

Immature stages of *Rhabdotocarabus melancholicus* ssp. *dehesicola* (García-París & París, 1995) (Coleoptera, Carabidae)

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Immature stages of Rhabdotocarabus melancholicus ssp. *dehesicola* (García-París & París, 1995) (Coleoptera, Carabidae).— In this paper, data on the immature stages (first, second and third larval instars and pupa) of *Rhabdotocarabus melancholicus dehesicola* (García-París & París, 1995) are given. Descriptions are based on morphological and biometrical studies made of individuals (four of each instar) obtained from cultures reared in laboratory with imagoes caught in the Arenosillo stream (S Iberian peninsula). Comments about the outstanding differences between the immature stadia of *R. melancholicus* and those of other sympatric *Carabus* (s.l.) species are also included. The following morphological structures were sufficient to identify this species: epistome, supraocellar tubercle, mandibles and *urogomphi* for larvae, or prothoracic pubescence for pupae.

Key words: Immature stages, Coleoptera Carabidae, *Rhabdotocarabus melancholicus dehesicola*.

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Introduction

Studies in past decades on *Carabus* (s.l.) focused almost exclusively on adult beetles, their taxonomy, distribution, biology and ecology. More recently however, a certain number of rigorous studies have been published paying more attention to the larvae and/or their ecology (i.e. HURKA, 1961, 1971; STURANI, 1962; RAYNAUD, 1975-76). This is because the knowledge of more than one life stage provides important information in different ways: several developmental forms (larva, pupa and imago) are different expressions of the same genotype and also, as immature stages can be more or less differentiated or conservative than adults, so some evolutionary relationships could be inferred.

In summary "immature stages evolve in their own way at their own rate, providing an array of characters that complement or supplement those more commonly studied adults" (GOULET, 1979).

Even though the *habitus* of the larva of *Carabus* is fairly uniform, they have some structural characteristics (spines, setae and pore position, microsculpture type and distribution, cephalic sclerites shape or head appendages, *urogomphi* morphology) that determine their variability. The typification of these characters may solve systematic problems and confirms or rejects the present classification of some taxa.

Very few studies related to the pupal morphology have been found. It is easy to presuppose that this lack of data is mostly due to the fact that it is improbable that the pre-imaginal stage will be found in the field as they are subterranean and immobile. Nevertheless, when pupal features are available they have a great interest from a biological point of view since as mentioned in the first paragraph.

In order to provide some information about this subject, research on the immature stages of Andalusian fauna of ground beetles was started several years ago (CÁRDENAS & BACH, 1992; CÁRDENAS, 1993; CÁRDENAS et al., 1994). *R. melancholicus* is the only species of *Rhabdotocarabus* genus and its geographic range includes the Rif mountains and the adjacent areas of northern Morocco, the Iberian peninsula and the French Pyrenees. Moreover, on the basis of the morphological variation between

populations from southern Spain and north-western Africa, GARCÍA-PARÍS & PARÍS (1995) recognized three subspecific taxa of *R. melancholicus*: *R. m. submeridionalis* (Breuning, 1975) distributed throughout south-eastern Spain, *R. m. dehesicola* (García-París & París, 1995) from south-western Spain and southern Portugal and *R. m. melancholicus* (Fabricius, 1798) geographically restricted to north-western Africa.

On this occasion, a detailed description corresponding to the larval instars of *R. m. dehesicola* is given, also including biometrical data of several larval structures and features of the pupa stage. In the literature, several references on the larval stages of *R. melancholicus* can be found (RAYNAUD, 1940; 1975-76), but they are very old and/or incomplete, so more information is necessary to provide a reliable description for this species. On the other hand, larval descriptions of sympatric species (*Hadrocarabus lusitanicus*, Fabricius, 1801; *H. dufouri*, Dejean, 1931; *Macrothorax rugosus*, Fabricius, 1792) are available, so a comparative discussion and specific diagnosis can be made.

Material and methods

Cultures with adults of *R. melancholicus dehesicola* from the Arenosillo stream (S Iberian peninsula, 30SUH7715 UTM coordinates) were carried out in the spring of 1997. Larvae were reared under environmental conditions and the methodology (from the adults' capture until the emergence of the new generation) is fully described in CÁRDENAS & HIDALGO (1995).

Pertinent biometrical data were recorded, and several specimens from each physiological stage were preserved. Larvae were dissected for anatomical study and (according to CASALE et al., 1982) the following structures were removed: epistoma, stemmata, antenna, mandible, maxilla, *labium*, leg, tergite, sternite, pygopod and *urogomphi*.

Biometrical data correspond to four specimens of each larval instar fixed 24 hours after hatching or after moulting. The following morphological data were considered: total and antennal longitudinals, cephalic, prothoracic, mesothoracic and metathoracic width and longitudinals, mean width and lon-

gitude of first, second, eight and ninth abdominal tergites and mean width and longitude of first, second, eighth and ninth abdominal sternites.

Results

General description of the larvae

Campodeiform larva, oligopode, black with greenish metallic brightness dorsally and shiny testacean-brown ventrally.

Head

Subquadrate in the first instar, becoming transverse in the next stages. *Labrum* and cervical groove are not distinct. Epistome typical of a quadricuspid larva (DEUVE, 1994), with a variable number of pores, often bearing setae, in a fairly fixed position in the consecutive stages (figs. 1-3). The *nasalia* teeth exceed the level of the lateral lobes of the anterior margin of the epistome. Hypodon dorsally evident in all the larval instars. Antennals sclerites forming at their hind extremity a strong and longitudinal supraocellar tubercle each (fig. 4). Ocellar protuberances, slightly prominent, with six *stemmata* and three setae each: one small seta between the first pair of *ocelli*, another anterior, stronger and larger over a setigerous pore, and the last one, also umbilicated and of an intermediate size, between the *ocelli* of the third pair of *stemmata* (figs. 1-3). Epicranial sclerites ventrally jointed throughout the gular seam, but the gula is indistinguishable. Pedicel constituted by four antennomeres: the first cylindrical and with several dorsal pores; the second with a setigerous pore; the third with three slender setae and a not very evident hyalin vesicle at the end; the fourth short, thin and with three apical and slender setae.

Strong and stout mandibles, wide at their base, strongly curved, with an inner blade, a sharp and curved *retinaculum*, a basal protuberance and a tuft of rigid setae -penicillus-well defined. Marginal edge of the mandible with a seta, at the same level as the *retinaculum*. A sensilla can also be seen at the base of the mandible.

Maxillae showing a reduced *cardo* and a voluminous, subcylindrical and pubescent stipes. Palp with three segments, decreasing

in its diameter towards the end and finishing in a single cupule. It is articulated with a short and fusiform palpifer which occasionally bears a seta on its inferior and external margin. Galea formed by two slender, cylindrical segments; the proximal with a ventral seta and the distal narrower and fusiform. Reduced lacinia consisting of a short tubercle on the inner angle of the stipes and bearing a distal tuft of ridge setae.

Labium composed of a *prementum* with two long setae dorsally inserted over a small tubercle and of a pair of two-segmented palps: the proximal segment is short, stout, glabrous and enlarged at the end, and the distal segment is longer and divided into two truncated cupules reaching the same level.

Thorax

The tergites are flattened and divided in symmetrical halves by a median line; *scutum*, *prescutum* and *postcutum* differentiated. Prothorax longer than the other thoracic segments, subtrapezoidal, laterally margined, with the hind-angles truncated. Mesothorax and metathorax shorter and a little wider than prothorax and laterally margined. On each half of the thoracic tergites an anterior series of "3,2,2" and another posterior one of "3,3,3" setae are present. In the pleural region of the mesothorax, at the ventrolateral position, the first pair of spiracles can be noted.

All three pairs of legs are very similar, stout, strong and heavily sclerotized, increasing in size from the first to the third pair. Coxa with some longitudinal rows of rigid setae and trochanter bearing two external series of spiny setae. Size and arrangement of setae and spines on the rest of the articles is somewhat variable depending on the larval stage; the tendency is an increase in the number and hardness of hairs and spines in the consecutive instars. Size relationships between the leg articles are also variable for each larval phase. Between the two claws of the pretarsus there are other dorsally inserted smaller claws and a pair of setae on the ventral side.

Abdomen

Composed of 10 segments. Tergites with a highly noticeable median line and differentiated in *prescutum*, *scutum* and *postscutum*, firstly narrower than the metatergite and

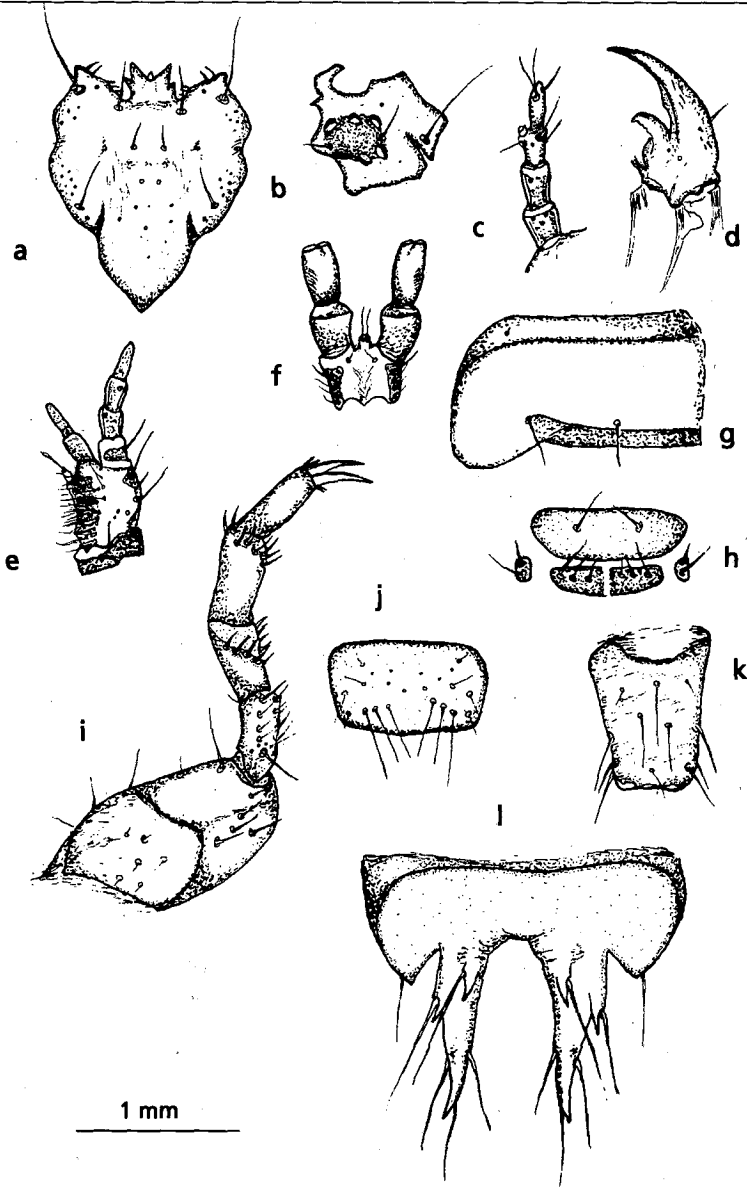


Fig. 1. First-instar larval structures of *R. melancholicus*: a. Epistome; b. Stemmata; c. Left antenna, dorsal view; d. Right mandible, dorsal view; e. Right maxilla, dorsal view; f. Labium, dorsal view; g. Fifth abdominal hemitergite; h. Fourth abdominal sternite; i. Right leg in third pair, dorsal view; j. Last abdominal sternite; k. Pygopod, dorsal view; l. Urogomphi, dorsal view.

Estructuras correspondientes al primer estadio larvario de R. melancholicus: a. Epistoma; b. Área ocelar; c. Antena izquierda, vista dorsal; d. Mandíbula derecha, vista dorsal; e. Maxila derecha, vista dorsal; f. Labio, vista dorsal; g. Quinto hemiterguito abdominal; h. Cuarto esternito abdominal; i. Pata derecha del tercer par, vista dorsal; j. Último esternito abdominal; k. Urópodo, vista dorsal; l. Urogonfos, vista dorsal.

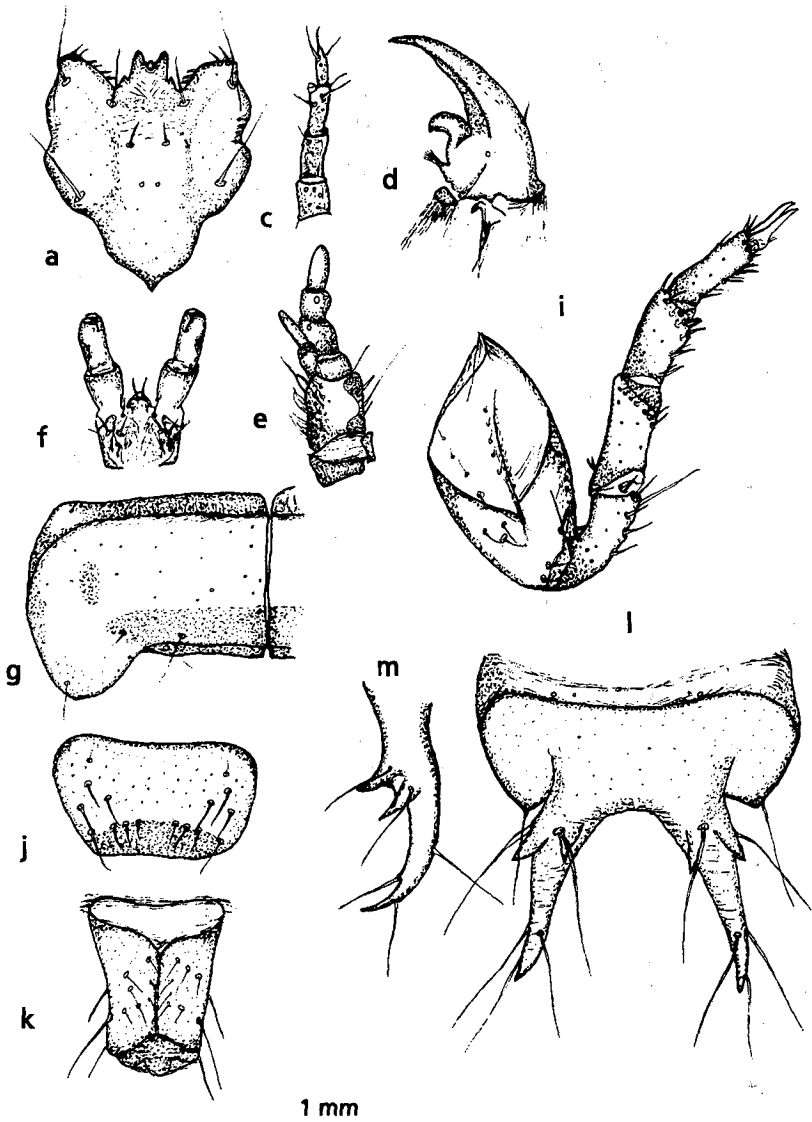
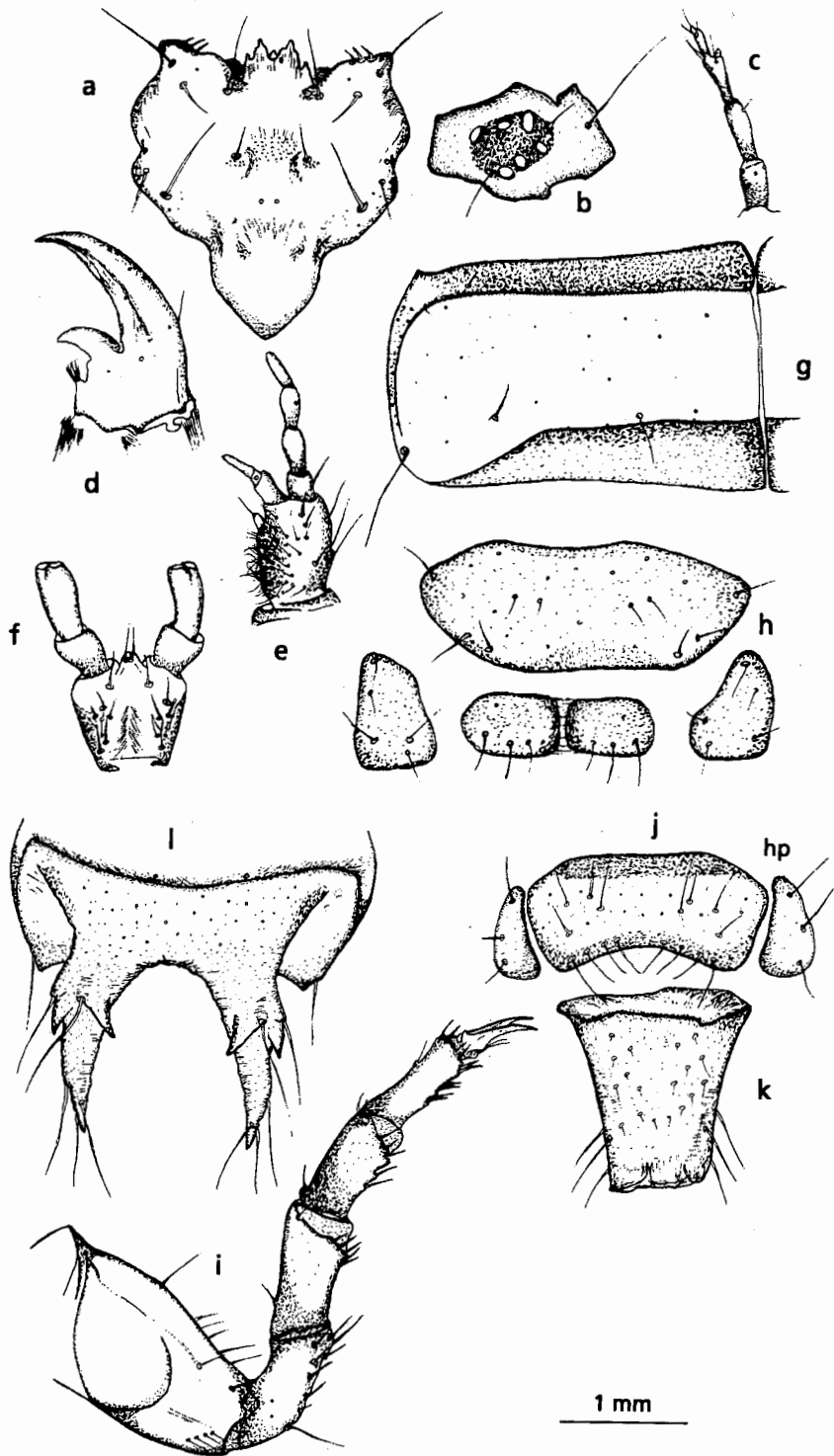


Fig. 2. Second-instar larval structures of *R. melancholicus*: a. Epistome; c. Left antenna, dorsal view; d. Right mandible, dorsal view; e. Right maxilla, dorsal view; f. *Labium*, dorsal view; g. seventh abdominal hemitergite; i. Right leg in third pair, dorsal view; j. Last abdominal sternite; k. Pygopod, ventral view; l. *Urogomphi*, dorsal view; m. *Urogomphi*, lateral view.

Estructuras correspondientes al segundo estadio larvario de R. melancholicus: a. *Epistoma*; c. *Antena izquierda, vista dorsal*; d. *Mandíbula derecha, vista dorsal*; e. *Maxila derecha, vista dorsal*; f. *Labio, vista dorsal*; g. *Séptimo hemiterguito abdominal*; i. *Pata derecha del tercer par, vista dorsal*; j. *Último esternito abdominal*; k. *Urópodo, vista ventral*; l. *Urogonfos, vista dorsal*; m. *Urogonfos, vista lateral*.



progressively enlarged towards the middle of the abdomen, becoming narrower and convex at the end. Discal region rather flattened, dispersely and finely punctuated, slightly striated, lacking in granulation and with, at least, two posterior setae on each hemitergite. The ninth segment is reduced, dorsally punctuated and granulated, taking the *urogomphi*. These are rather long, subparallel, curved upwards, and each one has two subequal, spiniform and well developed tubercles on its base, each with a rigid macrosetae. Last segment also laterally margined and with a seta on the posterior angles. At the end of each *urogomphus* there are three setae, two ventrally and, between them, one dorsal seta (figs. 1-3).

Pleural region of each segment showing two epipleurites, two hypopleurites and a pair of spiracles opened on the epipleura (from tergite I to VIII).

Sternites, longitudinally undivided, composed at first of five *sternella* (a *sternum*, two internal *sternella* and two external *sternella*) whose chaetotaxy and morphology vary depending on the larval stage. Internal *sternella* not merged before the fifth sternite, in the eighth segment all the sternal sclerites are fused forming a single plate and the ninth sternite and the respective hypopleurites are joined. The pygopod is conical, very robust and with setae whose size and location vary according to the larval instar.

These features correspond to the "*quadricuspides aequalispinulées*" larves Group A of RAYNAUD (1975-76).

In the next sections, the morphological

characteristics associated with each larval phase and their respective biometrical data (table 1) are given.

First larval instar (L-I, fig. 1)

The following structures can be emphasized: Epistoma more elongated and subtriangular than in posterior instars, central teeth of the nasal more divergent and with a pore. Ecdysial cleavage line very short. *Ruptor ovi* situated in the *pars aboralis frontalis* and consisting of a broad keel, anteriorly pointed, situated on each side. *Retinaculum* with some auxiliary basal teeth. Dorsal surface of the epistoma with four pairs of conspicuous symmetrically-situated macrosetae and a pair of central pores. Chaetotaxy of legs as follows: coxa with several lined rigid setae but without a clear crown of stout spines, trochanter with two lateral rows of spines, femur and tibia only with a crown of spines at the ends, and a glabrous tarsus. Only the ninth segment has a seta on the margin of the posterior lobes.

Second larval instar (L-II, fig. 2)

The following features can be mentioned: Epistome more transverse and subpentagonal than in L-I, with tendency to increase its sculpture. Leg chaetotaxy similar to L-I excepting the tibia and the tarsus, which bear an apical crown of rigid setae and two longitudinal rows of spines. All the abdominal tergites have a seta on the margin of the posterior lobes.

Third larval instar (L-III, fig. 3)

The following structures are highlighted: *Clypeus* more transverse and engraved than

Fig. 3. Third-instar larval structures of *R. melancholicus*: a. Epistome; b. *Stemmata*; c. Right antenna, dorsal view; d. Right mandible, dorsal view; e. Right maxilla, dorsal view; f. *Labium*, dorsal view; g. Second abdominal hemitergite; h. Third abdominal sternite; i. Right leg in third pair, dorsal view; j. Last abdominal sternite, hp. Hypopleurite; k. Pygopod, ventral view; l. *Urogomphi*, dorsal view.

Estructuras correspondientes al tercer estadio larvario de R. melancholicus: a. *Epistoma*; b. *Área ocelar*; c. *Antena derecha, vista dorsal*; d. *Mandíbula derecha, vista dorsal*; e. *Maxila derecha, vista dorsal*; f. *Labio, vista dorsal*; g. *Segundo hemiterguito abdominal*; h. *Tercer esternito abdominal*; i. *Pata derecha del tercer par, vista dorsal*; j. *Ultimo esternito abdominal, hp. Hipopleurito*; k. *Urópodo, vista ventral*; l. *Urogonfos, vista dorsal*.

in L-II and with six pairs of setae instead of the four pairs characteristic of the previous stadia. Location of setae and spines for the rest of the anatomical structures similar to L-II but with tendency to increase in size.

Pupa

Showing simultaneously archaic characters typical of larvae (maintenance of stemmata, or conformation of the expansions of the abdominal tergites) and adult features (dilata-tions of the male podothecas). Nevertheless, there are characters exclusive of the pupa: chaetotaxy of the abdominal tergites, morphology of the labrum (from trilobated to unilobated), mandibles always of archaic type (calosomian) or parastigmas related to the spiracles (CASALE et al., 1982).

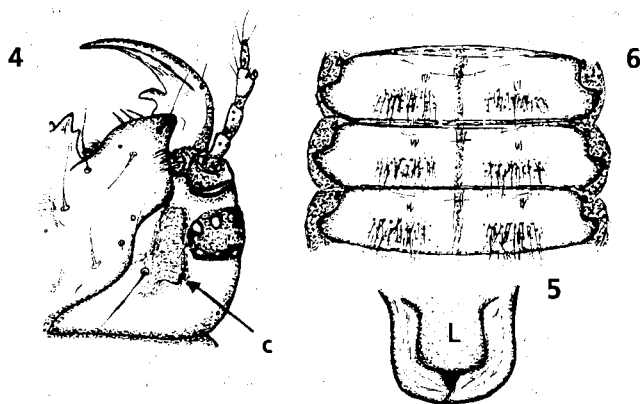
At the beginning, the pupa of *R. melancholicus* has a thin and slightly pigmented cuticle. It shows a unilobated labrum (fig. 5), not functional mandibles (adecticous pupa), appendages freely projected from the body (exarate pupa), mobile abdomen and mesothoracic and metathoracic pterothecas always laterally arranged.

The protergum is glabrous, lacking hairs

in the lateral margins, and the pleural region of the thoracic segments is also glabrous.

Bilobated tergal expansions are present in abdominal segments II-VI, with the anterior lobe reduced with respect to the posterior, which is more salient, sclerotized and slightly directed upwards. The lobes become progressively more evident towards the end of the body. Spiracles located in the pleural region of segments I-VII, under the anterior lobe of each tergal expansion, when the expansion exists. Parastigmas are not conspicuous. Chaetotaxy of the tergal plates consisting of a series of long, sparse, brown-testaceous hairs inserted in their respective setigerous pores. These bands of long hairs, are located at each side of the median line, in the posterior half of abdominal segments I to V and VIII (fig. 6); segments VI and VII are glabrous. The pleural region of the abdominal segments lack hairs and salient lobes. The ninth segment, also glabrous, bears a pair of short, but clearly noticeable *urogomphi*, each one with a small spiniform protuberance at the base.

Morphometrical mean data for the larval instars are given in table 1.



Figs. 4-6. 4. Cephalic sketch of larva of *R. melancholicus*, dorsal view: c. Supraocellar tubercle. 5. Sketch of the Labrum (L) of pupa of *R. melancholicus*. 6. Sketch of the abdominal tergal plates of pupa of *R. melancholicus*.

4. Dibujo esquemático de la cápsula cefálica de la larva de *R. melancholicus*, vista dorsal: c. Tubérculo supraocular. 5. Dibujo esquemático del labro (L) de la pupa de *R. melancholicus*. 6. Dibujo esquemático de las placas tergales abdominales de la pupa de *R. melancholicus*.

Table 1. Morphometrical mean data (mm) and standard deviations for each larval instar of *R. melancholicus* (N= 4).

Datos morfomètrics mitjans expressats en mm y sus correspondientes desviaciones para cada estadio larvario de R. melancholicus (N= 4).

	Larva I		Larva II		Larva III	
	\bar{x}	σ	\bar{x}	σ	\bar{x}	σ
Total length	10.12	0.88	14.87	0.99	22.17	1.69
Cephalic length	1.50	0.36	1.67	0.13	2.65	0.24
Cephalic with	1.95	0.13	2.22	0.10	3.22	0.36
Antennal length	0.82	0.15	1.07	0.10	1.72	0.15
Prothorax length	1.75	0.33	2.22	0.22	3.30	0.27
Prothorax max. width	2.35	0.13	3.10	0.08	4.62	0.29
Mesothorax length	1.17	0.17	1.45	0.13	2.55	0.13
Mesothorax max. width	2.52	0.09	3.45	0.21	4.96	0.34
Metathorax length	0.97	0.21	1.45	0.17	2.30	0.14
Metathorax max. width	2.52	0.19	3.35	0.21	5.10	0.29
Mean length of urotergites	0.69	0.09	1.07	0.03	1.52	0.16
Mean width of urotergites	2.25	0.07	2.95	0.16	4.20	0.27
Mean length of urosternites	0.50	0.05	0.78	0.03	1.16	0.13
Mean width of urosternites	2.14	0.23	2.69	0.16	3.97	0.85

Discussion

As the larva has been described, no significant changes in the morphology occur when the structures belonging to each larval instar of *Rhabdotocarabus melancholicus dehesicola* are compared. Therefore, the general description is sufficient to diagnose larvae of this species, independently of their stage. On the basis of the biometrical data, it could be deduced that the size increase during the first intermolt time is lower than the size increase from second to third stages, expressed in both absolute and relative terms.

A comparative diagnosis between *Rhabdotocarabus melancholicus* and other sympatric *Carabus* species in the area (*Macrothorax rugosus*, *Hadrocarabus lusitanicus* and *H. dufouri*) can be given.

With respect to the larva, a high number of distinctive characters are observed if larvae

belonging to the four species considered are examined jointly. The larval *habitus* of each species is sufficiently indicative in order to differentiate them. In a detailed comparison several clear features allow the identification of the different taxa: the larva of *Rostrilabre* type (*sensu* LAPOUGE, 1905-08) characterizes the *Macrothorax* genus; while the larva of *Quadricuspide* type characterizes genera *Hadrocarabus* (supraocular tubercle normally conformed, mandibles without a protuberance under the retinacle and external spine of urogomphi underdeveloped) and *Rhabdotocarabus* (supraocular tubercle being a conspicuous carina, mandibles with a protuberance under the retinacle, external spine of urogomphi well-developed).

Finally, the experience in rearing the *Carabus* (s.l.) permits the assumption that between the pupae of the different species studied there are sufficient differences to

assign them to their respective species. The prothoracic pubescence is sufficient to separate the *Rhabdotocarabus* pupa (prothorax glabre) from those other *Carabus* living in the area, *Macrothorax* and *Hadrocarabus* genera (prothorax with pubescence, disperse on the disc and forming a dense band of hairs at each side).

Resumen

Estadios inmaduros de Rhabdotocarabus melancholicus ssp. dehesicola (García-París & París, 1995) (Coleoptera, Carabidae)

Se describen los estadios inmaduros de *Rhabdotocarabus melancholicus dehesicola* (García-París & París, 1995), que se obtuvieron mediante cultivos de laboratorio con imagos procedentes del arroyo Arenosillo (tributario del Guadalquivir por su margen derecha). Los datos morfológicos y biométricos aportados (figs. 1-6, tabla 1) corresponden a cuatro individuos de cada fase del desarrollo preimaginal, y permiten caracterizar las formas inmaduras de esta especie y diferenciarlas inequívocamente de las de otros *Carabus* (s.l.) con los que cohabita en el sur de la península ibérica. La morfología del epistoma, de los tubérculos supraoculares, de las mandíbulas o de los urogonfos, en el caso de las larvas, o la pubescencia protorácica en el de las pupas han resultado claros rasgos diagnósticos.

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