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The larval stages of the variegated mud-loving beetle *Heterocerus similis* Grouvelle (Coleoptera: Heteroceridae)

Los estados larvales del escarabajo variegado amante del barro *Heterocerus similis* Grouvelle (Coleoptera: Heteroceridae)

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Resumen: Se describen e ilustran en detalle las larvas del último estadio del escarabajo ripario *Heterocerus similis* Grouvelle y se incluyen notas comparativas con los estadios anteriores. Sobre la base del ancho de la cápsula cefálica y del protórax se lograron identificar cuatro estadios larvales; considerando que los heterocéridos presentan cinco estadios larvales, los estadios identificados probablemente correspondan a los últimos cuatro. El largo de las larvas no permite distinguir entre estadios. La larva de *H. similis* se compara con la larva de *H. freudei* (Pacheco), la única otra larva de heterocérido sudamericana descrita.

Palabras clave: Argentina, Escarabajos riparios, Estados preimaginales, Morfología, Taxonomía.

Abstract: Last instar larvae of the variegated mud-loving beetle *Heterocerus similis* Grouvelle are described and illustrated in detail. Comparative notes with earlier instars are also included. Based on the width of the head capsule and the prothorax four different instars were identified; considering that heterocerids show five larval instars, the four instars identified probably correspond to the last four. Larval length was not useful to recognize among different larval stages. The larva of *H. similis* is compared to that of *H. freudei* (Pacheco), the only other described South American heterocerid larva.

Keywords: Morphology, Preimaginal stages, Riparian beetles, Taxonomy.

Introduction

Heteroceridae is a small family of riparian beetles with a worldwide distribution (except Antarctica). It includes small to medium sized Coleoptera commonly known as variegated mud-loving beetles. Both adults and larvae construct and inhabit tunnels in wet sand or mud at the margins of lotic and lentic habitats (Vanin *et al.*, 2016).

The family is subdivided in two subfamilies (Sazhnev, 2022), the monotypic Elythomerinae and Heterocerinae, which includes 366 species. Heterocerinae contains five genera: *Augyles* Schiødte, *Micilus* Mulsant & Rey, *Haraia* García & Jiménez-Ramos, *Heterocerus* Fabricius, and *Tropicus* Pacheco. In Argentina 22 species have been reported, 13 belonging to *Heterocerus* and nine to *Tropicus* (Skalický, 2021; Torres *et al.*, in press).

Heterocerus similis Grouvelle, 1896, originally described from Chile, is one of the most widely distributed species in Argentina, and probably

South America, ranging from Patagonia to Venezuela (Grouvelle, 1896; Trémouilles *et al.*, 1995; Trémouilles 1998, 2002; Skalický, 2021). Knowledge of preimaginal stages of heterocerids is very scarce in South America, only the larva and pupa of the Brazilian species *H. freudei* (Pacheco, 1973) have been described (Vanin *et al.*, 1995, as *Efflagitatus freudei*); for Argentina no larvae have been described so far (Torres *et al.*, in press). In this paper the last larval instar of *H. similis* is described and illustrated in detail, comparative notes with earlier instars are also presented; additionally, the larva of *H. similis* is compared to that of *H. freudei*.

Material and methods

Source of material. The specimens were collected in Chubut province, Percy river, north of Trevelin, 9.i.2004, 43° 04' 09" S, 71° 28' 22" W. Adults and larvae were collected by washing the shore where tunnels were observed. Both adults and larvae floated on the surface and were hand-picked with soft aluminum forceps and stored in 80% ethyl alcohol. The material was identified by Edgardo Trémouilles from the MACN (Museo Argentino de Ciencias Naturales). The material studied is kept in the larval collection of the author, additional material (adults and larvae) will be deposited in the Coleoptera collection of the LIESA (CIEMEP) and larval collection of the Laboratory of Entomology, Buenos Aires University, Argentina.

Morphology and Morphometry. Larval specimens were cleared in warm lactic acid, dissected, and mounted on glass slides with Hoyer's medium. Observations (up to 1000×), photographs and drawings were done with a Leica S6D dissecting microscope and Leica DMLB compound microscope both with camera lucida and a photographic camera attached. Ten larvae of different sizes were dissected for the morphological descriptions. Additionally, measurements of 27 specimens were taken with a Zeiss Stemi DV4 dissecting scope with micrometer. Measurements were used to compare larvae of different sizes in order to separate different instars. Measured structures were adjusted as parallel as possible to the plane of the objective. The following measurements were taken: head width (HW), pronotal width (PW) and total length (TL). Measurements of the head appendages, useful in instar identification of beetle larvae in other families (e.g. antennomere or maxillary palpomere lengths in Hydrophilidae or Dytiscidae) are not useful since they are very short.

Results

Description of the last instar larva of Heterocerus similis Grouvelle

Larva (Fig. 1a). Campodeiform, prognathous, subcylindrical in cross section, strongly pubescent; thorax the widest tagma. Measurements,

length (TL) 4.9-7.4 mm, head width (HW) 0.77-0.88 mm; pronotal width (PW) 0.96-1.19 mm.

Head capsule (Figs. 1b). Head capsule wider than long, broader at level of stemmata, slightly flattened dorsoventrally, densely covered by setae, those on frons shorter than those on parietale; dorsal and lateral surface bearing minute cuticular projections on distal two thirds. Frontal lines V-shaped, extending from base of head to base of antennae; coronal line absent. Five stemmata on each side of head, two closer to frontal line, two on lateral margin, one ventral; lenses well developed. Clypeus membranous, bare.

Labrum (Figs. 1b, 2c, 3a). Free, large, subrectangular, slightly wider than long, densely setose, lateral setae longer than those on disk; anterior corners rounded, bearing two stout setae slightly curved mesally. Anterior margin poorly sclerotized, dorsally with six short stout setae and one or two pairs of pores; ventrally with a pair of short sensoria and two pairs of pores. Epipharynx bearing two brushes of long microtrichiae oriented mesally. Tormae longer than labrum, longitudinally oriented.

Antenna (Fig. 3b). Minute, apparently three-segmented, basal antennomere the widest, closely attached to head capsule. Second antennomere with two small pores, one dorsal and one ventral, and bearing a stout subconical sensorium, larger than third antennomere. Third antennomere bearing a pair of short sensoria and distally projected as a sharp spine. Membrane between second and third antennomeres bearing a long seta basal to third antennomere, a short seta basal to subconical sensorium and three subtriangular sensoria behind third antennomere on outer margin.

Mandibles (Figs. 1b, 2a-b, 3c-d). Symmetrical, short and stout, bidentate apically, dorsal tooth longer than ventral. Inner margin with a small notch, behind it two rows of stout teeth, three dorsal and three ventral arranged as follows: dorsal row with distal tooth short, blunt, pointing forward, middle tooth larger, triangular, pointing mesally, basal tooth the largest, pointing posteriorly; ventral row with distal tooth long, pointing anteriorly, next two teeth similar in form and shape as dorsal ones. Basal four teeth form a molar area, with several short projections between dorsal and ventral rows; a long, stout, sharp seta pointing posteriorly originates at base of dorsal basal tooth.

Ventral mouthparts (Figs. 2d, 4a-b). Retracted within head capsule, forming a maxillo-labial complex. Maxilla with rather large, basal, subtriangular bare cardo; stipes twice as long as wide, densely setose ventrally, lateral setae longer; lacinia fixed, bearing several stout apical setae, distal ones stouter and apically branched; galea free, subcylindrical, as long as palpus, bearing six stout apical setae on distal and inner margins, a large distal bifid sensorium, and two small ventral pores closer to outer margin; palpus three-segmented, strongly bent outwards, basal palpomere the broadest, bare, with one small pore on inner margin, second palpomere the shortest, bearing one short seta on inner margin and one pore on outer margin, distal palpomere the longest, bearing several short apical sensoria and one long blunt basal sensorium

originating at membrane connecting with second palpomere. Labium elongate, mentum as long as stipes, densely pubescent, with two large pores basally; prementum shorter than mentum, with several setae on disk and a pair of distal stouter setae between base of palpi; ligula short, triangular in shape; palpi two-segmented, short and stout, basal palpomere shorter, bare, distal palpomere slightly longer, bearing several short apical sensoria; ligula as a short triangular plate; hypopharynx large, as two lateral lobes formed by dense blunt setae.

Thorax (Figs. 1a, 5a-b). Well developed, the widest tagma; prothorax wider than meso- and metathorax; prothorax wider at midlength, meso- and metathorax wider at basal third; dorsal plates with sagittal line and covered by dense pubescence. Legs well developed, five-segmented; prothoracic leg more robust and setose, fossorial; meso- and metathoracic legs slender, similar in size. Coxa large, subtriangular; trochanter short, closely attached to femur; femur the stouter segment, covered by numerous setae, wider apically; tibia shorter than femur, narrowing distally, bearing a few stout spiniform setae on distal half, stouter in prolegs; pretarsus slightly shorter than tibia, sharply pointed, with strong ventral spiniform seta, more basally placed in prolegs than in meso- and metathoracic legs.

Abdomen (Figs. 1a, 1c). Ten-segmented, narrowing towards posterior end, terga strongly sclerotized, lacking sagittal lines. Tergum of segment one with a short lateral notch on anterior third which bears a small sclerotized lateral lobe; segments two to eight similar in shape, wider than long; segment nine rounded apically, lacking urogomphi; segment ten short, ventrally oriented, forming a short conical pygopod. All segments with distal half bearing long setae intercalated with shorter ones.

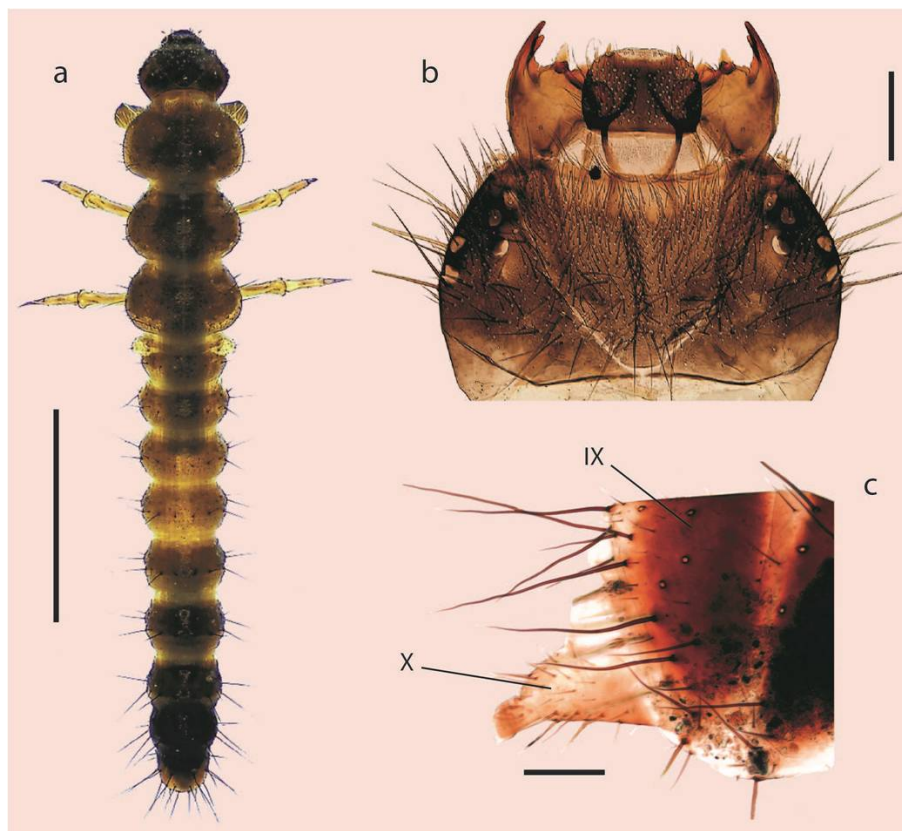


Fig. 1.

Heterocerus similis, last instar larva.

a) habitus, dorsal view; b) head capsule, dorsal view; c) abdominal segments nine and ten, lateral view. Scale bars: a = 2 mm; b = 0.1 mm; c = 0.2 mm.

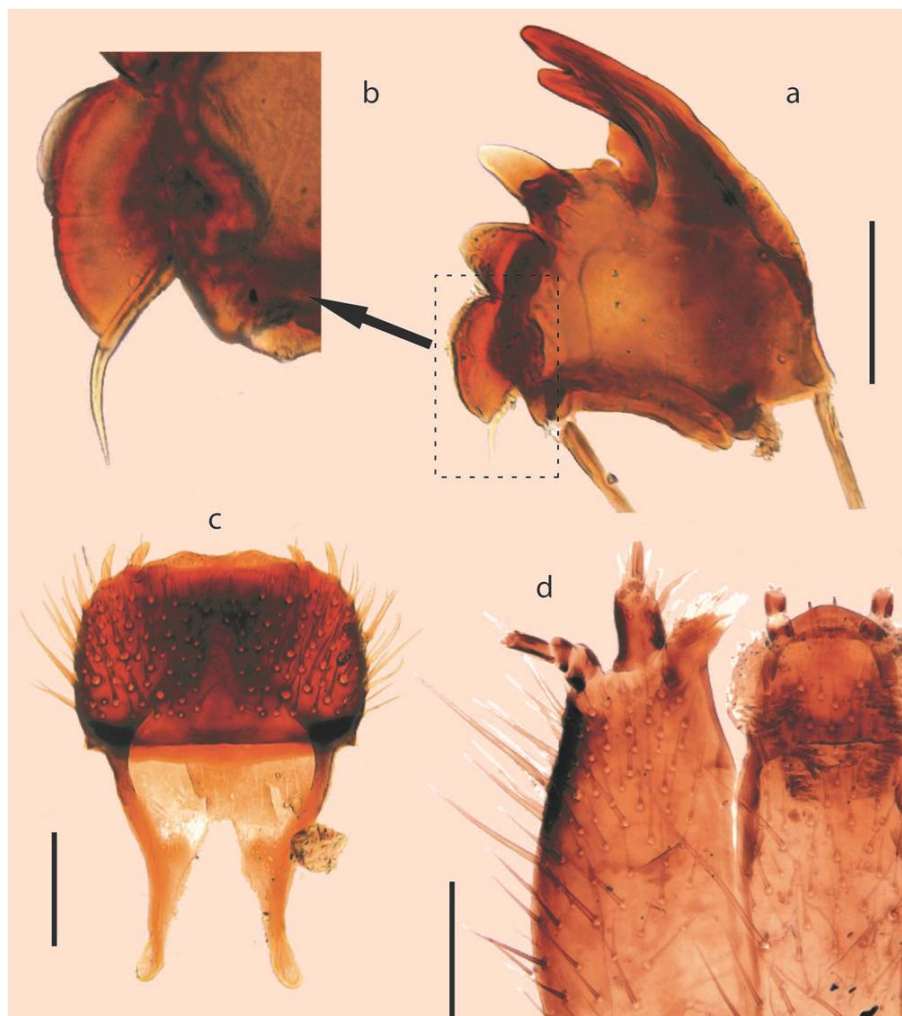


Fig. 2.

Heterocerus similis, last instar larva.

a) right mandible, dorsal view; b) detail of mandible; c) labrum, dorsal view; d) maxilla-labial complex, ventral view. Scale bars: 0.1 mm.

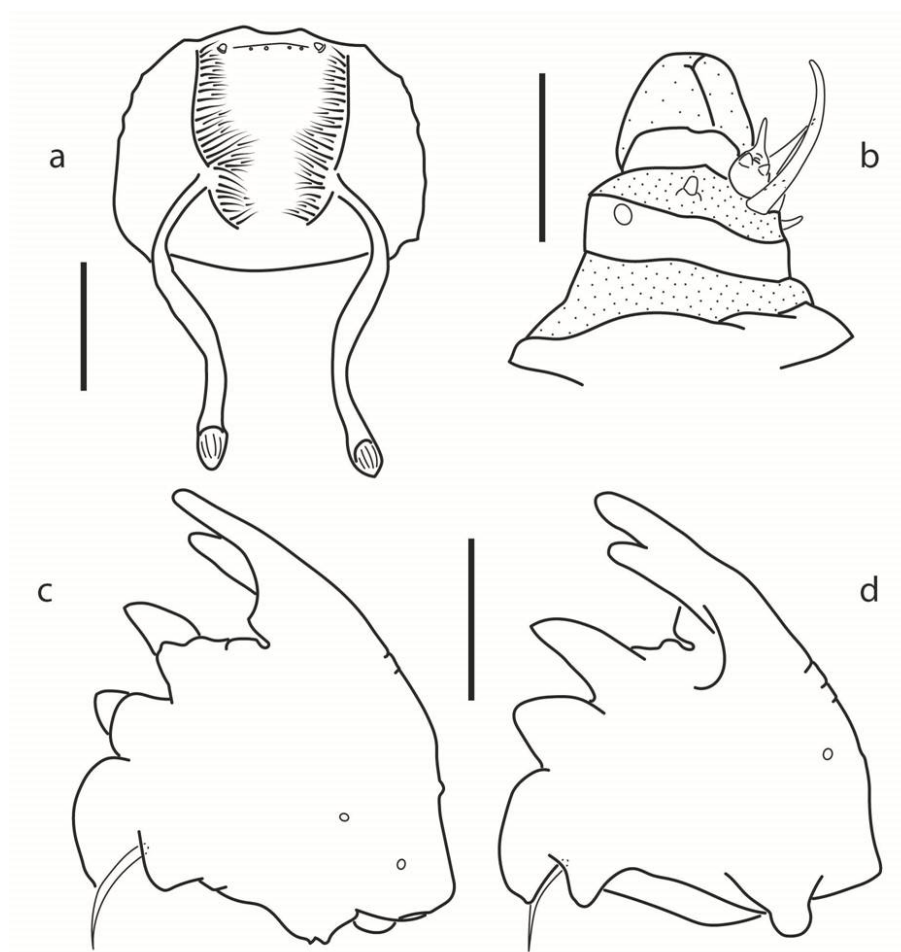


Fig. 3.

Heterocerus similis, last instar larva.

a) labrum, ventral view; b) right antenna, dorsal view; c) right mandible, dorsal view; d) right mandible, ventral view. Scale bars: a, c-d = 0.1 mm; b = 0.02 mm.

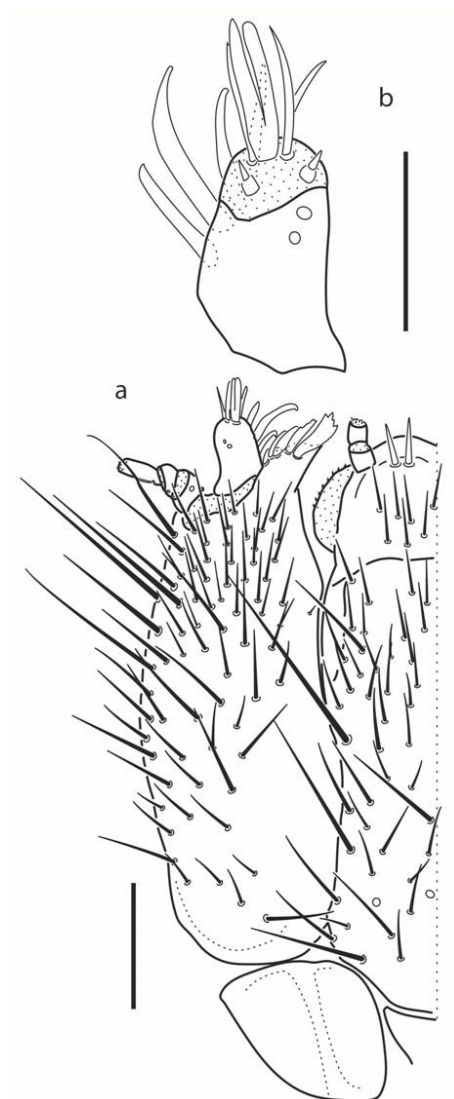


Fig. 4.

Heterocerus similis, last instar larva

a) maxillo-labial complex, ventral view; b) detail of right galea, ventral view. Scale bars: a = 0.1 mm; b = 0.05 mm.

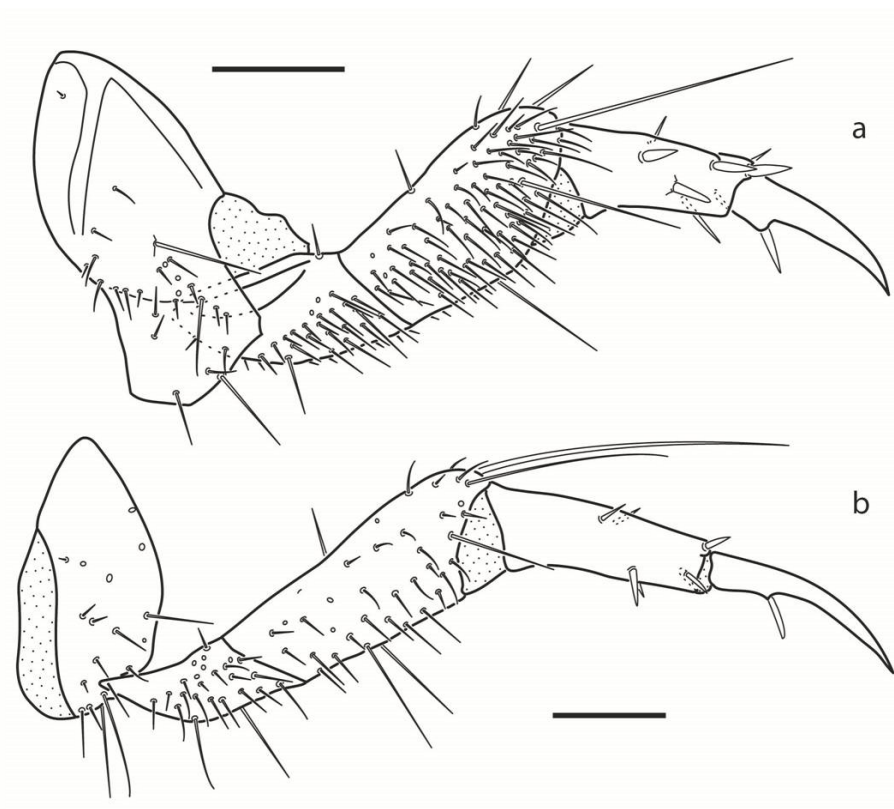


Fig. 5.

Heterocerus similis, last instar larva.

a) prothoracic leg; b) mesothoracic leg. Scale bars: 0.2 mm.

Earlier instars. Besides the morphometric measurements (Table I), no strong differences between mature larvae and earlier instars can be mentioned, they are all very similar in shape and morphology. Smaller larvae (putative second instar, see discussion) are less sclerotized, the head capsule is alike in shape and the distribution of stemmata is similar, both the frons and parietals are less setose and the minute cuticular projections are sparser and restricted to the basal half; labrum and maxillo-labial complex also less setose; the thoracic segments are also less setose; legs also similar; the abdominal segments have setae restricted to the posterior margin. Remaining instars similar, becoming more strongly sclerotized and more setose.

Measure	Instar 2 (n = 3)	Instar 3 (n = 4)	Instar 4 (n = 11)	Instar 5 (n = 9)
HW	0.33-0.39	0.47-0.49	0.58-0.63	0.77-0.88
PW	0.38-0.52	0.59-0.62	0.73-0.81	0.96-1.19
TL	2.1-2.8	2.8-3.4	3.5-4.9	4.9-7.4

Table I.

Measurements (in mm) for probable last four larval stages of *H. similis*.

Abbreviations: HW (head width); PW (pronotal width); TL (total length).

Discussion

Morphometry and instar determination. Two of the measurements, HW and PW, yielded similar results, clearly differentiating four larval sizes without overlap (Table I). On the other hand, TL showed some overlap and does not seem a good metric to distinguish among instars (Table I). These results are in accordance with studies on instar determination for other insects (Chimney et al., 2007; Frątczak & Matuszewski, 2014; Jakubed et al., 2019; Mahlerová et al., 2021).

Since Heteroceridae is reported to have five larval instars (Gwynn Silvey, 1935; Kaufman & Stansly, 1979), it was considered possible that either the first or last larval instar is missing. The other species for which data on head capsule width have been published is the Nearctic *Augyles auromicans* (Kiesenwetter, 1851) (Gwynn-Silvey, 1935). Adults of this species are, in average, larger (3.5-5.5 mm) than those of *H. similis* (3.5-4.5 mm). Gwynn-Silvey (1935) reports of HW for *A. auromicans* are: 0.32-0.34 mm (L1), 0.41-0.45 mm (L2), 0.53-0.58 mm (L3), 0.66-0.74 mm (L4) and 1.0-1.5 mm (L5). Considering the difference in size between the adults of both species, it should be expected that larvae of *H. similis* must be smaller than those of *A. auromicans*, therefore the measurements of *H. similis* (Table I) most likely correspond to the last four developmental instars. Additionally, adults of *Heterocerus freudei* are 3.4-4.2 mm long and the mature larva of this species has been reported to be 7.0 mm long (Vanin et al., 1995) which is also in accordance with the size of *H. similis* larvae and adults (4.9-7.4 mm and 3.5-4.5 mm respectively). Finally, since first instar larvae are smaller, they tend to be more difficult to collect, therefore it is not surprising that they could have been overlooked considering the collecting technique (hand-picking).

Comparative notes. Very few descriptions of heterocerid larvae are available in the literature. Böving & Craighead (1931) illustrated with rather good detail the larva of *H. pallidus* Say, 1823 (as *H. ventralis* Melsheimer), nonetheless there is no formal description of this larva in the text. Pierre (1946) described and sketched the larva of *H. aragonicus* Kiesenwetter, 1851. Peterson (1957), briefly described and illustrated some characters of an unidentified *Heterocerus* larva. Lawrence (1991) provided a diagnosis for heterocerid larvae and illustrated a few characters of the larva of *H. gemmatus* Horn, 1890 (as *Lanternarius gemmatus*). Finally, Vanin et al. (1995) published a description of the larva of *H. freudei* (Pacheco) (as *Efflagitatus freudei*); this is the most detailed description of a variegated mud-loving beetle larva, therefore the comparative notes presented below are mostly between the larvae of *H. similis* and *H. freudei*, and for some characters also with *H. pallidus*.

Mature larvae of both species are of a similar size, nonetheless some characters from the head appendages are useful to differentiate between both species. The labrum in *H. similis* has two stout, short and blunt setae on each anterolateral corner (apparently absent in *H. freudei*); additionally, the distal membranous portion of the labrum is small in *H. similis*, leaving an almost straight anterior margin (the membranous

portion is large in *H. freudei*, producing a large convex anterior margin). The mandibles are quite different, *H. freudei* has the inner teeth much sharper and curved, there are also a pair of sharp projections above the distal teeth (absent in *H. similis* and *H. pallidus*), in the position of these projections *H. similis* shows a small notch (absent in *H. freudei*, present in *H. pallidus*). The lacinia in *H. similis* is stouter, distally ending in a strong spine and bearing a pair of stout branched setae distally (lacinia rounded in *H. freudei*, and lacking branched setae; *H. pallidus* has a lacinia similar to that of *H. similis*). The galea in *H. freudei* is broad, apparently not articulated (narrower and articulated in *H. similis*) and bears many strong curved setae (*H. similis* has at most six setae and a bifid sensory appendage). The maxillary palp is proportionally longer in *H. similis* and *H. pallidus*, as long as or longer than the galea (slightly shorter in *H. freudei*).

The gut contents of the larvae consisted of partially digested substrate, rich in small sand particles and diatoms; probably small algae, zooplankton and organic material mixed with the substrate are also part of their diet. This makes larvae of *H. similis* non-selective detritivores (Trémouilles, 1998; Sazhnev, 2018).

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