Bulletin of Insectology **64** (1): 43-52, 2011
ISSN 1721-8861

Description of the larva and female genitalia of *Trechus gamae* with data on its ecology

Ana Sofia P. S. REBOLEIRA[1], Vicente M. ORTUÑO[2]
[1]*Centro de Estudos do Ambiente e do Mar & Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, Aveiro, Portugal*
[2]*Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain*

Abstract

The third instar larva and the female genitalia of *Trechus gamae* Reboleira et Serrano (Coleoptera Carabidae Trechini) are described, illustrated and commented. Larvae and imagos were collected in deep parts of caves from Estremenho karstic massif in the centre Portugal. The work provides the first study on hypogean beetle larvae from Portugal, increasing the knowledge about hypogean microendemic species from the Lusitanic district of the Iberian Peninsula. The morphological diagnostic characters of the female genitalia corroborate the inclusion of *T. gamae* in the "*T. fulvus*-group". Some new data on the ecology of this species are also given.

Key words: Trechini, larval morphology, pre-imaginal stages, troglobiont, Estremenho karstic massif, Portugal, Iberian Peninsula.

Introduction

Historically, the morphology of imagos represents the major source of phylogenetic information of beetles, with a certain absence of emphasis on larvae morphology. This is strongly related to the problems that arise when surveying chaetotaxy in preimaginal stages and to the scarce knowledge and interest about beetle larvae (Bousquet and Goulet, 1984; Grebennikov and Maddison, 2005; Solodovnikov, 2007).

Pre-imaginal stages may supply addictional and important information on ecological details. The larva is a different phenotypic expression of the same genotype of the imago and thus provides a good source of information and morphological diagnostic characters in taxonomy (Grebennikov and Maddison, 2005).

Obtaining adults from larvae at breeding laboratory in the case of hypogean species, is rare due to the difficulty of recreating the underground environmental conditions, so caves are unique places for studying preimaginal stages of beetles (Casale and Marcia, 2007).

Several studies about morphology of hypogean Trechini larvae and also about the genus *Trechus* Clairville 1806, have been performed (Casale and Marcia, 2007; Giachino, 1985; 1989; Jeannel, 1920; Luff, 1993; Makarov and Koval, 2003; Ortuño and Reboleira, 2010).

Female genitalia and more specifically the spermatecal complex is one important anatomical trait of the imago, aforetime neglected in taxonomic and systematic studies of Coleoptera, more particularly Carabidae. These structures contribute with very interesting information about phylogenetic relations (Liebherr and Will, 1998), therefore such studies have been included in recent decades (Liebheer, 1986; 1991; Freitag and Barnes, 1989; Roig-Juñent, 2000; among others).

The female genitalia may provide relevant information about relations between species of the genus *Trechus* (Ortuño, 2004; Ortuño and Toribio, 2005) and therefore may contribute to reinforce the "species groups", *sensu* Jeannel, 1927.

Nowadays, there are four known species of hypogean beetles in karstic caves in Portugal and their knowledge is limited to the description of imagos habitus, aedeagus and some activity recorded during winter and spring (Bellés, 1987; Jeannel, 1941; Reboleira *et al.*, 2009; 2010). Details about their biology and preimaginal stages are still unknown.

The present paper describes the third-instar of the larva and the female genitalia of *Trechus gamae* Reboleira et Serrano 2009 (Reboleira *et al.*, 2009), included in the *T. fulvus*-group (Jeannel, 1927). This species is endemic from the central subunit of Estremenho karstic massif - Planalto de Santo António, a Jurassic (Dogger) limestone, in the centre of Portugal. All the adults and larvae where obtained in deep parts of caves, where they seem to be the only hypogean beetle inhabiting (Reboleira *et al.*, 2009).

Materials and methods

Two larvae from Algar da Arroteia, 18.III.2007, S. Reboleira leg. and one from Algar do Pena, 10.III.2007, and one female from Algar das Gralhas VII, 24.III.2007, S. Reboleira leg., were selected from the field work conducted in five caves within the Santo António Plateau, of Estremenho karstic massif, centre Portugal (table 1). During six months, each cave was monitored in three different zones: near the entrance, in the deeper/terminal part of the cave and in one intermediate zone, to a maximum depth of 95 m.

Sets of five pitfall traps (each trap: 6 cm diameter, 7 cm depth, with a 1 cm diameter tube fixed inside, at the centre) were used in each selected zone. Substrate type

Table 1. Abiotic parameters of the studied caves. Entrance level and maximum depth sampled in meters, and mean temperature in depth zone in °C.

Cave	Locality	Altitude	Depth	Temperature
Pena	Barrerinhas	340	95	13.5
Gralhas VII	Pé da Pedreira	350	75	15
Marradinhas II	Casais da Moreta	250	30	17
Ladoeiro	Alvados	485	70	15.5
Arroteia	Chão de Pias	450	60	16

alternated between clay, limestone and bat guano. Traps were 1/3 filled with 1.2-propanodiol, and pork liver was used as attractive bait. Traps were covered with small stones to prevent flooding and disturbance by small vertebrates. The traps were checked and material collected monthly.

The largest larva was selected to describe, unequivocally, the larval instar-III. The larva was cleaned and preserved in Scheerpeltz solution (70% ethanol, 29% distilled water, 1% acetic acid and glycerine). After examination, the selected larva was prepared in Hoyer's solution (see Anderson, 1954: 30 g gum arabic, 20 ml glycerine, 50 ml distilled water, 200 g chloral hydrate) and mounted on microscope slide. Chloral hydrate was used as clearing agent of larval integument.

Notation of the primary setae and pores according to the homology method proposed by Bousquet and Goulet (1984) and used by Makarov (1994) in the key to the genera of the Palearctic larvae of the Carabidae. The presence of additional setae and pores were compared to the archetypal model of Bousquet and Goulet (1984). Setae are numbered with roman numerals, and pores with greek letters following the same lines as Giachino (1989). Several microsetae that do not express bilateral symmetry were not encoded, this feature is particularly noted in the thorax and urogomphi.

The usual procedure was used to prepare the female genitalia for microscopy studies (Ortuño *et al.*, 1992; Ortuño and Arillo, 2005). The terminal abdominal appendices were immersed in a saturated solution of KOH during 8 hours, then washed with Scheerpeltz solution and colored Chlorazol Black E®. The female genital was prepared with DMHF (Dimethyl Hydantoin-Formaldehyde Resin) and put on an acetate sheet.

Detailed analysis was made using an optical microscope and stereomicroscope, both equipped with drawing tubes.

The permanent microscope slides and others specimens are deposited in the first author collection and in the second author collection in Alcalá de Henares University (Spain).

Results

Description of the third instar larva of *T. gamae*

Habitus (figure 1): Larva slightly sclerotized; whitish with testaceous head; body shape very slender, subparallel-sided; larval length (from mandible to urogomphi apex, macrosetae excluded) 6.6 – 7.1 mm and legs with only one claw.

Figure 1. *T. gamae* habitus of the third instar larva, dorsal view. Larva size: 6.6 mm.

Cephalic capsule (figure 2A, 2B): subquadrate; longer than wide; flattened; narrowing at the base with a very narrow constriction in the middle of posterior lateral region; stemmata absent. Epicranial suture long (epicranial suture length / head length ratio = 0.17). Frontale sutures clearly visible, deeply curved and sinuous. Anterior margin of epistome (nasale) (figure 3A) tri-lobed; slightly asymmetrical; with variable number of teeth on each lobe; median lobe slight protruding. Frontale sclerite with isodiametric microsculpture of the integument, and parietal sclerite with more or less transverse microsculpture (figure 3B, 3C).

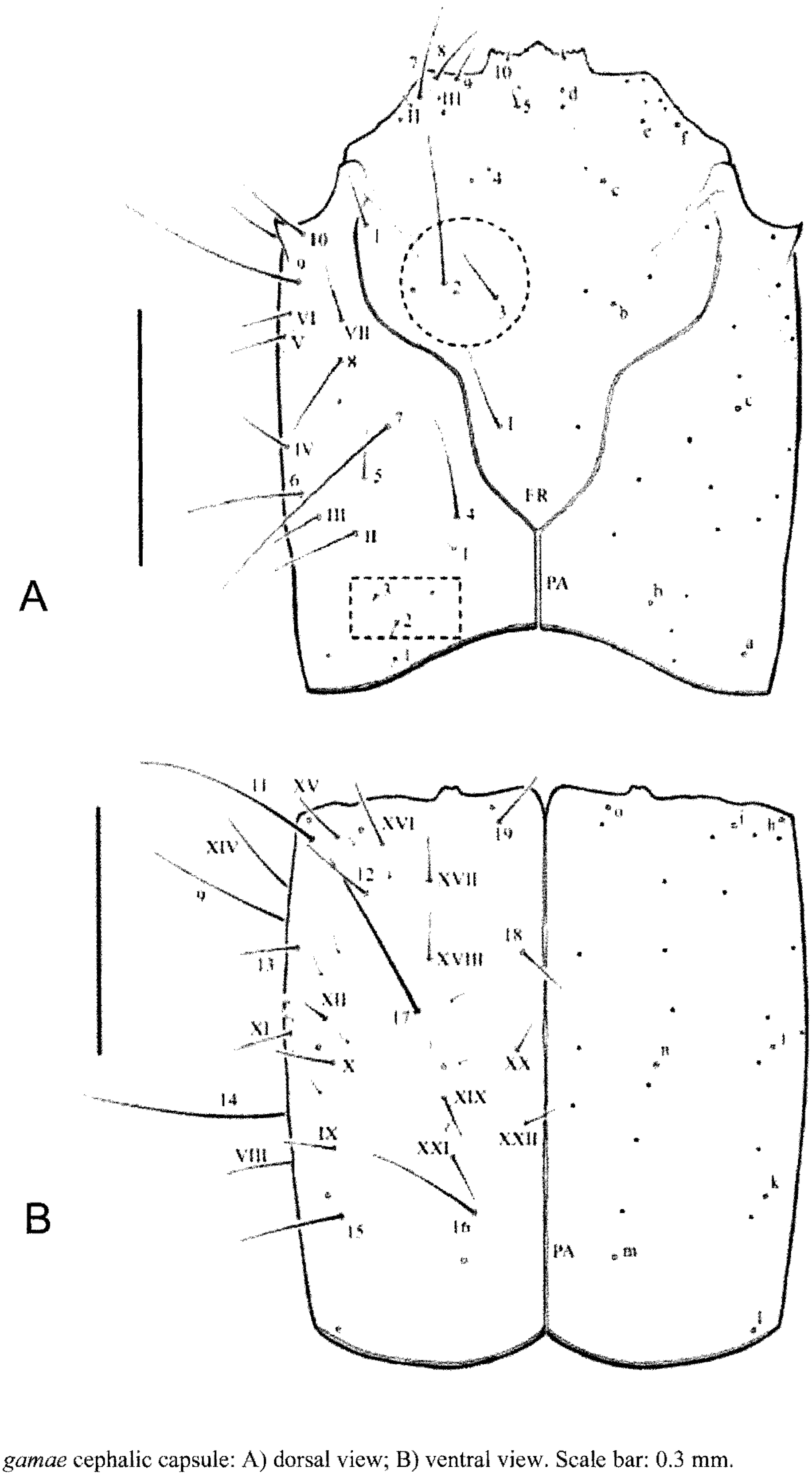

Figure 2. *T. gamae* cephalic capsule: A) dorsal view; B) ventral view. Scale bar: 0.3 mm.

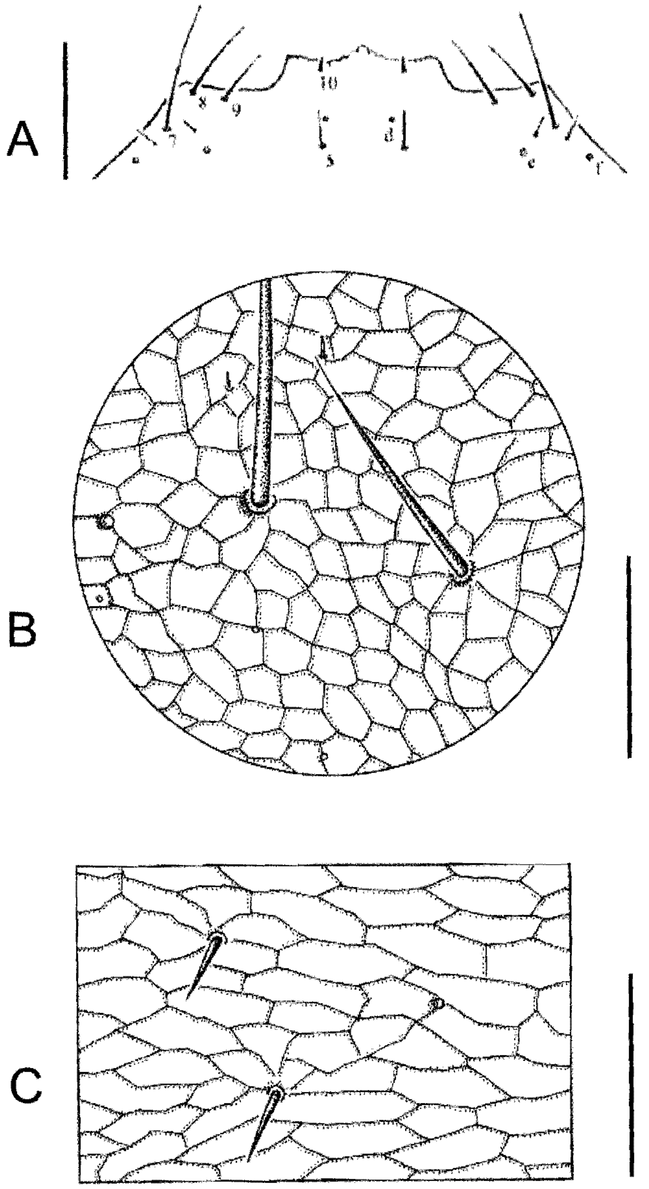

Figure 3. *T. gamae* details of the cephalic capsule: A) nasale and anterior margin of front; B) microsculpture of the frontal sclerite in dorsal view; C) microsculpture of the parietal sclerite in dorsal view. Scale bar: 0.1 mm.

Chaetotaxy of cephalic capsule: frontale [FR] (figure 2A): with 9 setae (FR_1-FR_5 ; FR_7-FR_{10}) + 3 setae (FR_I-FR_{III}) and 5 pores (FR_b-FR_f) on each side; FR_2 very long and FR_8 slightly longer than FR_9. Parietal [PA] (figure 2A, 2B): with 19 setae (PA_1-PA_{19}) + 21 setae (PA_I-PA_{XII}; PA_{XIII}-PA_{XXII}); 11 pores (PA_a-PA_c; PA_h-PA_o) on each side; PA_4, PA_7, PA_9, PA_{14}.

Mandible [MN] (figure 4A): falciform without additional teeth on terebra; moderatly curved; with 2 setae (MN_1-MN_2) and 2 pores (MN_b-MN_c); penicillum moderately developed.

Labium [LA] (figure 4B): labial palps with 4 articles, prementum with 6 setae (LA_1-LA_6) and one pore (LA_a) on each side; setae LA_5 small and LA_6 shorter than the first palp article; second articles with one pore (LA_c) and a complex of sensors; fourth articles with a sensory apex.

Maxilla [MX] (figure 4C, 4D): lacinia absent. Cardo with one seta (MX_1). Stipes with 4 setae (MX_2-MX_5) + 2 setae (MX_I-MX_{II}) and 2 pores (MX_a, MX_c) and a variable number of setae ($_gMX$); setae MX_4 elongate, compared with the archetypal model (Bousquet and Goulet, 1984). Galea with 2 articles (half as long as maxillary palps); second one largest than the first; first article of galea with 1 setae (MX_7) and one pore (MX_d); second article with 1 setae (MX_8) + 1 setae (MX_{III}) and a apical membranous sensilla. Maxillary palps with 5 articles; first article is the largest palp, with 1 setae (MX_{10}); second article with 2 pores (MX_e-MX_f); third article with 1 seta (MX_{11}) and 1 pore (MX_g) and fifth article with an apical membranous sensilla.

Antenna [AN] (figure 4E): with 4 articles, all with greater length than width; first article with 3 pores (AN_a-AN_b and AN_e); second article with 1 seta (AN_l); third article with 3 setae (AN_1-AN_3) and a conic sensorial appendage; fourth article with 4 setae (AN_4-AN_7), one pore (AN_g) and a sensorial appendage on the apex.

Thorax (figure 5): with a heavily sclerotized area with numerous additional setae; prothorax subquadrate; mesothorax and metathorax transverse. Pronotum [PR] (figure 5A): 1.1 times longer than wide; notum with 12 setae (PR_1-PR_6 and PR_8-PR_9 and PR_{11}-PR_{14}) and 4 pores (PR_b-PR_c; PR_g, PR_i) on each side. Mesonotum [MS] and metanotum [MT] (figure 5B, 5C): wider than large. Mesonotum with all primary setae (MS_1-MS_{14}) and 4 pores (ME_a- ME_b and ME_f- ME_g). Metanotum with all primary setae (MT_1-MT_{14}) and 5 pores (ME_a-ME_c; ME_f-ME_g) on each side.

Leg (figure 6A) with one claw; claw [CL] with one short seta (CL_1); tarsus [TA] with 3 setae (TA_1-TA_2 and TA_7); tibia [TI] with 7 setae (TI_1-TI_7) + 2 setae (TI_I-TI_{II}) and 1 pore (TI_a); femur [FE] with 5 setae (FE_1-FE_2 and FE_4-FE_6) + 11 setae (FE_I-FE_{XI}); trochanter [TR] with 7 setae and 4 pores (TR_b-TR_e).

Abdomen: urogomphi [UR] (figure 6B), in dorsal view, with 9 setae (UR_1-UR_9) + 5 setae (UR_I-UR_V) and 4 pores (UR_a, UR_d-UR_e, UR_g) on each side; UR_3 very long compared with the archetypical model (Bousquet and Goulet, 1984). Pygidium [PY] (figure 6C, 6D) with conical shape, 6 setae (PY_1-PY_4 and PY_6-PY_7).

Description of the female genitalia of *T. gamae*

Female genitalia (figure 7A, 7B): External genitalia (figure 7A) formed by dimerous IX gonopods (gonocoxites and gonosubcoxites) and IX laterotergites. Both gonocoxites are unguiform, with two thorn-shaped setae of considerable size on its dorsal surface. Small groove near apex and above ventral surface, with two fine, sensorial setae (= organe sétulé subapical *sensu* Deuve, 1993). Gonosubcoxite as long as wide, with approximately 5 or 6 large, thorn-shaped setae in the internal margin. Wing-shaped, slightly sclerotized IX laterotergite with one group of setae over the basal margin (approximately 20) and one more internal group (with very few setae, approximately six). Internal geni-

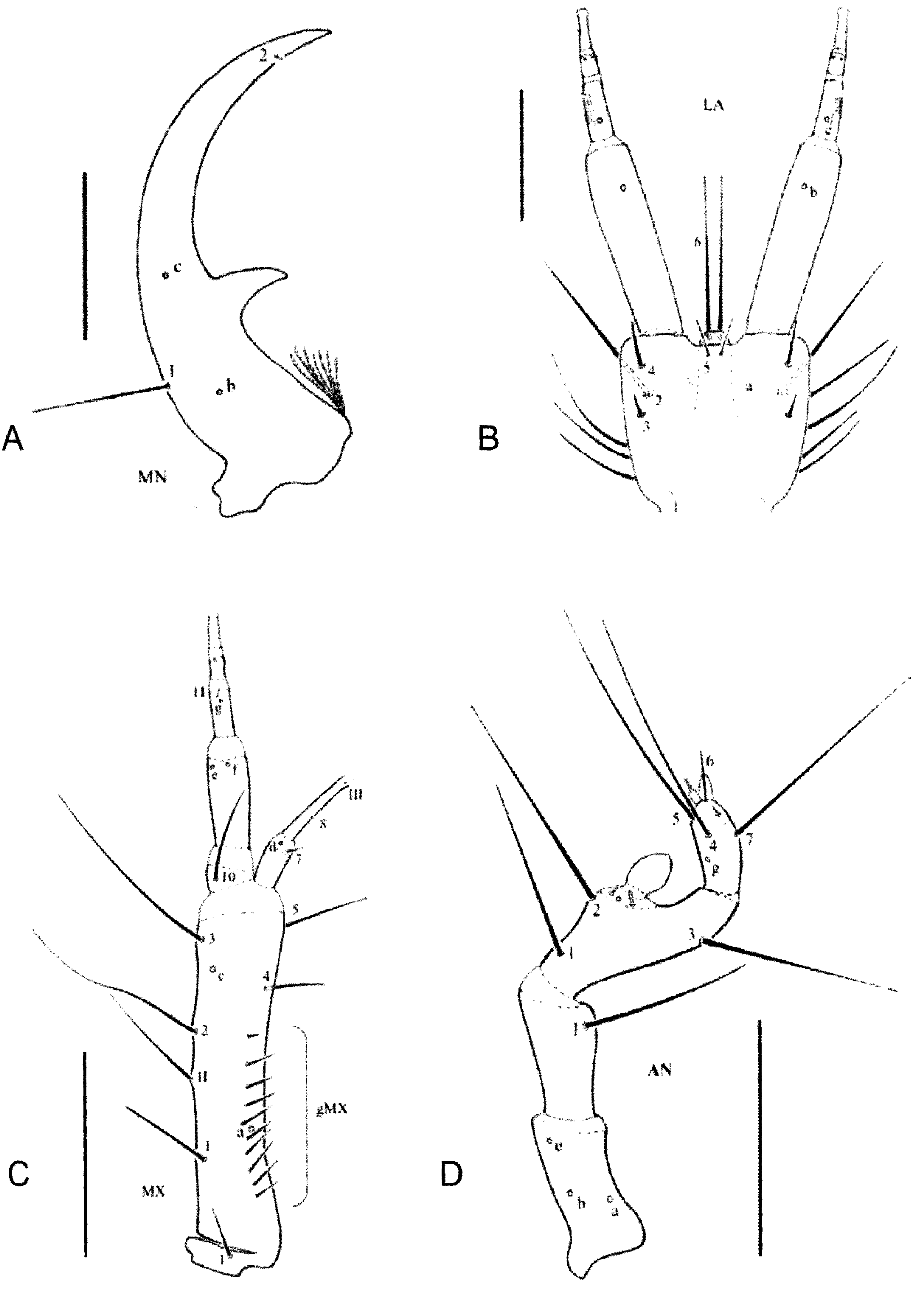

Figure 4. *T. gamae* cephalic capsule details: A) mandible in dorsal view; B) labium in dorsal view; C) maxilla in ventral view; D) left antenna in dorsal view. Scale bar, A) and B): 0.1 mm; C) and D): 0.2 mm.

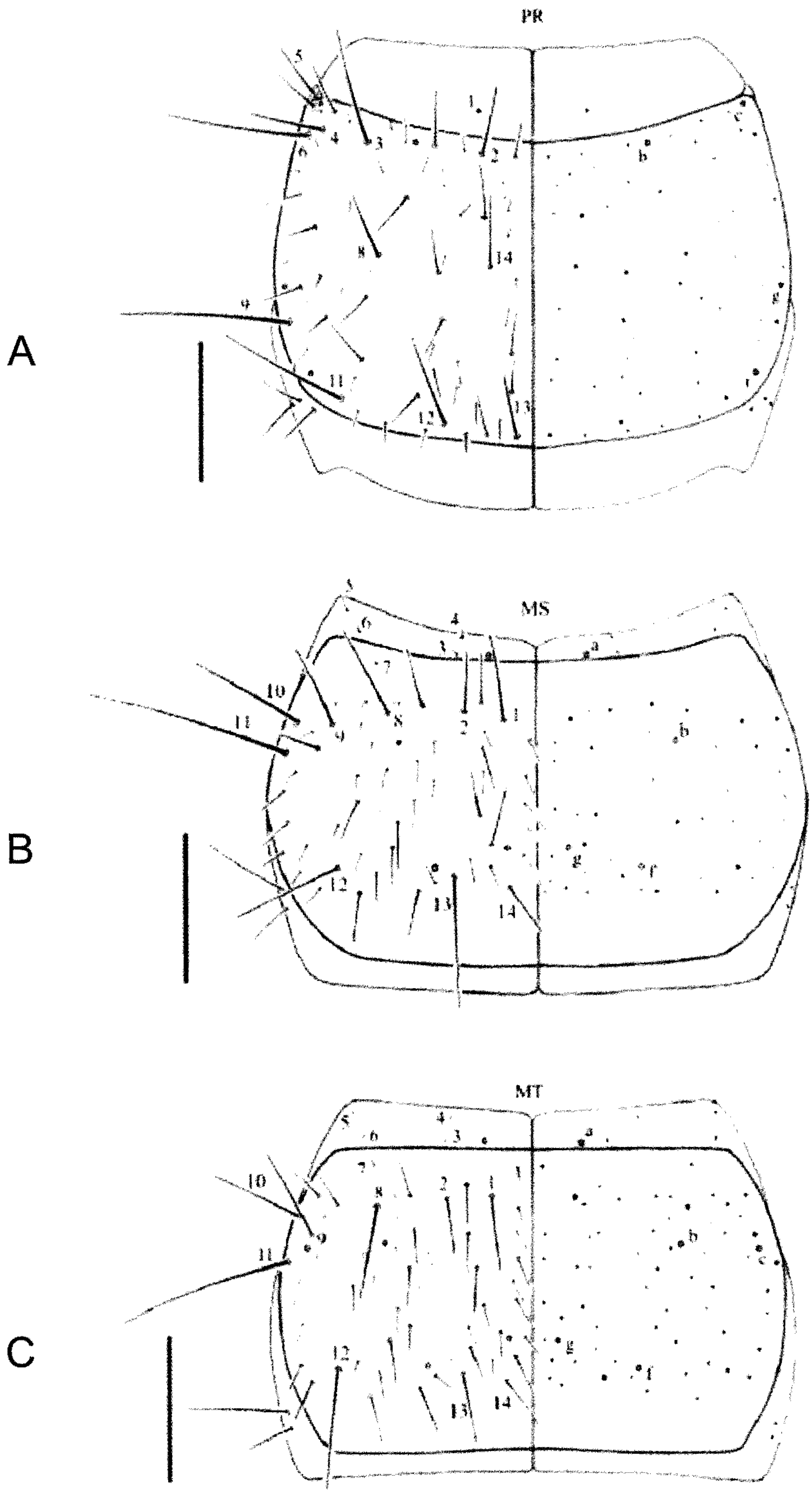

Figure 5. *T. gamae* thorax in dorsal view: A) Pronotum; B) Mesonotum; C) Metanotum. Scale bar: 0.2 mm.

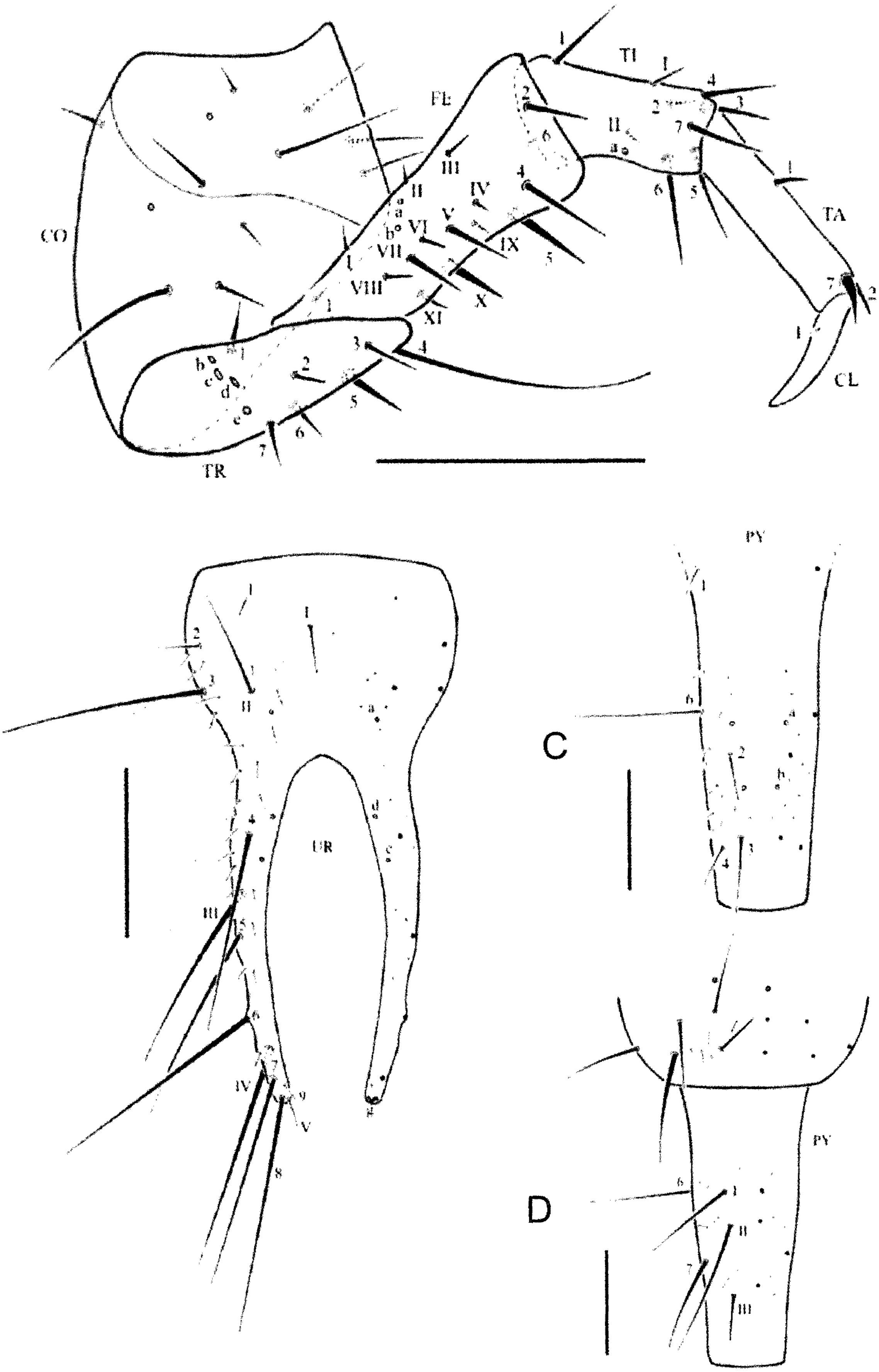

Figure 6. *T. gamae* A) Leg; B) Urogomphi in dorsal view; C) Pygidium in dorsal view; D) Pygidium in ventral view. Scale bar: A) C) and D) 0.2 mm; B) 0.3 mm.

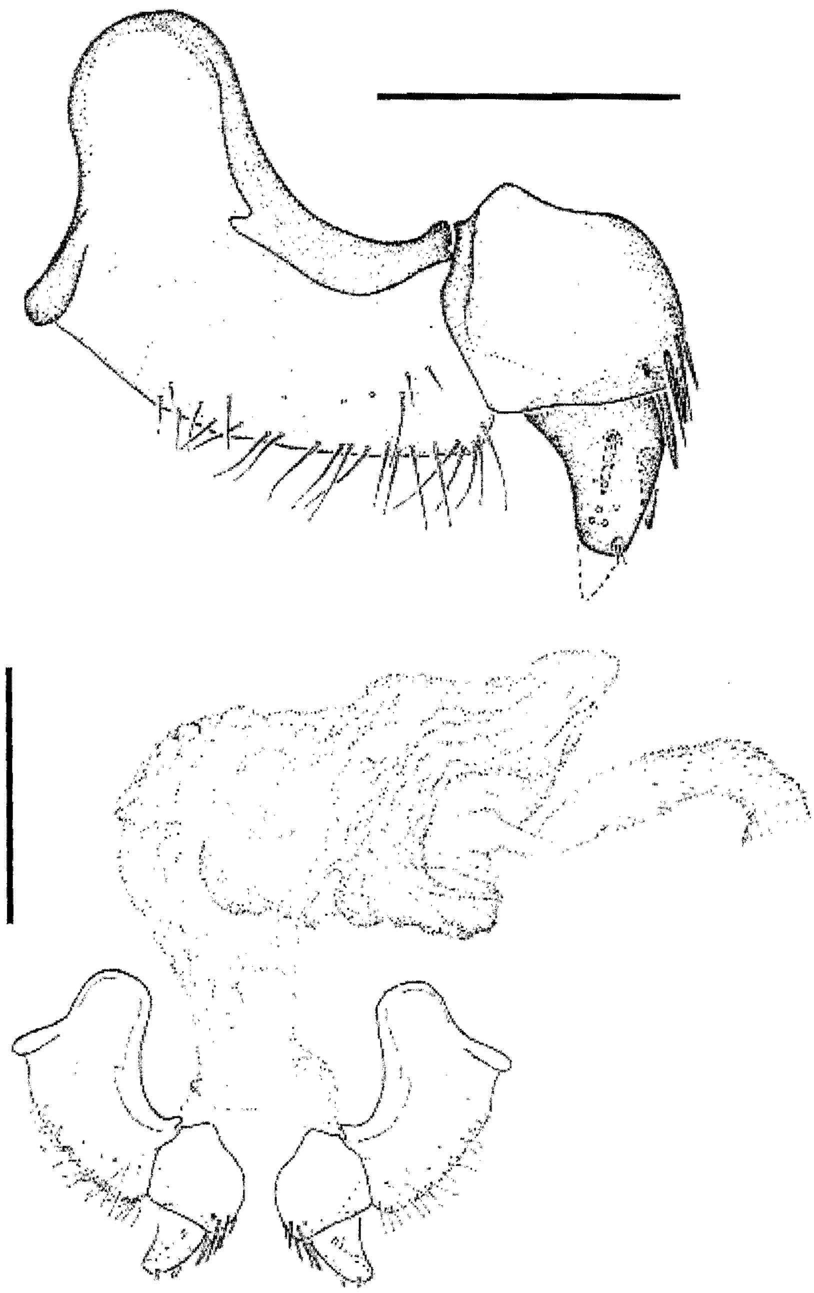

Figure 7. Female genitalia of *T. gamae* A) External genitalia and B) Internal genitalia. Scale bar: A) 0.2 mm and B) 0.4 mm.

talia (figure 4) completely membranous; short and large tubular-shaped vagina-bursa ending in spermatheca sacciform with densely folded walls. The spermatheca is located to the right in ventral view, perpendicular to the sagittal plan. The odd oviduct makes a contact with the spermathecal complex at the invagination on the distal area of the spermatheca; interior densely covered by microfringes.

Ecology

In the centre of Estremenho karstic massif, *T. gamae* was found in five caves and seems to be the only hypogean beetle dwelling in the deep part of these caves. Between the twilight zone and the deep oligotrophic areas of these caves, sometimes *T. gamae* shares its habitat with the troglophile ground beetle *Laemostenus (Pristonychus) terricola* (Herbst 1783), and larvae of both species where found in the same pitfall traps, but they are easily recognized by their different body shape and the presence of one claw (*Trechus*) or two claws (*Laemostenus*) (Grebennikov and Maddison, 2005; Makarow, 1994).

In six months of biological monitoring, preimaginal stages of *T. gamae*, appeared only in deep zones of oligotrophic caves, never associated with large amounts of bat guano, circumstance that is typical of species that show a strict hypogean lifestyle, unlike what happens with the troglophile *L. terricola*.

The larvae of *T. gamae* exhibits the same distribution as the imagos given by Reboleira *et al.* (2009), being only found in deep and apparently oligotrophic parts of the sampled caves, with a humidity of 100% all the year round. This is evidence that the larva has the same troglobiont lifestyle as the imago, reinforcing the hypogean behaviour of the species.

In these parts of the caves, both larva and imagos share habitat with several species of: Gastropoda *Oxychilus draparnaudi* (Beck 1837); Collembola *Onychiurus circulans* Gisin 1952 and *Arrhopalites pygmaeus* Wankel 1980; Diplura *Podocampa* cf. *fragiloides* Silvestri 1932; Coleoptera *L. (Pristonychus) terricola*; unidentified hypogean Isopoda; Chilopoda *Lithobius forficatus* (L. 1758) and *Lithobius* sp.; unidentified Symphyla and the Araneae *Nesticus lusitanicus* Fage 1931, the last species is very abundant in the caves of this massif.

The female dissected for study, despite being in good condition (apparently a young imago), showed very worn apexes in both gonocoxits (figure 7A), reaching the vicinity of the subapical sensory groove. This may suggests that for ovipository purposes this specimen digs in hard and abrasive substrates.

Discussion

T. gamae larvae show the general characteristics of the tribe Trechini. The third instar larvae displays, among other characters, the absence of lacinia in the maxilla, only one claw showing one short seta on the leg and the presence of secondary setae on the frontale part of the cephalic capsule (Bousquet and Goulet, 1984; Grebennikov and Maddison, 2005).

As other larvae of hypogean/endogean carabids, the *T. gamae* lavae exhibit dense pubescence along the body, interpreted by some authors as an adaptation to enable flotation in flooding conditions (Laneyrie, 1974; Carabajal *et al.*, 1999; Casale and Marcia, 2007), but very important for enhancing the mechanoreceptive function.

Small differences are noted compared to larvae of other species of the genus *Trechus*. These differences are mainly on the length/width ratio of the cephalic capsule, the shape of the nasale, slight differences in the development of setae and the lack of stemmata. The larva of *T. gamae* has a remarkable resemblance to the larva of *Trechus alicantinus* Espanol, also included in the "*T. fulvus*-group" (Ortuño and Reboleira, 2010).

The female genitalia of *T. gamae*, and especially the shape of the spermatheca complex, is very similar to *T. fulvus* and other species in its midst, and also the ones defined under the "*T. martinezi*-lineage" (*sensu* Ortuño and Arillo, 2005). The main characters of the female genitalia corroborate the close relation of *T. gamae* with the other species of the "*T. fulvus*-group". However, it can be observed different degrees of development of the sacciform spermatheca in this species group. While in *T. gamae* the spermatheca does not exceeds the length of the whole vagina-bursa (with tubular appearance), in *Trechus torressalai* Ortuno et Arillo (Ortuño and Arillo, 2005) it is hypertrophied, being more developed than in *T. gamae*.

Acknowledgements

We would like to express our gratitude to Fernando Gonçalves and Artur Serrano for the support in the work which allowed the collection of larvae, to the Portuguese caving clubs CEAE-LPN, NEUA, GPS, NEL and AES, for their constant support in the field work and to Parque Natural das Serras de Aire e Candeeiros (PNSAC/ICNB) for all the logistic support. We are grateful to Alberto Sendra (Diplura), Alberto Lopez Pancorbo (Araneae), and Rolanda Matos (Gastropoda) for specimens' identification and to Xavier Riesco for the English revision. All specimens were collected under the permit of Instituto de Conservação da Natureza e da Biodiversidade.

References

ANDERSON L. E., 1954.- Hoyer's solution as a rapid permanent mounting medium for bryophytes.- *The Bryologist*, 57: 242-244.

BELLÉS X., 1987.- *Fauna cavernícola i intersticial de la Península Ibérica i les Illes Balears*.- Conseil Superior d'Investigacions Científiques, Editorial Moll, Mallorca, Spain.

BOUSQUET Y., GOULET H., 1984.- Notation of primary setae and pores on larvae of Carabidae (Coleoptera: Adephaga).- *Canadian Journal of Zoology*, 62 (4): 573-588.

CARABAJAL E., GARCÍA J., RODRÍGUEZ F., 1999.- Descripción de un nuevo género y una nueva especie de Trechini (Coleoptera: Caraboidea: Trechidae) de la cordillera Cantábrica.- *Elytron*, 13: 123-131.

CASALE A., MARCIA P., 2007.- Larval morphology of *Sardaphaenops adelphus* Casale, 2004, a highly specialized troglobiont beetle endemic to Sardinia (Coleoptera, Carabidae).- *Subterranean Biology*, 5: 35-42.

DEUVE T., 1993.- L'abdomen et les genitalia des femelles de Coléoptères Adephaga.- *Mémoires du Muséum National d'Histoire Naturelle*, 155: 1-184.

FREITAG R., BARNES B. L., 1989.- Classification of Brazilian species of *Cicindela* and phylogeny and biogeography of subgenera *Brasiella, Gaymara* new subgenus, Plectographa and South American species of *Cylindera* (Coleoptera: Cicindelidae).- *Quaestiones Entomologicae*, 25: 241-386.

GIACHINO P. M., 1985.- Morfologia larvale di alcuni *Trechus* alticoli delle Alpi Lepontine.- *Bollettino della Società Entomologica Italiana*, 117 (8-10): 161-171.

GIACHINO P. M., 1989.- Contributo alla conoscenza della morfologia larvale del genere *Trechus*. La larva di *Trechus schatzmayri* Focarile, 1949 (Coleoptera, Carabidae).- *Rivista Piemontese di Storia Naturale*, 10: 131-135.

GREBENNIKOV V. V., MADDISON D. R., 2005.- Phylogenetic analysis of Trechitae (Coleoptera: Carabidae) based on larval morphology, with a description of first-instar *Phrypeus* and a key to genera.- *Systematic Entomology*, 30: 38-59.

JEANNEL R., 1920.- Les larves des Trechini (Coleoptera, Carabidae).- *Archives de Zoologie Expérimentale et Générale*, 59 (3): 509-542.

JEANNEL R.,1927.- Monographie des Trechinae. Morphologie comparée et distribution géographique d'un groupe de Coléoptères (II livraison).- *L'Abeille*, 33: 1-592.

JEANNEL R., 1941.- Premières explorations des grottes du Portugal par M. A. de B. Machado. Coléoptères.- *Anais da Faculdade de Ciências do Porto*, 26 (2): 5-15.

LANEYRIE R., 1974.- Sur la systématique des Trechinae (Coleoptera: Trechidae).- *Nouvelle Revue Française d'Entomologie*, 4: 3-21.

LIEBHERR J. K., 1986.- *Cladistic analysis of North American Platynini and revision of the* Agonum extensicolle *species group (Coleoptera: Carabidae)*.- University of California Press, Berkeley, USA.

LIEBHERR J. K., 1991.- Phylogeny and revision of the *Anchomenus* Clade: The Genera *Tetraleucus*, *Anchomenus*, *Sericoda*, and *Elliptoleus* (Coleoptera: Carabidae: Platynini).- *Bulletin of the American Museum of Natural History*, 202: 1-163.

LIEBHERR J. K., WILL K. W., 1998.- Inferring phylogenetic relationships within Carabidae (Insecta, Coleoptera) from characters of the female reproductive tract, pp. 107-170. In: *I. Phylogeny and classification of Caraboidea (Coleoptera: Adephaga), XX international congress of entomology* (BALL G. E., CASALE A., VIGNA TAGLIANTI A., Eds), Florence, Italy, 28 August 1996. Museo Regionale di Scienze Naturali, Torino, Italy.

LUFF M. L., 1993.- The Carabidae (Coleoptera) larvae of Fennoscandia and Denmark.- *Fauna Entomologica Scandinavica*, 27: 1-187.

MAKAROW K. V., 1994.- A key to the genera of the Ground-beetle larvae (Coleoptera, Carabidae) of the Palearctic region.- *Bollettino del Museo Regionale di Scienze Naturali di Torino*, 12 (1): 221-254.

MAKAROV K. V., KOVAL A. G., 2003.- A contribution to the knowledge of the biology of a troglobiont Carabid species, *Jeannelius birsteini* Ljovuschkin, 1963 (Coleoptera, Carabidae, Trechini).- *Entomological Review*, 83 (7): 819-826.

ORTUÑO V. M., 2004.- An enigmatic cave-dwelling ground beetle: *Trechus barratxinai* Español 1971 (Coleoptera, Carabidae, Trechinae, Trechini).- *Revue suisse de Zoologie*, 111 (3): 551-562.

ORTUÑO V.M., ARILLO A., 2005.- Description of a new hypogean species of the genus *Trechus* Clairville, 1806 from eastern Spain and comments on the *Trechus martinezi*-lineage (Coleoptera: Adephaga: Carabidae).- *Journal of Natural History*, 39 (40): 3483-3500.

ORTUÑO V. M., REBOLEIRA A. S. P. S., 2010.- Description of the third instar larva of a hypogean ground beetle, *Trechus alicantinus* (Coleoptera: Carabidae: Trechinae).- *Entomologica Fennica*, 21 (1): 33-42.

ORTUÑO V. M., TORIBIO M., 2005.- Descripción de un nuevo *Trechus* Clairville, 1806 (Coleoptera, Carabidae, Trechini) de los montes cantábricos orientales (Norte de España).- *Graellsia*, 61 (1): 115-121.

ORTUÑO V. M., OUTERELO R., ALONSO J., 1992.- Estudio taxonómico comparativo de las especies ibéricas de *Chlaeniellus* Reitter, 1908 (Coleoptera, Caraboidea, Callistidae).- *Boletín de la Real Sociedad Española de Historia Natural (Sección de Biología)*, 88 (1-4): 147-163.

REBOLEIRA A. S. P. S., GONÇALVES F., SERRANO A., 2009.- Two new species of cave dwelling *Trechus* Clairville, 1806 of the *fulvus*-group (Coleoptera, Carabidae, Trechinae) from Portugal.- *Deutsche Entomologische Zeitschrift*, 56 (1): 101-107.

REBOLEIRA A. S. P. S., ORTUÑO V. M., GONÇALVES F., OROMÍ P., 2010.- A hypogean new species of *Trechus* Clairville, 1806 (Coleoptera, Carabidae) from Portugal and considerations about the *T. fulvus* species group.- *Zootaxa*, 2689: 15-26.

ROIG-JUÑENT S., 2000.- The subtribes and genera of the tribe Broscini (Coleoptera: Carabidae): cladistic analysis, taxonomic treatment, and biogeographical considerations.- *Bulletin of the American Museum of Natural History*, 255: 1-90.

SOLODOVNIKOV A. Y., 2007.- Larval chaetotaxy of Coleoptera (Insecta) as a tool for evolutionary research and systematics: less confusion, more clarity.- *Journal of Zoological Systematics and Evolutionary Research*, 45 (2): 120-127.

Authors' addresses: Ana Sofia P. S. REBOLEIRA (corresponding author, sreboleira@ua.pt), Centro de Estudos do Ambiente e do Mar & Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, 3810 Aveiro, Portugal; Vicente M. ORTUÑO (vicente.ortuno@uah.es), Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain.

Received May 26, 2010. Accepted February 22, 2011.