Biological notes and description of egg and first instar larva of *Carabus (Oreocarabus) ghilianii* La Ferté-Sénectère 1847 (Coleoptera: Carabidae)

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Abstract. In this work, the authors describe the egg and first instar larva, hitherto unknown, of *Carabus (Oreocarabus) ghilianii* La Ferté-Sénectère 1847, a threatened and protected species endemic to the Iberian Peninsula. With respect to the larval morphology, a comprehensive study of the chaetotaxy of the three tagmata is presented, accompanied by a detailed iconography. In addition, data on the biology of imagoes are provided, taken in its natural habitat and in captivity, highlighting the novel fact that this species produces winter larvae. Thus, reproduction begins in late spring. Both the eggs and the larvae were obtained after captive rearing of nine specimens collected in the Sierra de Guadarrama (Madrid, Spain).

Résumé. Notes biologiques et description des oeufs et des premiers stages larvaires de *Carabus (Oreocarabus) ghilianii* La Ferté-Sénectère 1847 (Coleoptera : Carabidae). Dans ce travail, les auteurs décrivent l'oeuf et le premier stade larvaire, jusqu'ici inconnus, de *Carabus (Oreocarabus) ghilianii* La Ferté-Sénectère 1847, espèce menacée et protégée, endémique de la Péninsule Ibérique. En ce qui concerne la morphologie larvaire, une étude complète de la chétotaxie des trois tagmata est présentée, accompagnée d'une iconographie détaillée. De plus, des données nouvelles sont fournies sur la biologie des imagos à l'état sauvage et en captivité, mettant en évidence le fait nouveau que cette espèce produit des larves hivernales. Ceci permet à la reproduction de débuter à la fin du printemps. Les oeufs et les larves ont été obtenus grâce à l'élevage en captivité de neuf spécimens collectés à la Sierra de Guadarrama (Madrid, Espagne).

Keywords: Larval description, chaetotaxy, egg, biological notes, reproduction, Sierra de Guadarrama.

*C*arabus (Oreocarabus) ghilianii La Ferté-Sénectère 1847 is a species endemic to the Iberian Peninsula, namely to the Sistema Central Range. It inhabits areas near streams; in broom scrubs (*Cytisus purgans* L.*, Juniperus communis* L. subsp. *nana* Syme); and beech (*Fagus sylvatica* L.) and pine (*Pinus sylvestris* L.) woods in the Guadarrama Mountains (between 1300 and 1940 metres above sea level); Ayllón, Pela, Gredos and Béjar Mountains (reaching an altitude of 2400 m.a.s.l.) (García-París & Ortuño 1988; Ortuño & Toribio 1996; Ortuño & Toribio 2002). However, the natural vegetation between 1200 and 1700 m.a.s.l. in the Guadarrama Mountains includes oak forests (*Quercus pyrenaica* Willd), which are nowadays restricted to some slopes, due to livestock pressure and massive afforestation with *P. sylvestris* L. (Costa *et al.* 2005).

Carabus (Oreocarabus) ghilianii is considered threatened (Viejo & Sánchez Cumplido 1995), and it has been catalogued as vulnerable by the IUCN; thus, it appears in the *Red Book of Invertebrates of Spain* (Serrano & Lencina 2006). It is also protected by the

law M. 2/91 (see Boletín Oficial de la Comunidad de Madrid 1991) according to a series of reasons: a) its endemic and stenotopic nature; b) its predatory behaviour, and hence, its low population densities; c) the anthropic pressure to which its populations have been subjected, because of indiscriminate captures by collectors, use of pesticides, habitat degradation caused by massive deforestation and urban and tourist pressure (García-París & París 1993).

Despite all of the above, there is a large gap in the knowledge about the biology of this species, since historically more attention has been paid to its distribution (Jeanne 1969; Serrano 1989; García París & París 1993; Zaballos 1994) and morphology (Ortuño & Hernández 1992) than to other aspects of its biology, such as its ecological preferences (Novoa 1975; García-París & Ortuño 1988) or certain intrinsic features like its cytotaxonomy (Serrano 1979-80). This is the reason why, until now, no data have been available on preimaginal stages of this species.

However, in recent decades, a major effort has been devoted to study the preimaginal morphology and biology of other *Carabus* L. 1758 species, both in Spain (Cárdenas *et al.* 1994; Cárdenas & Hidalgo 1995; Cárdenas & Hidalgo 1998) and the rest of Europe

(Hůrka 1971; Raynaud 1975–76; Casale *et al.* 1982; Luff 1993; Makarov 1994). Today, more information is available on the larvae of *Carabu*s than any other Coleopteran genus (Arndt & Makarov 2003).

On the other hand, the importance of larval characters for building more precise beetle systematics has been demonstrated (Beutel 1993; Solodovnikov 2007). Selective pressures on larvae are not the same as in imagoes; therefore preimaginal morphology is able to evolve in a different way to the imaginal, as they demonstrate different expressions of the same genotype (Goulet 1979; Luff 1993). Hence, the study of larval morphology provides a wealth of information that complements the previously available information about the imagoes, as well as in the case of *Carabus (Oreocarabus) ghilianii*. This is the reason why several authors have proposed models or systems of nomenclature for larval chaetotaxy in different groups of Coleoptera (Thomas 1957; Ashe & Watrous 1984; Bousquet & Goulet 1984; Makarov 1992; May 1994).

Nevertheless, the study of preimaginal morphology presents some major difficulties: a) the small number of specimens available in collections, b) the need to dissect and make microscopic preparations for detailed observation of some structures, c) existence of some intraspecific variability in some characters, like chaetotaxy, and d) difficulty in establishing if a character is homologous or not in various taxa (Solodovnikov 2007).

For the reasons above, we believe that this study helps to fill an important gap of knowledge about such an emblematic species in the Iberian entomology as *Carabus (Oreocarabus) ghilianii*. This new information has vital importance from a taxonomic and ecological point of view, and it also has applications in the management and conservation of this species.

Material and Methods

Nine imagoes of *Carabus (Oreocarabus) ghilianii* La Ferté-Sénectère 1847, made up of five females and four males, were collected at the beginning of July in a pine forest of *P. sylvestris* L., which is located in the municipality of Lozoya (Madrid, Spain), and whose UTM coordinate is 30TVL33.

Moss, stones and substrate were collected in the same place in order to prepare the terrariums. The substrate and the moss were sterilized by subjecting them to heat in a microwave at 900W for five minutes to eliminate nematodes, mites, fungi and other organisms that might damage the eggs. The temperature of the substrate where imagoes were collected was measured with a thermometer, a "QUARTZ digi-thermo", to set up the temperature of the breeding chamber.

Imagoes were placed in pairs, except for the fifth female, which was placed with a male and a female, in terrariums, in a breeding chamber, Radiber S. A. model EC-360, with a temperature of 12.5 °C, to which a luminous device was adapted, and with a summer photoperiod of 14 hours of light and 10 hours of darkness. They were kept together for two weeks, with the aim of promoting the copula, before separating them into individual terrariums. The terrariums were built with plastic containers of 20 × 20 cm and whose height was 8 cm, with a hole at the top with a diameter of 5 cm covered with a grid with a mesh size of 1.5 mm. The bottom of the terrariums was covered with 2.5-cm-thick substrate, some moss cuts in order to keep humidity and a stone or a piece of pine bark, as a shelter.

Imagoes were fed with approximately 2-cm-long larvae of *Tenebrio molitor* L. 1758, which were previously damaged, due to the hardness of their cuticle, in order to facilitate the emission of fluids and stimulating the voracity of imagoes of *C. (O.) ghilianii*. Furthermore, the diet was supplemented with raw calf liver, lumbricid oligochaetes, banana and apple.

From the moment males and females were separated, eggs deposited on the substrate were sought by turning over the earth carefully. Eggs were handled with a paintbrush and were placed individually in small glass jars, 6 cm in height and 2.5 cm in diameter, with a small amount of substrate and moss. The aim was to have enough humidity, but avoiding excess moisture in order to prevent the development of fungi. These jars were placed in the breeding chamber at the same temperature of the imagoes. The eggs destined for observation and morphological study were conserved in Scheerpeltz's solution (60% ethanol 96° 38% distilled water, 1% acetic acid, 1% glycerine).

The chorion was prepared by dissecting one of the eggs conserved in Scheerpeltz. Chorion fragments were extended over a microscope slide and soaked in Hoyer's solution (gum arabic, glycerine, chloral hydrate and distilled water). These fragments were studied with an optical microscope, ZEISS 474620-9900, with *camera lucida* and a calibrated ocular micrometer, to measure the reticulation, size and shape of the cells and ridges and the configuration of the micropile. That measure of density was determined according to the criterion of Kaupp *et al.* (2000), by calculating the average length of ten adjoining cells.

Once the eggs hatched, and after their cuticles had sclerotized, four larvae were sacrificed and conserved in Scheerpeltz.

One of these four larvae 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) and placed on acetate sheets for use as microscope slides, which measured 1.5 × 0.5 cm. Another small acetate sheet was placed over each slide as a cover slip for observation under the microscope.

Another larva was placed in a small jar with lactic acid (90%) and at a temperature of approximately 22 °C for 10 days, in order to discolour the cuticle slightly. After that, the whole specimen was prepared for optical microscopy, on a microscope slide in DMHF with chloral hydrate. The aim was to compare the whole specimen with the dissected one, in order to reveal distortions or artefacts because of the dissection.

The habitus was drawn by studying a Scheerpeltz-conserved larva, by using a stereoscopic binocular microscope, Nikon SMZ1000. Each preparation was observed and measured with the optical microscope. The habitus and preparations were drawn in ink. The drawings were scanned and labelled using the informatic application Adobe Photoshop Cs 8.0. Instead

of drawing the tergum of the abdominal segment I, which was a little damaged in the dissected specimen, the tergum of the abdominal segment IV was drawn, pointing out the observed differences with the first one. Nevertheless, there are very few differences.

The nomenclature adopted for the setae and pores is the one proposed by Bousquet & Goulet (1984), later reinterpreted by Makarov (1992) for the *Carabus* larvae. According to this criterion, setae are named with numbers and pores with letters. In the case of supernumerary setae, they are named with Roman numbers; and supernumerary pores, with Greek letters.

With the idea of getting the larval instar II, five larvae were kept alive in the breeding chamber, in small individual terrariums with diameters of 8 cm and heights of 6 cm, with 3 cm of substrate. They were fed with *Tenebrio monitor* larvae, *Musca domestica* L. 1758 larvae, *Drosophila melanogaster* Meigen 1830 larvae, captivity-bred collembola *Heteromurus nitidus* (Templeton 1835), Thysanura, Embioptera, small lumbricid oligochaetes, and small pieces of apple and banana. In the winter, the photoperiod was changed to 10 hours of light and 14 of darkness, and the temperature was progressively reduced over two weeks, ultimately reaching a minimum of 4 °C, in order to induce diapause. In the spring, the temperature was raised to 12.5 °C and a photoperiod of 12 hours of light and 12 of darkness was established.

Imagoes were returned to their natural habitat in the autumn, in order to minimize the impact on that population, given its status as a protected species.

Results

Laying of eggs and larval development

Sixteen eggs were obtained between August 4 and September 1 in 2008; the females laid them separately, burying them into the substrate. The number of eggs per female varied between 1 and 9. Of all eggs obtained, one was directly conserved in Scheerpeltz for the study of chorion; six did not hatch and started to develop fungi. Therefore, they were conserved in Scheerpeltz in order to prevent deterioration and study them; nine
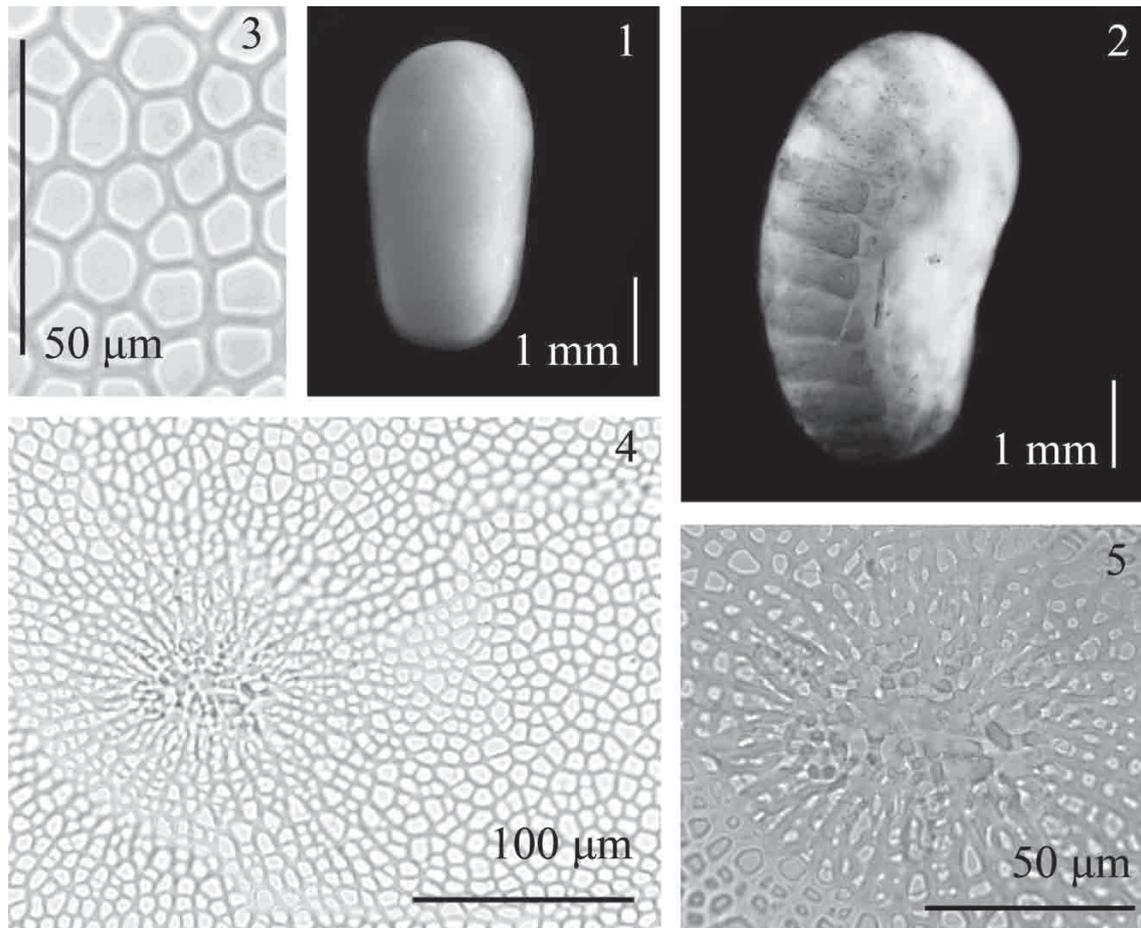
Figures 1–5
Egg of *Carabus (O.) ghilianii*. 1, egg in first stages of development*; **2,** egg in the latest stages of development*; **3,** detail of microreticulation of chorion; **4,** detail of micropile and reticulation of chorion*; **5,** detail of micropile.

finally hatched out. It took them between 10 and 17 days to complete their embryonic development.

Five larvae were selected to continue their development. Despite efforts, they did not reach the next instar and died. During this process, they showed very little activity and did not feed despite the quantity and variety of prey available. They burrowed themselves between 1 and 3 cm deep in a small chamber, where they remained bent, in a position similar to the one they adopt during their embryonic development. Terrariums were inspected periodically in the first weeks of winter, but in order to reduce the stress caused to the larvae, it was decided to stop these inspections, until the moment the temperature was raised. In the spring, it was found that they had not survived.

Biology of imagoes

Imagoes were more active during the hours of darkness, although they were occasionally active during the light hours too. They showed a clear preference for *Tenebrio molitor* larvae, as live food, and for banana, although they did not reject liver either. Unlike other *Carabus* species, they did not feed on lumbricids and showed very little preference for apple.

Gregary tendency was confirmed, both in the place of capture and in the laboratory, by finding several specimens of both sexes under the same stone.

Egg morphology

Egg size increases during ontogeny, from 3.5 mm in length and 2.5 mm in width in the first stages (fig. 1), to 5 mm in length and 2.7 mm in width just before hatching (fig. 2). There is also a slight variation in egg colour, starting with a greyish-white ivory tone at the time of laying that gradually changes to a more cream colour until hatching.

Chorion reticulation has an approximate density of 10.5 cells/0.1 mm. The average length of cells is 8.8 μm, and ridges have an approximate width of 2 μm (figs. 3, 4, 5).

Morphology of first larval instar

Visibly sclerotized and pigmented larvae, approximately 10.5 mm in length (from the nasale to the apex of the pygidium), and relatively robust aspect (fig. 6).

Cephalic capsule: slightly wider than long: 1.69 mm in width and 1.07 mm in length. Epicranial suture reduced, almost absent. Frontal suture slightly curved. Frontale is 1.08 mm in width and 1.12 mm in length (fig. 7). The nasale bears four teeth, each of which has a small apical sensillum. It presents two egg bursters on both sides of the basal region of the fron-

tal. These egg bursters consist of two elevated carinae incorporating several raised teeth that anteriorly end in a point, directed forward (fig. 8). There are nine setae of different sizes at each side of the sagittal plane. FR1 is absent, and FR3 is so reduced that only the alveole is seen. There are five pores: FRa, FRb, FRc, FRd, FRe, according to the model of Makarov (1992), and a supernumerary one, FRα. Parietale (figs. 9, 10) showing six stemmata, three anteriorly and three posteriorly, around the ocular protuberances. The basal half of the gular suture is beaded. It bears eleven setae, of which PA1, PA2, PA3, PA9 and PA19 are much reduced (less than 0.1 mm in length), while PA7 is hypertrophied (0.65 mm approximately). Setae PA5, PA4, PA8 and PA17 *sensu* Bousquet & Goulet (1984) are absent. The pore PAe is in the centre of the ocular protuberances. The pore PAi *sensu* Bousquet & Goulet (1984) is not absent, unlike in the model of Makarov (1992). The pores PAa, PAb, PAc, PAf, PAg, PAh, PAl, and PAk are absent. There is a dorsal supernumerary pore, PAα, and a ventral supernumerary pore, PAβ. Mandible robust and curved (fig. 11). It bears a well-developed and curved retinaculum, with small lobes that give it a serrulate aspect on the proximal edge. There are two setae, MN1 and MN2, and three pores; the third one (MNc) is present in the model of Bousquet & Goulet (1984) and absent in the study by Makarov (1992). Labium (figs. 12, 13) without ligula in the prementum. Labial palpus with two palpomeres, the last of which shows an elliptical area, on the external side, approximately 0.12 mm in length, where the integument becomes a rough texture. It bears a total of fourteen to sixteen setae at each side of the sagittal plane. LA4 and LA5 reduced and LA6 well-developed; gLA7 consists of two or three setae that decrease in size towards the sides. In one of the specimens, a supernumerary seta next to LA6 has been observed. The pore LAc is absent, but there are two supernumerary pores in the ventral side: LAα, besides LAa; and LAβ, in the last palpomere. Maxilla (figs. 14, 15) with stipe subcylindrical and short. Cardo reduced. Four palpomeres in the palpus, progressively decreasing in size. The last one as long as the second and half of the third together. Galea as long as half the palpus or slightly longer. Seta MX1 very little developed; MX2 very well-developed (0.5 mm in length approximately). Lacinia with MX6 in the apex, whose length exceeds the joint between the two galeomeres. MX4 little developed. MX11 and MX12, apparently absent, are much reduced. MX9 and MX5 are absent. The last one is indistinguishable from gMX. Beside MX2, there are two supernumerary setae: MXI and MXII. The pores MXa, MXb, MXc, MXd and MXg are observed. The pore MXe is absent, but there

are six supernumerary pores: MXα, MXβ, MXγ, MXδ, MXε and MXζ. Antenna (figs. 16, 17) with four sub-cylindrical antennomeres, the first of which is slightly longer than wide. The second and third antennomeres

are longer than the first one, although similar in width. The last antennomere is approximately half as wide and almost as long as the third. It bears nine setae, six of which are well-developed: AN1, AN2, AN3, AN4,
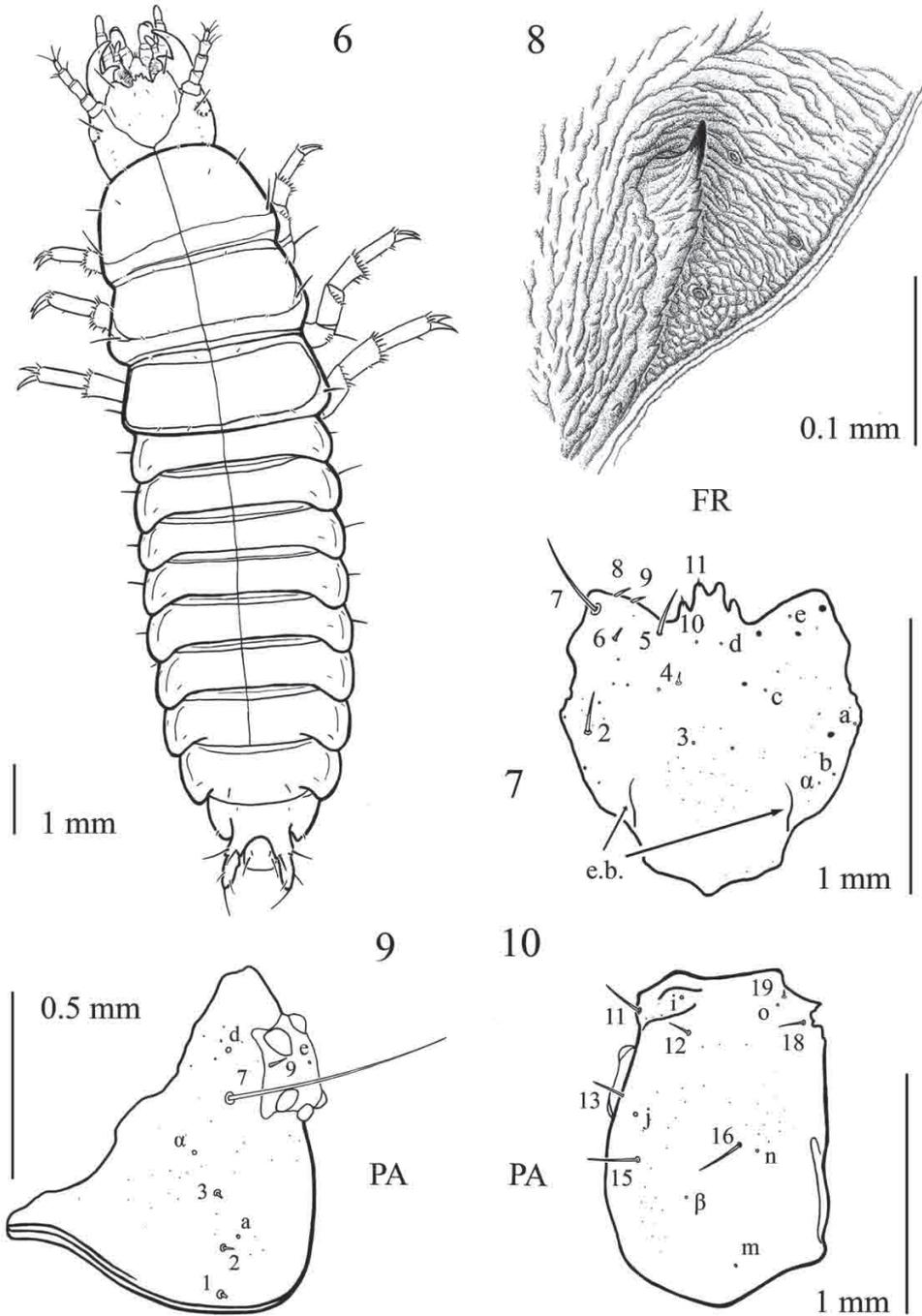
Figures 6–10
First instar larva of *Carabus (O.) ghilianii*. 6, *habitus;* **7,** frontale; **8,** detail of the frontale: egg bursters; **9,** parietale in dorsal view; **10,** parietale in ventral view.

AN5 and AN7, while gAN6 bears only three short setae. The first antennomere shows the pores ANc, ANd and ANe, but not the ANa or ANb. In the third antennomere, the pore ANf is seen and ANg in the fourth. There are three supernumerary pores: ANα, ANβ and ANγ in the first, second and third antennomeres, respectively. Dorsally, beside the sensorial appendage, there is a pore and two very short sensillae.

Thorax: A medial suture, which coincides with the sagittal plane, is observed in all tergites. In them, there are also three areas with different levels of sclerotization: pretergum, slightly sclerotized; tergum, very sclerotized; and postergum, less sclerotized. The sternum includes the following sclerotized structures: prosternite (PS), mesosternite (MS), episternum (ES), epimeron (EM), and a pleurite (PL) in the pleuron (fig. 18) Prothorax
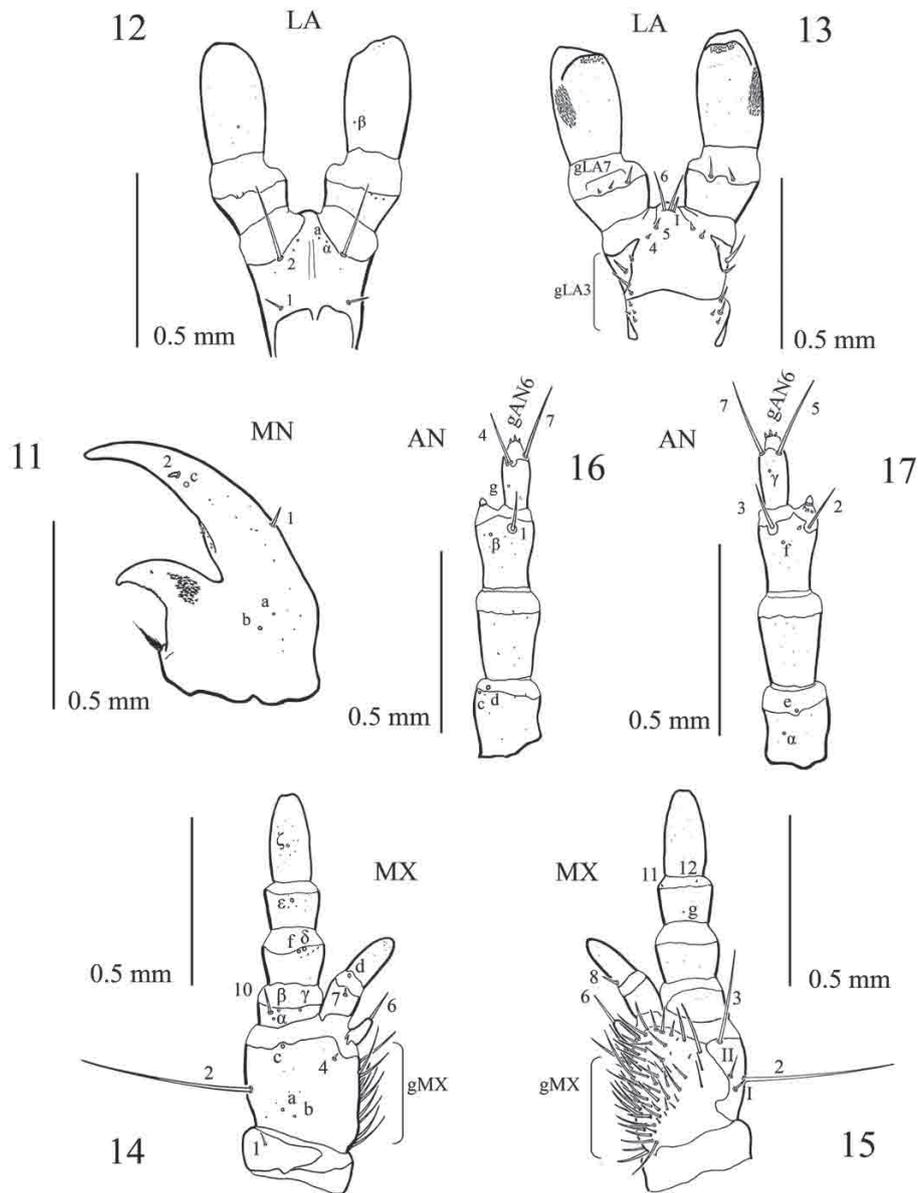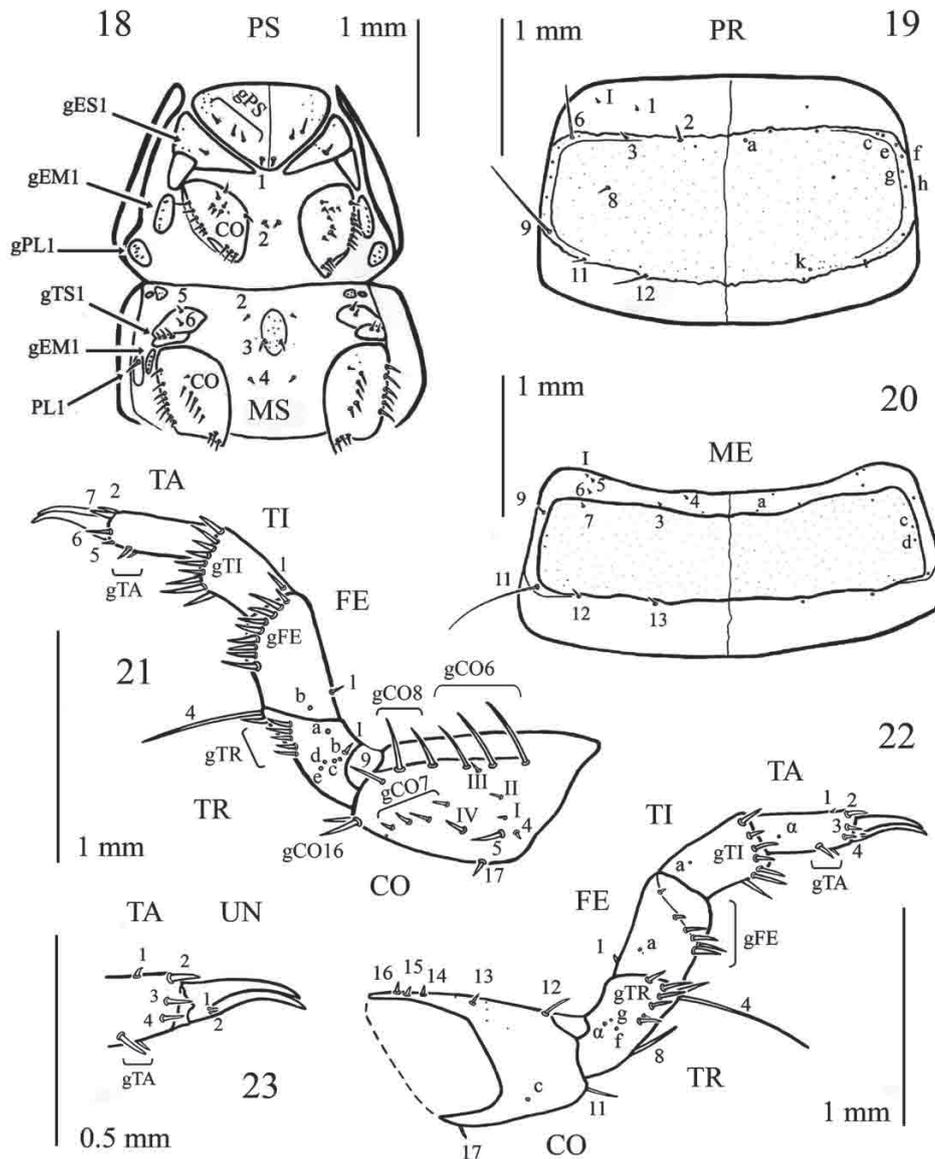

Figures 11–17
First instar larva of *Carabus (O.) ghilianii*. 11, right mandible in dorsal view; **12,** labium in ventral view; **13,** labium in dorsal view; **14,** right maxilla in ventral view; **15,** right maxilla in dorsal view; **16,** right antenna in ventral view; **17,** right antenna in dorsal view.

is the longest thoracic segment, widening towards the rear, with a maximum width of 2.6 mm and a maximum length of 1.05 mm. Pronotum (fig. 19) bears eight setae at each side of the sagittal plane. Seta PR1 is much reduced; PR2, PR3, PR8 and PR11 are very short. PR6 andPR12 are well-developed, and PR9 is the longest of all of them. PR4, PR5, PR7, PR10, PR13 and PR14 are absent. A small supernumerary seta may appear at one side, in the pretergum. The pores PRi, PRj and PRL are absent. Prosternum and pleuron with gPS, PS1 and PS2 present; gEM1 consists of a small group of short setae, and gPL1 consists of only one seta. Mesothorax slightly wider than prothorax, but a little shorter. Maximum width 2.9 mm. Maximum length: 1.3 mm. Mesonotum (fig. 20) bears ten setae (including a short supernumerary one) at each side


Figures 18-23
First instar larva of *Carabus (O.) ghilianii*. 18, prothorax and mesothorax in ventral view; **19,** pronotum; **20,** mesonotum; **21,** prothoracic right leg in ventral view; **22,** prothoracic right leg in dorsal view; **23,** detail of leg: claws.

of the sagittal plane. ME11 is the most developed. ME9, ME12 and ME13 are slightly developed, and ME3, ME4, ME5, ME6 and ME7 are much reduced. Setae ME1, ME8, and ME14, which appear in the model of Makarov (1992), are absent. The pores MEb, MEe, MEf and MEg are absent. In mesosternum and pleuron, setae MS2, MS3 and MS4; PL1; ES5 and ES6 are observed. gTS1 consists of three setae and
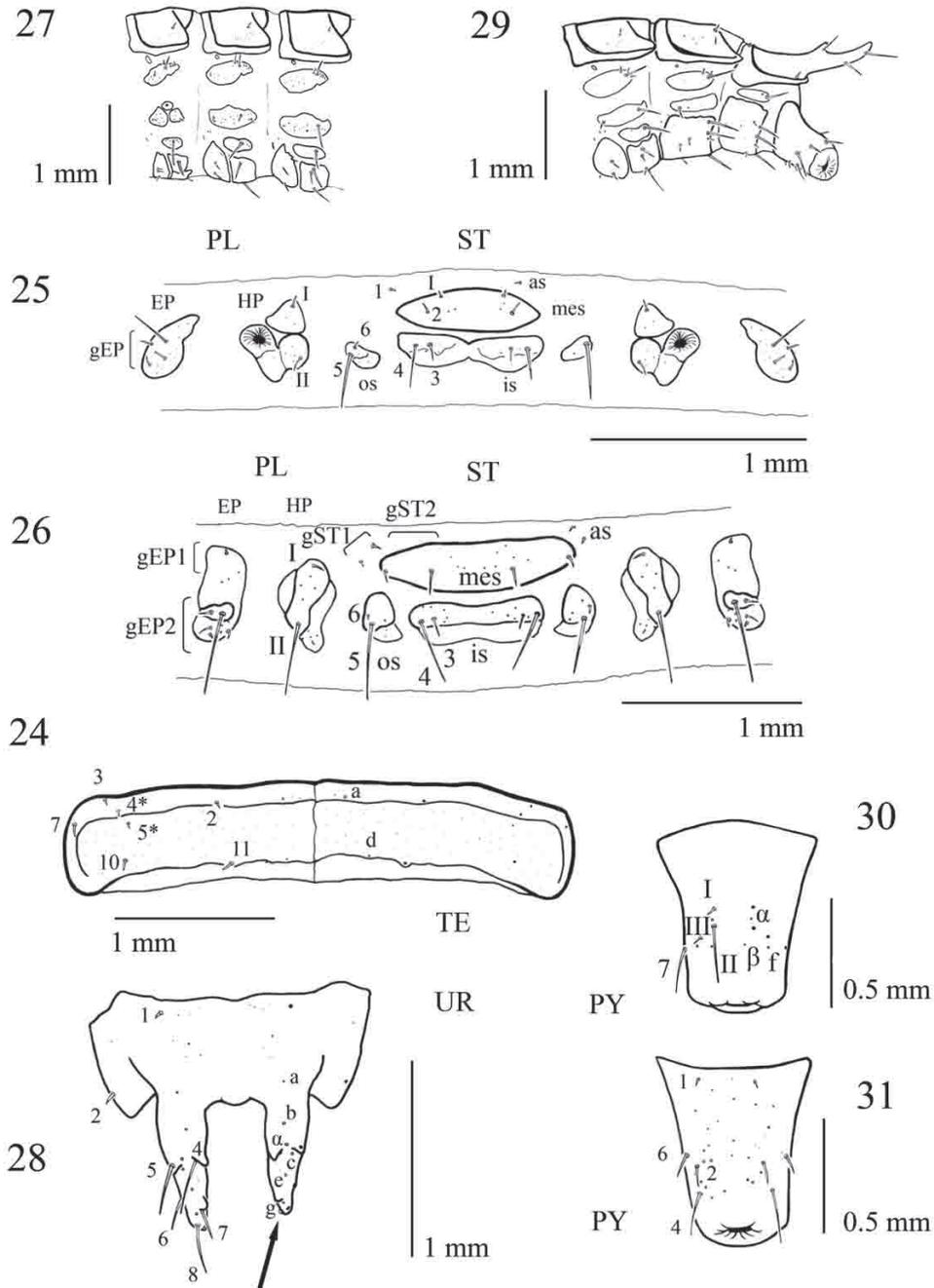
Figures 24–31
First instar larva of *Carabus (O.) ghilianii*. 24, tergite of abdominal segment IV, setae corresponding to abdominal tergite I are marked with an asterisk; **25,** extended pleura and sternon of abdominal segment I; **26,** extended pleura and sternon of abdominal segment IV; **27,** abdominal segments I, II and III in lateral view; **28,** urogomphi in dorsal view; **29,** abdominal segments VII, VIII and IX in lateral view; **30,** pygidium in ventral view; **31,** pygidium in dorsal view.

gME1 is very reduced. MS1 and gES1 *sensu* Makarov (1992) are not observed, as they are much reduced in size. With respect to the metathorax, the chaetotaxy of the metanotum is similar to that of the mesonotum. Maximum width of 3.0 mm and maximum length of 1.1 mm.

Legs (figs. 21, 22, 23) less sclerotized than tergites. Pro-, meso-, and meta-thoracic legs are similar in chaetotaxy and size (2.8 mm from coxa to claws). The description is based on the right prothoracic leg. Coxa: 0.85 in length (0.4 mm in the posterior and dorsal side) and 0.55 mm in width. It bears a total of twenty-five setae. gCO11 *sensu* Makarov (1992) consists of only one seta CO11. CO10 is absent; there are four small supernumerary setae: COI, COII, COIII and COIV. The only distinguishable pore is COc. Trochanter: 0.5 × 0.27 mm. It bears fourteen setae. The longest is TR4, reaching a length of 0.5 mm. The second-biggest seta is TR8. Setae TR7, TR5 and TR6 are indistinguishable, as they appear surrounded by supernumerary setae, forming a homogeneous group, called gTR *sensu* Makarov (1992). In addition to the pores described by Makarov (1992), there is another supernumerary one: TRα. Femur: 0.5 × 0.3 mm. It bears a group of fifteen setae, which, as a whole, are called gFE, except for FE1, which is isolated from the rest. These setae are arranged in a semicircular line around the ventral and distal extremes of the femur. Two supernumerary pores, FEa and Feb, are observed. Tibia: 0.4 × 0.27 mm. It bears fourteen setae, one of which (TI11) is not located near gTI. This group is also arranged in a semicircular line around the distal end of the tibia. Tarsus: 0.45 × 0.18 mm (a little narrower). It bears nine well-differentiated setae and a supernumerary pore, TAα. Claws: Two claws (fig. 23), 0.26 mm in length, curved towards the end. Two setae, UN1 and UN2.

Abdomen of 6.4 mm in length approximately. The average length of abdominal segments is 0.7 mm, and the maximum width of the abdominal segments is 2.9 mm. With respect to the tergites, the abdominal tergite I bears seven setae, although the abdominal tergite IV bears five. Only the segment I bears setae TE4 and TE5 (fig. 24). Setae TE1, TE6, TE8 and TE9 are absent, although, except for TE8, they appear in the model of Makarov (1992). The pores TEa and TEd are observed. With respect to the sternites and pleurites, abdominal segments I and IV (figs. 25, 26), and a lateral view of the three first segments are drawn (fig. 27), in order to observe the differences between them because of the presence or absence of the spiraculum. In neither case are the pores drawn, for it is not necessary according to Bousquet & Goulet (1984) and Makarov (1992). With respect to the sternites, the anterior sternite (as) is not

sclerotized, but there is one seta ST1 in the abdominal segment I in its correspondent position that may also appear as a little group of setae gST1 in the abdominal segment IV. Median sternite (ms) is slightly sclerotized. It bears a group of two setae at each side of the sagittal plane, named gST2 according to Bousquet & Goulet (1984). Innersternite (is) is fused in the midline, the anterior half slightly more sclerotized than the posterior. Setae ST3 and ST4 are well-developed. Outersternite (os), with ST6 almost absent, and ST5 very developed. With respect to pleurites, epipleurite (EP) is relatively sclerotized. It bears four setae in segment I and six setae in the segment IV. Hypopleurite (hy) is slightly sclerotized too. There is a more sclerotized central stripe, anteroposteriorly, on which there are two supernumerary setae. In abdominal segment I, there is a conspicuous respiratory spiraculum, which is absent in the rest of the segments, in addition to the small spiraculum that is present above the epipleurite in all abdominal segments.

Urogomphi (figs. 28, 29) bear seven setae. UR1 and UR2 are much reduced. UR3 and UR9 are absent. The pores URa, URb, URc, URe, and URg are present, but URd *sensu* Makarov (1992) and URf are absent. There is a supernumerary one, URα, above UR5. Urogomphi also bear a small protuberance on the inner side of the distal extreme, in the position of UR9.

The pygidium (figs. 29, 30, 31) is short and wide, narrowing towards the end, reaching a width of 0,5 mm at the end. It bears eight setae at each side of the sagittal plane: PY1, PY2, PY4 and PY6 *sensu* Makarov (1992); a group gPY7 with a single seta and three other supernumerary setae. Ventrally, there are only three pores: PYf *sensu* Bousquet & Goulet (1984) and PYα and PYβ, supernumerary.

Discussion

Certain gregariousness has been observed in *C. (O.) ghilianii* imagoes, as happens in other species of the genus (Casale 1973). It is known that this behaviour in Carabidae depends upon olfactory stimuli (Wautier 1971), although the concrete pheromones affecting sexual attraction and/or aggregation within or between *Carabus* species are still unknown (Assman 2003). According to this, substantial data have been obtained in different species: it was observed that three times more males of *C. (Chrysocarabus) auronitens* Fabricius 1792 were found in female-baited pitfalls than in male-baited or unbaited ones (Baumgartner 2000); in *C. (Oreocarabus) hortensis* L. 1758; *C. (Oreocarabus) glabratus* Paykull 1790; *C. (Mesocarabus) problematicus* Herbst 1786 and *C. (Megodontus)*

violaceus L. 1758, pitfalls baited with females or males attracted significantly more individuals of either sex of the given species than did unbaited traps (Assman 2003). Therefore, the gregarious tendency observed in *Carabus (Oreocarabus) ghilianii* is not surprising, as it is common in the genus *Carabus*.

Females of *Carabus* lay eggs separately as a mechanism to reduce the incidence of cannibalism between larvae, as cannibalism is a well-known behaviour in this genus (Huk & Kühne 1999; Kern 1921). The number of eggs laid per female is only known from captivity and fluctuates greatly according to the individual and the species, from almost a dozen in *C. (Limnocarabus) clatratus* L. 1761 (Huk & Kühne 1999) to more than fifty in *C. (Tachypus) auratus* L. 1761 (Scherney 1957). In *C. (O.) ghilianii,* observations of ovoposition suggest a number of eggs laid per female from one to nine, although those data must not be taken categorically, as they are the result of the rearing of only five females in captivity.

The breeding of *Carabus* in captivity, as in any other Carabidae genus, always poses difficulties. In most *Carabus* breeding experiments, the mortality of larvae is very high (Hůrka 1972; Huk & Kühne 1999). In the case of *C. (O.) ghilianii,* despite the spoilage of six eggs and the death of five larvae selected to continue its development, achieving the lay of eggs and the first larval instar of such a stenoic species must be considered an important success.

The observed embryonic development in *C. (O.) ghilianii* takes between ten and seventeen days, which is within the known range for other *Carabus* species whose embryo development oscillates between five or six days for *C. (Tachypus) auratus* L. 1761 at 18 °C and twenty-one days for *C. (Archicarabus) nemoralis* O. F. Müller 1764 at the same temperature (Sturani 1962).

Despite the accuracy of the measures of egg microreticulation, it must be taken into account that Luff (1981) demonstrated that there is a significant intraspecific variability of up to 5% in the density of cells per 0.1 mm among different eggs. In a similar way, environmental conditions, such as the quantity of carbohydrate in the diet or temperature, can also affect not only the fertility of the female, but also egg size (Luff 2003), so we cannot yet know whether the eggs of *C. (O). ghilianii* in nature match exactly with the same characteristics as those obtained in captivity. Nevertheless, and despite that intraspecific variability, the description of the chorion of *Carabus (Megodontus) violaceus* L. 1758 given by Luff (1981) is very similar to that for *C. (O.) ghilianii*. Therefore, this information about the microreticulation could be relevant for completing the diagnosis of the subfamily, but it must

be studied if it could also be relevant for subspecific level.

The time needed for completing the development of the larvae of *C. (O.) ghilianii* still remains unknown, although it could be greater than 100 days, as has been observed in other species with winter larvae (Van der Drift 1958; Rijnsdorp 1980; Betz 1992; Busato & Casale 2004), suggesting an adaptation to cold climates (Busato 2009). This time could be even longer, if *C. (O.) ghilianii* had a biannual cycle of life, as other *Carabus* species that live in northern Europe, or at high altitudes (Houston 1981; Refseth 1984; Lindroth 1985), such as these: *C. (Megodontus) violaceus* L. 1758; *C. (Procrustes) coriaceus* L. 1758 or *C. (Oreocarabus) glabratus* Paykull 1790. Nevertheless, it has been demonstrated that the time of development in other Carabidae is not constant and depends on the quantity of food that the larva receives (Nelemans 1987a, 1987b, 1988).

C. (O.) ghilianii is adapted to a cold climate; therefore, the death of the larvae must not be related to the low temperature. Despite the observations of the inactivity of larvae during winter time, they could possibly interrupt diapause for feeding, as other *Carabus* species, imagoes (4–5 °C, according to Casale *et al.* 1982) or larvae (3 °C, according to Betz 1992). The cause of death of first instar larvae may probably be the absence of adequate prey during hibernation.

A series of observations of first instar larval morphology has been made, comparing those results with data on other known larvae. The nasale bears four teeth, which seems to be the primitive condition in the family Carabidae (Luff 1993), and it is a feature shared with other species of the same subgenus, like *C. (Oreocarabus) hortensis* L. 1758 and *C. (Oreocarabus) glabratus* Paykull 1790 [*sensu* Deuve 2004: *C. (Pachystus) glabratus*] and another species of different subgenera, like *Aulonocarabus* Reitter 1896, *Diocarabus* Reitter 1896 and *Orinocarabus* Kraatz 1878.

The difficulties in studying larvae, cited by Solodovnikov (2007), increase because of the great number of small pores in the thoracic and abdominal tergites, which complicate the comparison of them with the models of Makarov (1992) and Bousquet & Goulet (1984). Therefore, it is possible that its interpretation could be a little subjective.

The frontal bears two egg bursters, which consist of two elevated carinae incorporating several raised teeth, that anteriorly end in a point directed forward, just as they appear in other *Carabus* species, like C. (*Rhabdotocarabus) melancholicus* Fabricius 1798 (Cárdenas & Hidalgo 1998). Moreover, according to Luff (1993), this is the common position of egg bursters in

Carabidae. The shape of the egg bursters is, according to Luff (1993), important for specific identification of the first larval instar. Nevertheless, the description of egg bursters of *C. (Mesocarabus) lusitanicus* Fabricius 1801 given by Cárdenas & Hidalgo (1995) is surprising, as they are described in the form of two small *cornua* (=horns), centrally positioned in the frontal and not basally. These observations do not match those we have made of first instar larvae of that species.

With regard to the labium, it is similar to the model proposed by Makarov (1992), but with less setae in gLA3. The maxilla shows setae MX7 and MX8 well-developed, if compared with the model of Bousquet & Goulet (1984) and Makarov (1992). Setae AN1 of antenna seems to be in a different position with respect to the model of Bousquet & Goulet (1984), moved from the basal region of the antennomere to the distal region of the ventral side, and setae AN2 and AN3 are on the dorsal side. However, this interpretation of AN1 is debatable, since it could simply have shifted towards the distal region of the antennomere, and the AN2 could have shifted to the ventral side. Therefore, AN1 could be interpreted as AN2 and vice versa. Although both hypotheses seem plausible, this approach involves less changes with respect to the model of Bousquet & Goulet (1984). Seta AN6 is much reduced, and it is surrounded by two other small setae or sensilla of similar shape and size, already described in Bousquet & Goulet (1984); therefore, we consider that group gAN6. That groups was already seen by Makarov (1992), and described by Busato (2003). Prothoracic setae PR4 and PR5 are absent as in the model of Makarov (1992). It is not possible to distinguish the mesothoracic pore MEe, since there are several small pores in that position. The coxal seta CO9 could be one of the group gCO8 according to the model of Makarov (1992); however, we adopt the criterion of Bousquet & Goulet (1984). The two setae of the group gTA (*sensu* Makarov 1992) could be interpreted as supernumerary setae according to Bousquet & Goulet (1984). Abdominal tergites show a clear reduction in number and size of setae in relation to the model proposed by Bousquet & Goulet (1984); in regard to the pores, they are not drawn in Makarov (1992), and it is complicated to establish a correspondence with the general model of Bousquet & Goulet (1984). As in other *Carabus* species, the sclerotization of the abdominal anterior sternite is almost absent. The abdominal hypopleurite bears only two setae, HPI and HPII, unlike the model of Makarov (1992), who finds more than two setae in that position. With respect to the epipleurite of abdominal segment I, it is hard to distinguish the two groups of setae that Makarov (1992) proposes, although in the segment IV, five setae are observed in the posterior group and one in the anterior group. In both cases, there are more than two setae, unlike in the model of Bousquet & Goulet (1984). Urogomphi are similar in shape to other species, although they bear a small protuberance that could match the seta UR9 or they could be sensilla. Neither Makarov (1992) nor Bousquet & Goulet (1984) describe it. Finally, the pygidium bears a reduced gPY7 in comparison to the model of Makarov (1992), where a seta PY7 and three supernumerary setae are observed.

Conclusions

The stenotopic status of this species places a number of limitations on recreating natural conditions in the laboratory, issues that add to the intrinsic difficulties already cited by Solodovnikov (2007) concerning the study of preimaginal stages. Therefore, and in spite of not achieving the whole lifecycle of *C. (O.) ghilianii,* achieving reproduction in captivity and the first larval instar must be considered a success. Significant information on preimaginal anatomy has been provided, which is quite relevant in the taxonomy of *Carabinae*. Also, data about its biology are revealed, which may be important for the management and protection of species. In the same way, all contributions to the knowledge of the biology of the imago, or preimaginal stages, are really important for developing efficient management plans for its conservation.

It is worth noting two interesting novelties in the knowledge of larvae. The first one is that the results indicate that this is a winter larva. This fact seems to contradict Turin *et al.* (2003), as they state that this species is a spring breeder. It is worth mentioning the fact that the mountain environment they inhabit is characterized by cold temperatures and a short summer, which may promote development times longer than usual. Therefore, the hypothesis presented here is that copulation begins in late spring, as indicated by Turin *et al.* (2003), but both the egg-laying and hatching continue during the summer. They do not have enough time to complete their larval development during the summer, and, therefore, they enter diapause in the autumn in order to complete the cycle the following year. The second novelty is that, in terms of morphology, we found some peculiarities in this larva that have not been described in other *Carabus* larvae, such as the small protuberances on the insides of the ends of urogomphi, the presence of only two setae in the abdominal hypopleurite (HPI and HPII), and the absence of the two groups of setae expected in abdominal epipleurite I.

To compare these characteristics with those of other larvae, it is necessary to study larval chaetotaxy in depth and to also develop a good iconography that may help to interpret the results of those individual studies. This will be the essential basis for developing the identification keys for larvae of the subgenus *Oreocarabus*, keys that have not been developed yet due to lack of available information (Arndt & Makarov 2003).

Finally, it should be noted that the information generated by this new study will benefit the knowledge of *Carabus (Oreocarabus) ghilianii*, an emblematic species of the Iberian entomology, because of its stenotopic and endemic condition and its status as a vulnerable species.

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