Carabus (Oreocarabus) guadarramus La Ferté–Sénectère, 1847 (Coleoptera, Carabidae): first instar larva and reflections on its biology and chorology

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Abstract

Carabus (Oreocarabus) guadarramus *La Ferté–Sénectère, 1847 (Coleoptera, Carabidae): first instar larva and reflections on its biology and chorology*.— We provide information for the first time on the larval morphology of *Carabus (Oreocarabus) guadarramus* La Ferté–Sénectère, 1847, a species endemic to the Iberian peninsula. A detailed iconography is also provided. Three main diagnostic features are given which are particularly relevant to the goal of providing a useful and practical tool to help differentiate between the first instar larvae of this species and the related *Carabus (Oreocarabus) ghilianii* La Ferté–Sénectère, 1847, which lives in syntopy with *C. guadarramus*. Detailed maps with 10 x 10 km UTM of the geographic distribution of both species are provided in order to highlight their different chorology. Some of the possible environmental causes that might affect its current distribution are discussed.

Key words: Captivity breeding, Chaetotaxy, Competition, Endemic species, Iberian peninsula, Larval morphology.

Resumen

Carabus (Oreocarabus) guadarramus *La Ferté–Sénectère, 1847 (Coleoptera, Carabidae): primer estadio larvario y reflexiones sobre su biología y corología*.— Se proporciona, por primera vez, información sobre la morfología larvaria de *Carabus (Oreocarabus) guadarramus* La Ferté–Sénectère, 1847, una especie endémica de la península Ibérica, acompañada de una detallada iconografía. Se destacan tres caracteres diagnósticos especialmente relevantes con el objetivo de proporcionar una herramienta útil y práctica para facilitar el reconocimiento del primer estadio larvario de esta especie con respecto a otra que es filogenéticamente muy próxima y habita en sintopía: *Carabus (Oreocarabus) ghilianii* La Ferté–Sénectère, 1847, especie protegida y endémica del Sistema Central. Además, se aportan mapas detallados con las UTM de 10 x 10 km de donde se conoce cada especie, con el fin de poner de manifiesto su diferente corología. Se discuten algunas de las posibles causas ecológicas que podrían condicionar su actual distribución.

Palabras clave: Cría en cautividad, Quetotaxia, Competencia, Especies endémicas, Península ibérica, Morfología larvaria.

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Introduction

Carabidae populations have shown to be good bioindicators of the state of conservation of the ecosystems they inhabit, and a suitable tool to evaluate their recovery after anthropic alterations (Eyre & Luff, 1990; Kromp, 1990; De Vries, 1994; Spence et al., 1996; Davies & Margules, 1998; Duelli & Obrist, 1998; Venn, 2000; Irmler, 2003; Rainio & Niemelä, 2003). Within this family, the genus *Carabus* Linnaeus, 1758 is notable as it fulfils several characteristics that make it interesting in conservation biology: a wide distribution (holarctic), high specific diversity (almost 800 spp.), large size (between 12 and 50 mm), and diverse coloration. All these factors make it a conspicuous group that has awakened the interest of entomologists since the dawn of entomology, which explains why its taxonomy is well developed and why reliable data on its distribution has been available for more than a century (Turin et al., 2003; Assmann, 2003). The state of conservation of these species will therefore reflect the health of their habitats. For this reason it is vital to have information on the biology and ecology of the *Carabus* species, including their life cycle and larval development, which have been increasing in recent years (see for example Penev et al., 2008; Bousquet, 2010; Kotze et al., 2011).

Although there is more information available on the larvae of *Carabus* than on any other genus of Coleoptera, there is still much to learn about the larval morphology and biology of a large number of species of this genus (Arndt & Makarov, 2003). This lack of information is particularly serious in terms of endemic species of small or extremely reduced areas (micro–endemic species), as it makes it very difficult to attempt to design plans for environmental management and, therefore, to facilitate preservation of the species'.

One of the most feasible methods to study the biology of these species is the observation of their behaviour and their breeding in controlled environments, as well as the study of pre–imaginal instars. This protocol has been largely used in carabidology (Verhoeff, 1917; Hůrka, 1971, 1996; Raynaud, 1975–76; Malausa, 1977; Casale et al., 1982; Luff, 1993; Makarov, 1994; Arndt & Makarov, 2003; among others), supplying a lot of information. Nevertheless, it often poses several problems such as the low viability of the eggs, and the high mortality of larvae (Hůrka, 1972; Huk & Kühne, 1999). This might be due to the difficulty to recreate natural biotic and abiotic conditions. Nevertheless, since the mortality rates in different species of *Carabus* under natural conditions are unknown, it is possible that those reached in the laboratory are similar. The study of the morphology and chaetotaxy of larvae, once captured in nature or hatched from eggs in captivity, also poses several difficulties, such as the need for special microscopic preparations, the fact that some structures reach the limits of resolution of optic microscopy, and the lack of specimens in collections for establishing comparisons (Solodovnikov, 2007). Nevertheless, once these problems are addressed, studies on larval morphology provide very useful information to reinforce systematic and phylogenetic proposals (Beutel, 1993; Arndt, 1998; Solodovnikov, 2007).

There are 29 species of *Carabus* on the Iberian peninsula (Serrano, 2003), 16 of them endemic (Serrano et al., 2003; Jiménez–Valverde & Ortuño, 2007). The subgenus *Oreocarabus* Géhin, 1876 contributes three endemisms of unequal distribution. *Carabus (Oreocarabus) amplipennis* Lapouge, 1924 is distributed along a north–western arch (the Basque Mountains, Cantabrian mountain range, Mountains of León, Galicia, north of Portugal, and the mountain ranges of Bussaco and La Estrella); *Carabus (Oreocarabus) guadarramus* La Ferté–Sénectère, 1847 in the mountain ranges of the centre of the Iberian peninsula and peri–plateau reliefs, and the third species, *Carabus (Oreocarabus) ghilianii* La Ferté–Sénectère, 1847, is exclusive to the Central System mountain range, and lives in syntopy with *C. (O.) guadarramus*. There is a big gap in the knowledge on these species, but more attention has been paid to *C. (O.) ghilianii,* since it is a threatened (García–París & París, 1993; Viejo & Sánchez Cumplido, 1995; Serrano & Lencina, 2006) and protected species (see Boletín Oficial de la Comunidad de Madrid, 1991) that has a more restricted distribution area (Jeanne, 1969; García–París & Ortuño, 1988; Serrano, 1989; García–París & París, 1993; Zaballos, 1994). Regarding this species, some interesting contributions have been made to its imaginal anatomy (for example, Ortuño & Hernández, 1992), biology, and ecological preferences (Novoa, 1975; García–París & Ortuño, 1988; Gilgado & Ortuño, 2011) and, recently, to its previously unknown pre–imaginal morphology (Gilgado & Ortuño, 2011). Under this same perspective, it is now pertinent to tackle the study of *C. (O.) guadarramus* and its larvae. This will enable identification of these two species in their first instars, something that was not possible until now. This would be of great utility, not only for the systematic/phylogenetic aspects that would clarify the relations of the species within the subgenus *Oreocarabus* Géhin, 1876, but also for the management of this species for its conservation in the areas it inhabits.

This work contributes the first data on the larval morphology of *C. (O.) guadarramus,* allows us to identify the group of characteristics that facilitates the distinction in the larvae of both species. Data on the biology of pre–imaginal instars are provided. In addition, the chorology, and its spatial relation with the other two Iberian species of the same subgenus, are analysed.

Material and methods

Two larvae were studied. They were obtained from two gravid females of *C. (O.) guadarramus,* and collected in July of 2008 in a pine forest of *Pinus sylvestris* L. in the locality of Lozoya UTM: 30TVL33 (Madrid, Spain). Both females were deposited in the same terrarium containing a previously sterilised substrate. After 10 months, nine eggs were detected. The end result

was the study of two larvae that could be compared with an exuviae collected in the same location as the imagoes. After their study they were also assigned to this species.

The two imagoes of *C. (O.) guadarramus* were kept in captivity from the moment of capture to the following spring (10 months), under conditions of constant humidity, varying the photo–period and temperature by using a breeding chamber Radiber S.A. EC–360 with a luminous dispositive. A summer temperature was set (14 hours of light and 10 of dark) at 12.5ºC, but in the winter it was necessary to decrease the temperature to 4ºC and the light hours to 10, with 14 of dark. In spring the temperature was raised to 12.5ºC and the photo–period was set to 12 hours of light and 12 of dark.

Eggs and larvae obtained were conserved in Scheerpeltz (60% ethanol 96º, 38% distilled water, 1% acetic acid, 1% glycerine). One of them was dissected, extracting the labium, mandibles, maxillae, antennae, cephalic capsule sclerites, legs, and tergal and sternal sclerites by using dissection tweezers. These pieces were soaked in the water–soluble resin dimethyl hydantoin formaldehyde (DMHF) (Steedman, 1958) and placed on acetate sheets, which measured 1.5 × 0.5 cm, for use as microscope slides. Another small acetate sheet was placed on each slide as a cover slip for observation under the microscope. The microscope slides with the dissected larva are deposited at Vicente M. Ortuño's (VMO/AU) collection at the Department of Zoology and Physical Anthropology of the University of Alcalá. The habitus was drawn using a NIKON SMZ1000 stereoscopic binocular microscope. Each preparation was observed and measured with an optical microscope ZEISS 474620–9900, with a camera lucida and a calibrated ocular micrometer. The habitus and preparations were drawn in ink. The drawings were scanned and labelled using the application Adobe Photoshop Cs 8.0. The adopted nomenclature for the setae and pores is that proposed by Bousquet & Goulet (1984). According to this criterion, the setae are named with numbers, and pores with letters.

Results

A total of nine eggs were found in the terrarium of the two female *C. (O.) guadarramus*. Three of them were conserved and six were kept over the substrate, in different recipients, at a temperature of 12.5ºC. Finally, four of them failed, and therefore only two larvae were obtained.

The morphology (fig. 1), including the chaetotaxy, of *C. (O.) guadarramus*, is quite similar to *C. (O.) ghilianii* (see Gilgado & Ortuño, 2011), which is the reason why it would be redundant to give an exhaustive description. Nevertheless, although at first sight these larvae may seem indistinguishable, a detailed examination reveals important differences between them. There are three main differential characteristics: 1) the nasale is sharpened and narrow in *C. (O.) guadarramus* (figs. 2A, 2B); 2) the group of setae gTA of the protarsi of *C. (O.) guadarramus* includes five setae, while it includes only two in *C. (O.) ghilianii* (figs. 2C, 2D); and 3) the shape of the urogomphi is different in both species; in *C. (O.) guadarramus* they are more basal and the protuberances are more pronounced (figs. 2E, 2F).

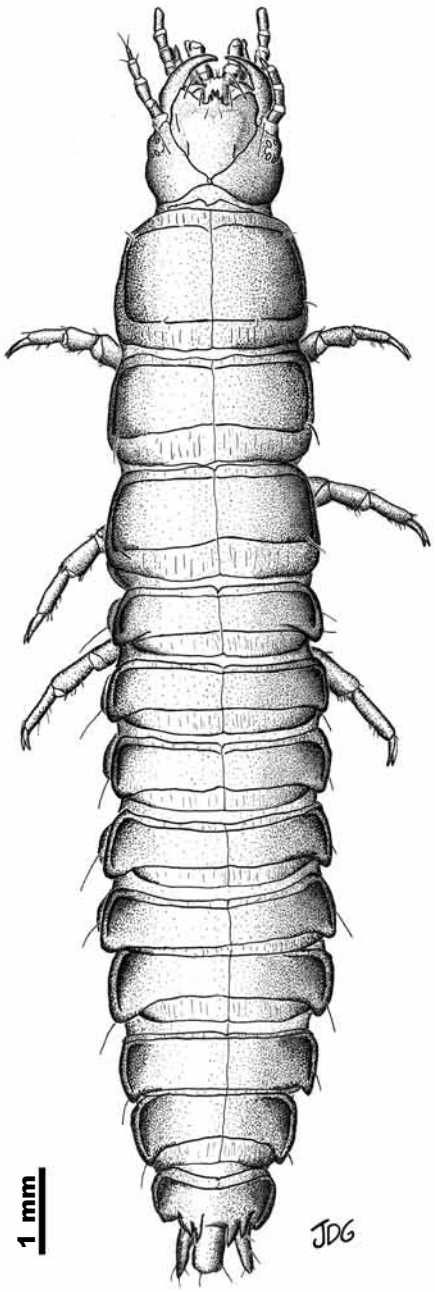

Fig. 1. Habitus of the first larval instar of *C. (O.) guadarramus*.

Fig. 1. Habitus del primer estadio larvario de C. (O.) guadarramus.

Integration of the information on the chorology of *C. (O.) guadarramus* places this species in several peri–plateau mountains (fig. 3A) (Novoa, 1975; Gimeno, 1982; Zaballos, 1986; Zaballos & Jeanne, 1994; Forel & Leplat, 1998; among others), although always under the protection of fresh forest, represented by Atlantic deciduous forests, montane perennial forests, and sub–atlantic or sub–mediterranean marcescent or deciduous forests. *Carabus (O.) guadarramus* is also present at the perimeter of the forest ecotone and, more rarely, in mountain meadows above the upper limit of forest (Novoa, 1975; Ortuño, pers. observ.). Ecological preference for moist forests is also seen from the results obtained by Ruiz–Tapiador & Zaballos (2001) and Serrano et al. (2005) in the Toledo Mountains, where this species is rare because the sclerophyllous forest is dominant, with small extensions of moist forest (marcescent forest of *Quercus pyrenaica* Willd.).

Discussion

The first especially interesting finding in this study in relation to obtaining the larvae of *C. (O.) guadarramus* is that females, already gravid, had no contact with males for 10 months, and then laid the eggs. This means that sperm can be kept viable in the spermatheca over the long term. This datum might be interesting for a possible management plan and captivity breeding, if necessary, directed to reintroducing this species or similar species in nature or reinforcement of their populations.

Regarding the interpretation of larval morphology, Bengtsson (1927) divided the genus *Carabus* into three species groups according to larval morphology: the '*Archeocarabus*–group', supposed to be the most primitive, with larvae of small tergites and short legs; the '*Neocarabus*–group', a more modern species with larvae of broad tergites and long legs; and the '*Metacarabus*–group', that would include the species with intermediate characteristics. In this last group, *C. (Oreocarabus) hortensis* Linnaeus, 1758 was included, and therefore *C. (O.) guadarramus* and *C. (O.) ghilianii* should also be; all of them are included within the subgenus *Oreocarabus*. However, a great disparity in relation to the number of setae of the gTA is observed in these three species, not matching the expected homogeneity of the subgenus. In many *Carabus* species the presence of three to five pairs of setae in the gTA is common (see Makarov, 2003); these are interpreted as supernumerary in relation to the model of Bousquet & Goulet (1984). However, the absence of those setae is considered a characteristic of the species of the group *Archeocarabus sensu* Bengtsson (1927), as indicated by Makarov (1993). In that sense, the larva of *C. (O.) hortensis* does not present setae of the group gTA (Bengtsson, 1927; Arndt, 1985, 1991), *C. (O.) ghilianii* has a gTA of two setae, and *C. (O.) guadarramus* has a gTA of five setae. This diversity shown by *Oreocarabus* underlines, once again, the inconsistency of the classification of Bengtsson (1927), which was later employed by Lapouge (1929). Molecular data suggest that these classifications do not match with the phylogeny and, therefore, they have been rejected (Arndt et al., 2003).

Regarding the distribution of *C. (O.) guadarramus*, the populations of La Sagra and Cazorla (Sub–Betic mountain range) (Jeanne, 1969), which are the most southern of this species, deserve special attention. There is also a single and small population at the north side of the river Ebro, in the massif of Andia (see Forel & Leplat, 1998), whose biogeographic significance is still undetermined since this plentiful river represents a severe natural barrier to the dispersal of this and other species. The possibility of anthropochory must be taken into account, for example, by means of an activity of reforestation or transport of materials, woods, etc. from side to side of the river. *Carabus (O.) guadarramus*, *C. (O.) ghilianii*, and *C. (Oreocarabus) amplipennis* constitute the whole of the groups of Iberian species of the subgenus *Oreocarabus*. The actual distribution of *C. (O.) guadarramus* barely overlaps with that of *C. (O.) amplipennis.* This last species is distributed along a north–western arch (the Basque Mountains, the Cantabrian mountain range, the mountains of León, some enclaves in the north of Castilla, Galicia, north of Portugal and the mountains ranges of Bussaco and La Estrella) and has three recognised subspecies (see Serrano, 2003), and a fourth of recent description (Mollard, 2006) of very doubtful validity. However, this distribution is different from that exhibited by *C. (O.) ghilianii,* whose distribution area is embraced by the presence of *C. (O.) guadarramus* in the Central System mountain range (figs. 3A, 3B). In this case, the strategies that may facilitate the existence of both species in the same area (for example, in the pine forests of the Guadarrama mountain range) might imply a micro–spatial segregation, according to their different micro–habitat preferences observed. *Carabus (O.) ghilianii* is more stenotopic (more hygrophilic) in relation to *C. (O.) guadarramus,* settling mainly in small, moister areas, but where *C. (O.) guadarramus* may also have access.

It is also likely that, as observed in other species, the presence of two syntopic species is a factor that might condition the relative abundance of one of them in certain areas (Lensky, 1982, 1984), by competition or opportunism. In relation to this latter supposition, it has been observed that in some areas with stable populations of *C. (O.) ghilianii*, after marked anthropic alterations, this stenoic species disappears in favour of *C. (O.) guadarramus*, which moves into its niche (Ortuño, pers. observ.). Although this is a single observation, possible competition between the two species should be taken into account when treating it as a vulnerable species, in addition to the anthropic factors already known. At least, it raises a number of questions about the possible strategies that enable the coexistence of both species in the same habitat.

It has been observed that specimens of *C. (O.) guadarramus* and *C. (O.) ghilianii*, kept captive and subjected to identical environmental conditions in the breeding chamber, laid their eggs at very differ-

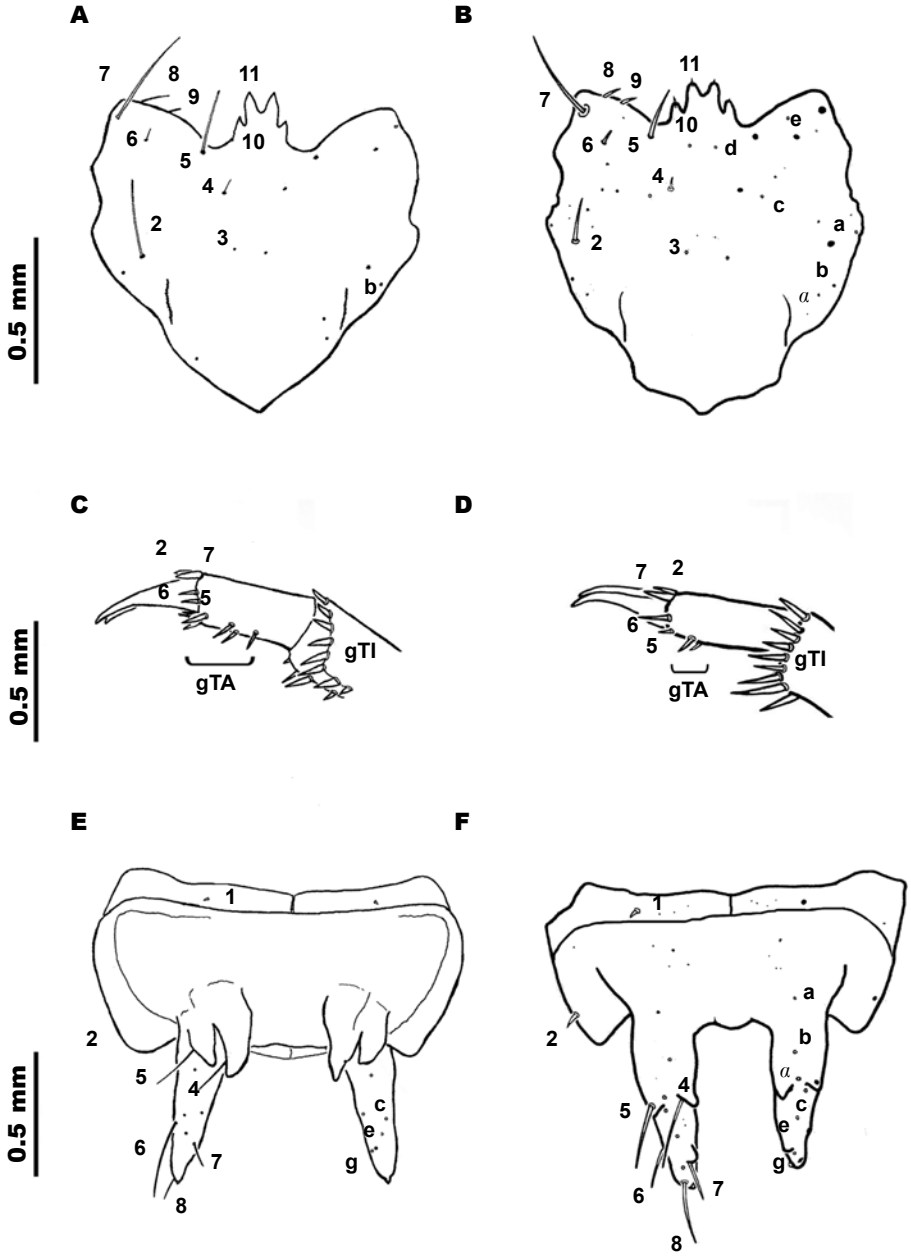

Fig. 2. Anatomical details of the first larval instar of *C. (O.) guadarramus* and *C. (O.) ghilianii* that present most differences: Frontale of: A. *C. (O.) guadarramus;* B. *C. (O.) ghilianii;* Detail of the leg of: C. *C. (O.) guadarramus;* D. *C. (O.) ghilianii*; Urogomphi of: E. *C. (O.) guadarramus;* F. *C. (O.) ghilianii*.

Fig. 2. Detalles anatómicos del primer estadio larval de C. (O.) guadarramus *y* C. (O.) ghilianii *que muestran la mayoría de diferencias. Frontal de: A.* C. (O.) guadarramus*; B.* C. (O.) ghilianii. *Detalle de la pata de: C.* C. (O.) guadarramus; *D.* C. (O.) ghilianii. *Urogonfi de: E.* C. (O.) guadarramus; *F.* C. (O.) ghilianii.

ent moments. Gravid females of *C. (O.) guadarramus* abstained from the hatchery in the summer and fall, laying eggs in early spring (when the temperature of the breeding chamber was raised) whereas, on the contrary, *C. (O.) ghilianii* laid the eggs in summer. One may hypothesise that this is due to a temporal segregation between them, as has already been described for other *Carabus* species cohabiting in one area and found to differ in the timing of reproduction and larval development (Lenski, 1982, 1984; Sota,

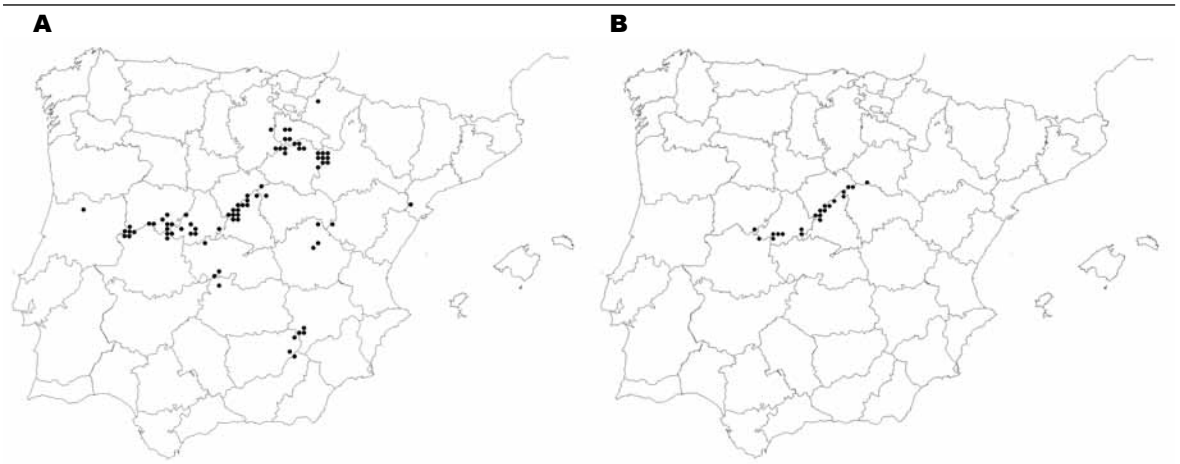

Fig. 3. Distribution of: A. *C. (O.) guadarramus* and B. *C. (O.) ghilianii* in the Iberian peninsula. The dots correspond to the 10 x 10 km UTM coordinates of where they have been sighted.

Fig. 3. Distribución de: A. C. (O.) guadarramus; B. C. (O.) ghilianii *en la península Ibérica. Los puntos corresponden a las coordenadas UTM de 10 x 10 km donde han sido observados.*

1985). This behaviour, if confirmed with the study of more specimens, should be taken into account for the monitoring and environmental management of natural populations.

Whether those species might have other strategies to avoid competition, such as different alimentary preferences, as observed by Sota (1985) in two different syntopic species of *Carabus,* remains unknown.

The syntopic condition of both Iberian species is not patent along the entire Central System mountain range, nor in the Guadarrama mountain range. *Carabus (O.) ghilianii*, in addition to its stenohygrobic condition and its almost riparian habits, is restricted to an altitudinal interval that goes from 1,400 to 1,950 m a.s.l. It lives only in forest mountain environments (García–París & Ortuño, 1988; Ortuño & Toribio, 1996, 2002; Serrano & Lencina, 2006; Gilgado & Ortuño, 2011), disappearing with the upper limit of the forest of the mountain ranges of Guadarrama, Ayllón, and Pela. This implies that its competition with *C. (O.) guadarramus* may be restricted to a very delimited altitudinal interval. In spite of this, even in optimal conditions, the population densities of *C. (O.) ghilianii* are low, perhaps as a result of the restricted strip of moist space available on both sides of small water courses. However, when *C. (O.) guadarramus* is found in optimal conditions, its population densities are much higher, since they have no such restrictions. In the case of the mountain ranges of Gredos and Béjar, in the western limit of the distribution area of *C. (O.) ghilianii,* forests are diminishing nowadays, a circumstance that might have limited the presence of *C. (O.) ghilianii* even more. This could be the reason why, in these areas, it is restricted to peat grasslands and broom and, according to the results of surveys conducted, also at very low population densities. All this suggests that these populations of *C. (O.) ghilianii* are not at their ecological optimum.

Conclusions

Carabus (O.) guadarramus shows a group of setae gTA consisting of five setae, whereas *C. (O.) ghilianii* and *C. (O.) hortensis* show two and zero setae, respectively. This reflects the inconsistency in the classification of Bengtsson (1927) and Lapouge (1929) which used the gTA as a diagnostic character for including the subgenus *Oreocarabus* in the '*Metacarabus*–group'.

The presence of *C. (O.) guadarramus* could be a limiting factor in the presence or abundance of *C. (O.) ghilianii* in localities where they cohabit; this possibility should be taken into account, therefore, when managing their habitats.

Data obtained from breeding in captivity suggest that there is a certain temporal segregation in the breeding schedules of both species, which would reduce the competition between them. In captivity, the females of *C. (O.) guadarramus* showed the ability to keep the sperm viable for 10 months inside the spermatheca, in order to fertilise and lay their eggs at the beginning of the spring when the temperatures were raised. Nevertheless, it is known that *C. (O.) ghilianii* lay their eggs mainly at the end of the spring and throughout the summer (Gilgado & Ortuño, 2011).

The first instar larvae of *C. (O.) guadarramus* can be easily differentiated from *C. (O.) ghilianii* by three diagnostic characteristics: the shape of the nasale, the group of setae gTA, and the shape of the urogomphi.

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