, FR – *Formica rufibarbis*, LG – *Lasius grandis*. Points are means, whiskers are 95% confidence intervals of  
442 the mean.

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**Table legends**

**Table 1.** Overview of conditions at dates of studying circadian activity of spiders and ants.

**Table 2.** List of ant species collected by pitfall traps in three non-invaded and one invaded citrus groves from April 2005 to April 2006. Total number of specimens of each species and ant diversity (Shannon-Wiener index) per orchard are presented.

470 **Supporting information**

471 **Video S1.** Video footage showing *Zodarion cesari* attacking an Argentine ant, *Linepithema humile* in the field

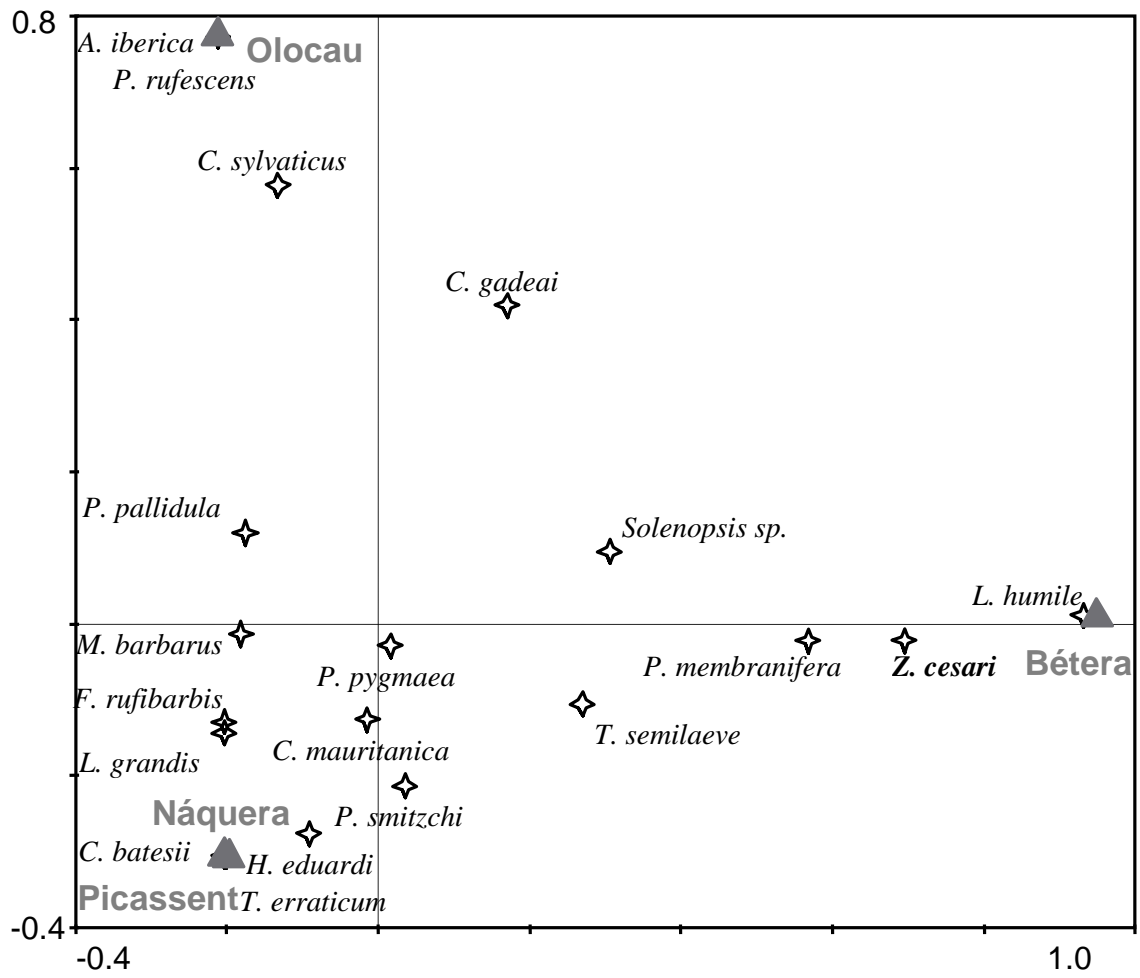
472 (Quicktime format).

473 **Video S2.** Video footage showing *Zodarion cesari* attacking an Argentine ant, *Linepithema humile* in the laboratory

474 (Quicktime format).

1 Fig. 1.

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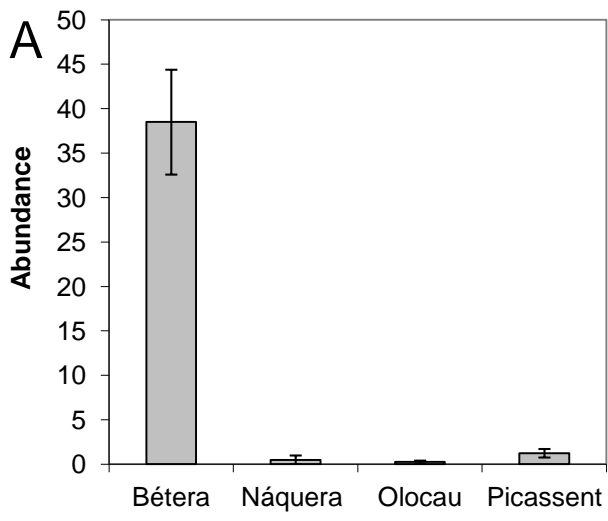
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5 **Fig. 2.**

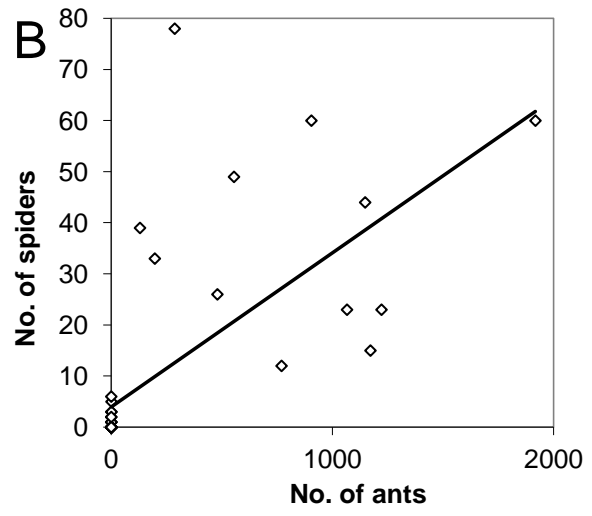
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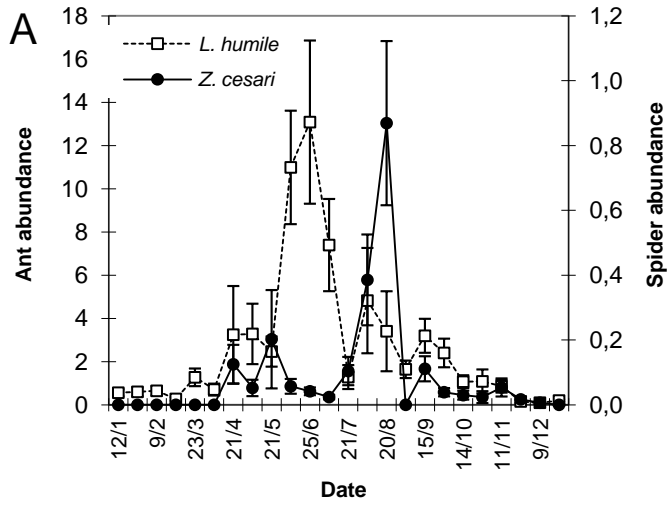


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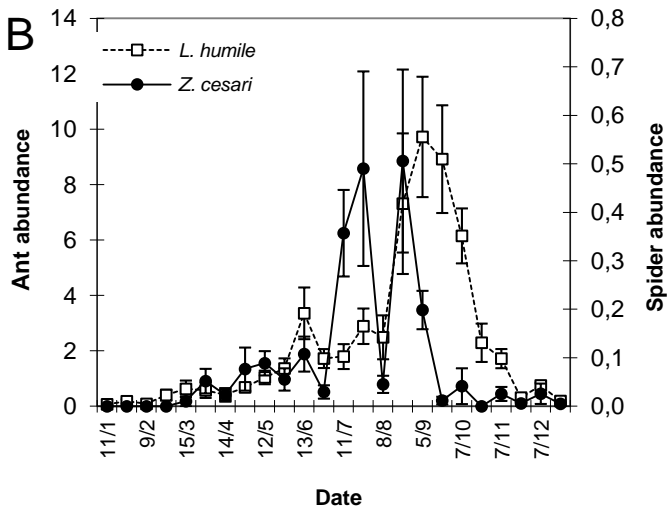
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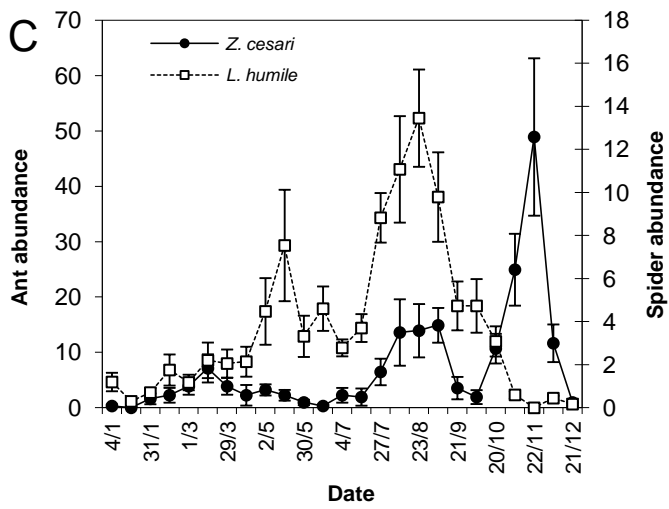
10 Fig. 3



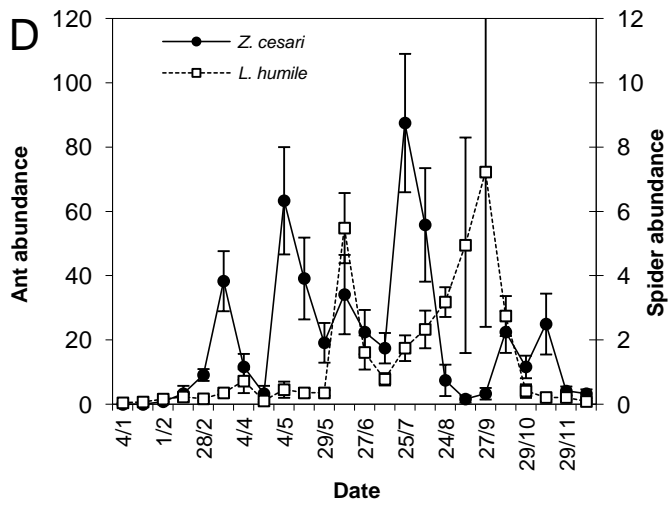
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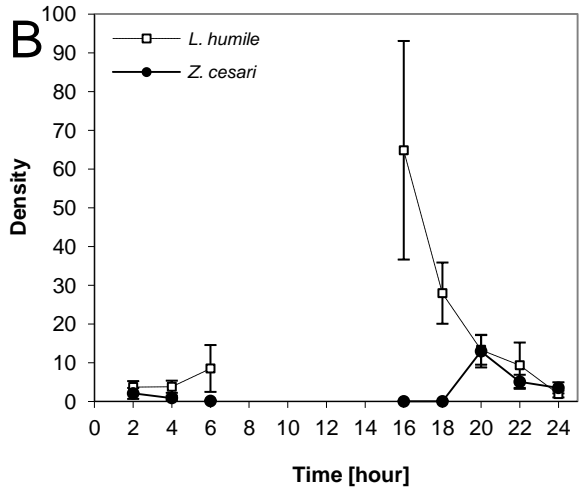
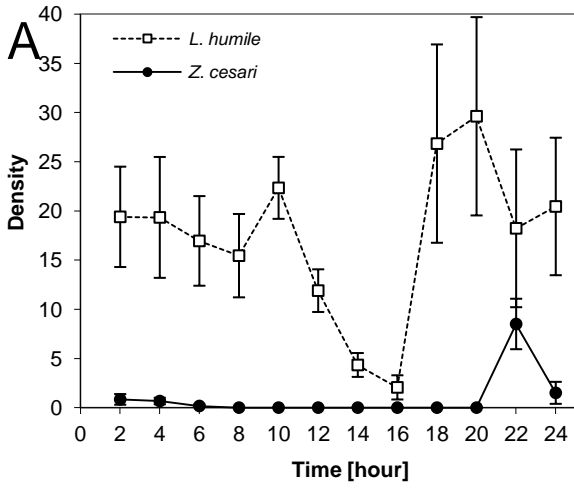
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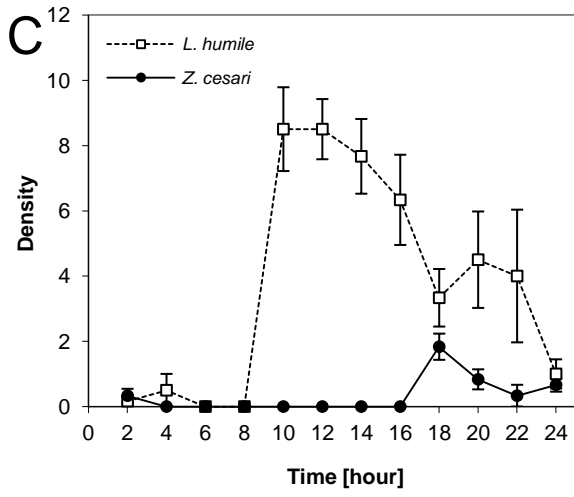
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29 **Fig. 4**

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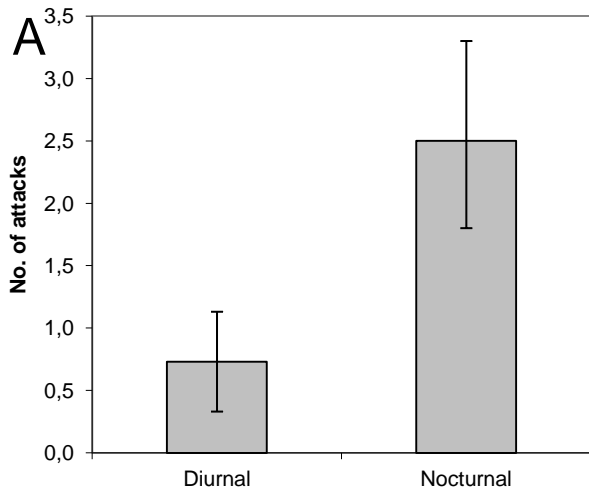


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34 Fig. 5

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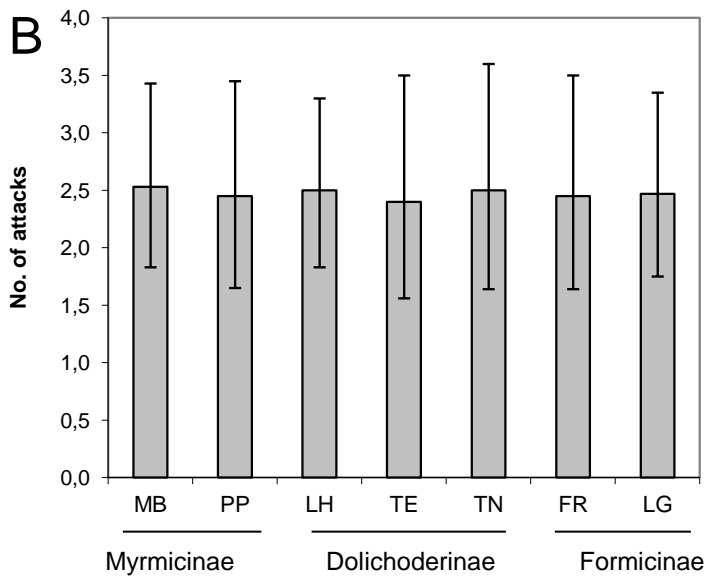


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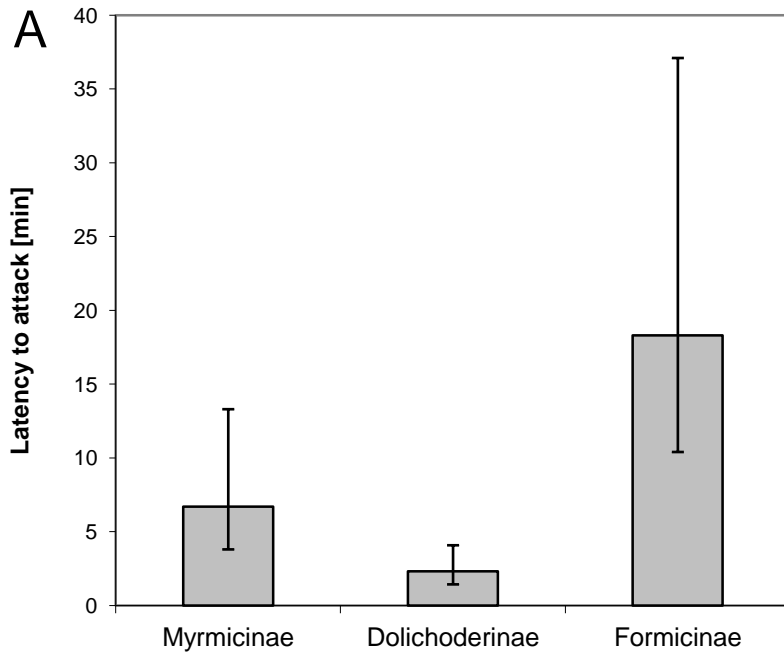


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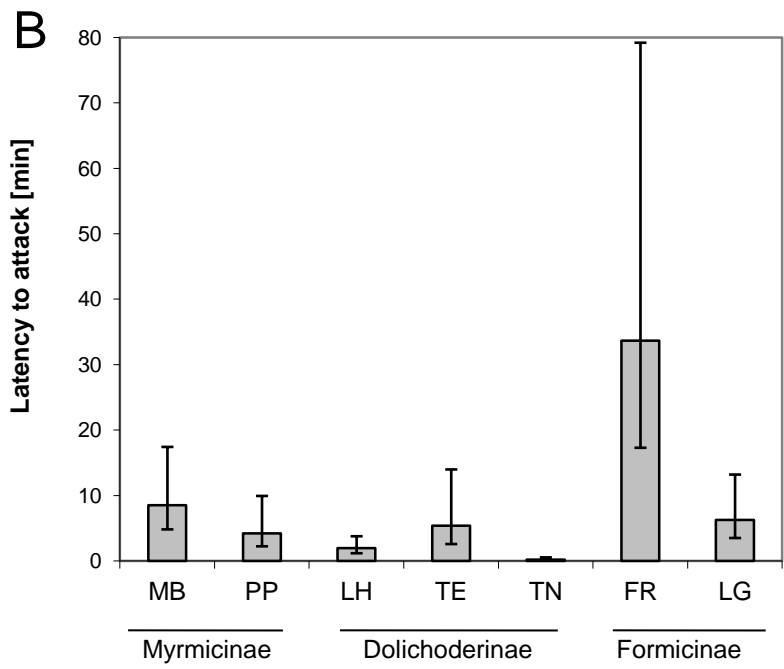
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42 **Fig. 6**



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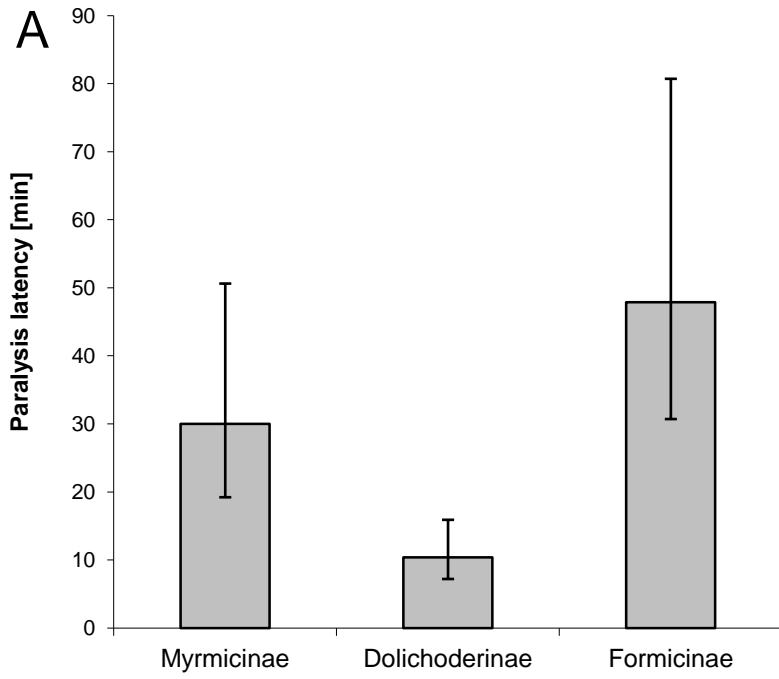


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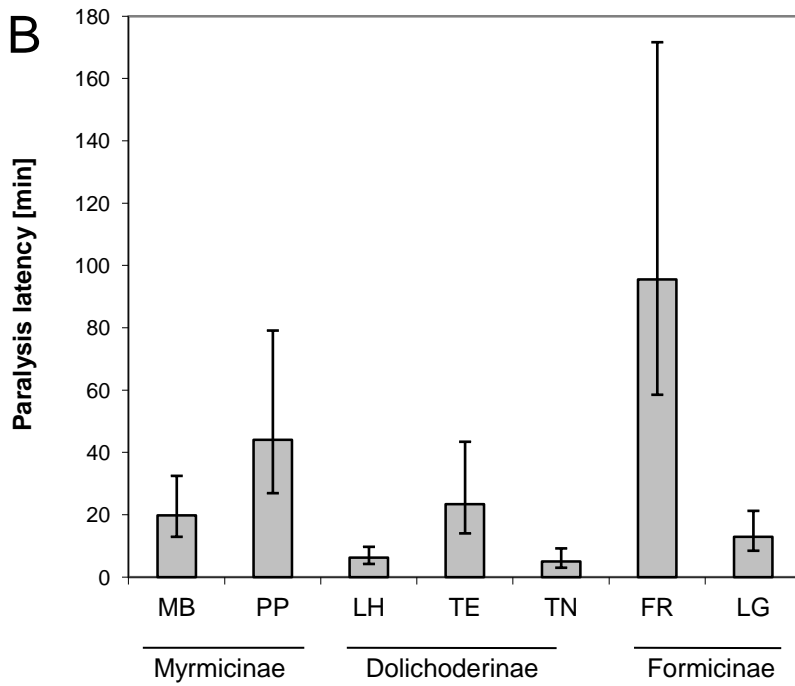
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46 **Fig. 7**

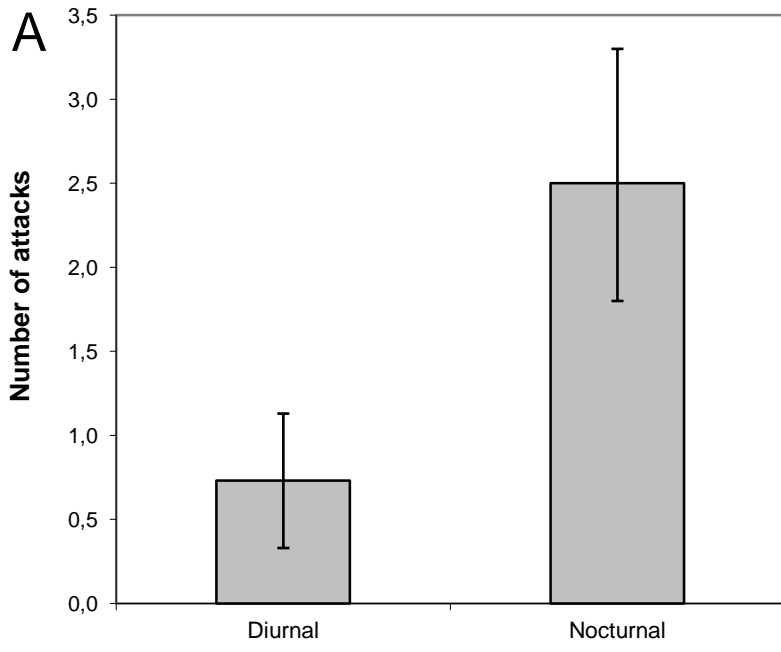
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**Table 1.** Overview of conditions at dates of studying circadian activity of spiders and ants.

Date	Sunrise	Sunset	Temperature [°C]*		
			Minimum	Maximum	Average
29 May	06:40	21:30	13.6	27.6	20.6
8 October	08:00	19:30	9.4	31.9	20,7
13 November	07:40	17:50	3.4	19.8	11,6

53 \* Data were obtained from the IVIA Irrigation Technology Service Montcada station (STR-IVIA), located  
54 approximately 2 km north of Bétera orchard.

55

56 **Table 2.** List of ant species collected by pitfall traps in three non-invaded and one invaded citrus groves from April  
57 2005 to April 2006. Total number of specimens of each species and ant diversity (Shannon-Wiener index) per orchard  
58 are presented.  
59

Subfamily/Species	Non-invaded			Invaded
	Náquera	Olocau	Picassent	Bétera
<b>Ponerinae</b>				
<i>Hypoponera eduardi</i> (Forel)	0	57	16	4
<b>Myrmicinae</b>				
<i>Aphaenogaster iberica</i> Emery	214	0	0	0
<i>Cardiocondyla batesii</i> Forel	0	26	0	0
<i>Cardiocondyla mauritanica</i> Forel	2	17	5	4
<i>Messor barbarus</i> (Linnaeus)	219	721	512	4
<i>Pheidole pallidula</i> (Nylander)	9,255	10,331	2,444	109
<i>Pyramica membranifera</i> (Emery)	6	17	2	123
<i>Solenopsis</i> sp.	18	7	31	27
<i>Tetramorium semilaeve</i> André	9	194	134	223
<b>Dolichoderinae</b>				
<i>Linepithema humile</i> (Mayr)	3	0	3	9,850
<i>Tapinoma erraticum</i> (Latreille)	0	21	19	0
<b>Formicinae</b>				
<i>Cataglyphis gadeai</i> De Haro & Collingwood	6	1	0	4
<i>Camponotus sylvaticus</i> (Olivier)	52	3	1	2
<i>Formica rufibarbis</i> Fabricius	129	916	299	0
<i>Lasius grandis</i> Forel	1,770	14,223	4,186	0
<i>Plagiolepis pygmaea</i> (Latreille)	98	339	130	174
<i>Plagiolepis schmitzii</i> Forel	1	35	38	17
<i>Polyergus rufescens</i> (Latreille)	30	0	0	0
<b>Total abundance</b>	11,812	26,908	7,820	10,541
<b>Diversity (mean and SE)</b>	0.58	0.98	0.96	0.45
	(0.10)	(0.05)	(0.09)	(0.13)

