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First larval description and chaetotaxic analysis of the neotropical whirligig beetle genus *Enhydrus* Laporte (Coleoptera, Gyrinidae)

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\textbf{A B S T R A C T}

The larva of the whirligig beetle *Enhydrus sulcatus* (Wiedemann, 1821) is described and illustrated for the first time, including detailed morphometric and chaetotaxic analyses of the cephalic capsule, head appendages and legs. Larvae of *Enhydrus* Laporte, 1834 exhibit the characters traditionally recognized as autapomorphies of the family Gyrinidae: well developed cardo, completely divided prementum, presence of lateral abdominal tracheal gills, and presence of four terminal hooks on the pygopod. The egg bursters located on the parietal, the presence of an additional sensorial plate on the third antennomere, and a well developed lacina may also represent autapomorphies of the family. *Enhydrus* larvae share with those of the other known Dineutini genera the presence of numerous minute additional setae on the mandible, the presence of additional setae on the cardo, the submedial position of the coxal seta CO12, the absence of the trochanteral seta TR2, and the presence of numerous pore-like additional structures on the ultimate maxillary and labial palpmers. On the other hand, *Enhydrus* can be distinguished from the other known dinute genera by the presence of pore-like additional structures on the basal maxillary and labial palpmers, the presence of ventral spinulae on the pygopod, and the bare tracheal gills, among other characters.

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\textbf{Introduction}

*Enhydrus* Laporte, 1834 is a small gyrinid genus composed of four species (Miller and Bergsten, 2012). It is included in the tribe Dineutini together with the extant genera *Andogyrus* Ochs, 1924, *Dineutus* MacLeay, 1825, *Macrogyrus* Régimbart, 1882 and *Porrorhynchus* Laporte, 1835, and several extinct genera (Gustafson and Miller, 2013). Members of this genus are relatively large (adult length about 20 mm) and occur mainly in South America, with two species (*E. sulcatus* (Wiedemann, 1821) and *E. tibialis* Régimbart, 1877) in Brazil, one species (*E. mirandus* Ochs, 1955) in Venezuela, and the remaining species (*E. atratus* Régimbart, 1877) in Ecuador and Colombia but reaching Panamá and Costa Rica in Central America. The known southern limit of the genus is a record of *E. sulcatus* in the state of Rio Grande do Sul, southern Brazil (Maňko, 1993). The adult members of *Enhydrus* were revised by Brinck (1978).

*Enhydrus sulcatus*, the type species, inhabits streams that run through hills of preserved gallery forest, and within this habitat the adults can be found, either isolated or in aggregations, in the streams or in small pools formed by the intricate geography of the stream borders, always in shaded places with some current (Maňko, 1997; Alvarenga et al., 2011). This species has been subject of detailed studies on the adult external and internal morphology (Maňko, 1993), morphometry and sexual dimorphism (Alvarenga et al., 2011), pygidial glands (Barth, 1960), and bionomy and habitat (Maňko, 1997). However, the study of the larvae has remained a gap in our knowledge, with this stage being unknown for the genus.

A system of nomenclature for the primary sensilla of larvae of the family Gyrinidae is presently under development (Archangelsky and Michat, 2007; Michat et al., 2010; Michat and Gustafson, 2016). Although incomplete and subject to improvement based on the discovery of more gyrinid larvae, this system provides a descriptive template to which larvae of more genera can be incorporated. In this contribution we study the first-instar larva of *E. sulcatus* to provide, for the first time, a detailed description of the larval morphology and primary chaetotaxy of the genus *Enhydrus*. We also compare the morphological and chaetotactic...
characters of this genus with those of other gyrinid genera for which the larvae have been described in detail, and discuss remarkable or interesting findings.

**Material and methods**

**Source of material**

The description provided in this paper is based on five instar I specimens obtained from eggs laid by adults collected in June 2009 at the following locality: Brazil, Minas Gerais State, Ingaí, 21° 20'47"S 44° 59'27"W. Females were kept alive in plastic water tanks and fed with small insects. The oviposition occurred two days after confinement, and the eggs hatched 28 days after oviposition. The larvae could not be successfully reared in the laboratory due to their unknown requirements.

**Methods**

The larvae were cleared in lactic acid, dissected, and mounted on glass slides in polyvinyl-lacto-glycerol. Microscopic examination at magnifications up to 1000× and drawings were made using an Olympus CX31 compound microscope equipped with a camera lucida. Drawings were scanned and digitally inked using a Genius PenSketch tablet. The material is held in the collections of Y. Alarie (Department of Biology, Laurentian University, Sudbury, Ontario, Canada) and M.C. Michat (Laboratory of Entomology, Buenos Aires University, Argentina).

**Morphometric analysis**

We employed the terms used in previous papers dealing with the larval morphology of Gyrinidae (Archangelsky and Michat, 2007; Michat et al., 2010). The following measurements were taken (with abbreviations shown in parentheses): Total length (excluding terminal tracheal gills) (TL); maximum width (excluding tracheal gills) (MW); head length (HL) (total head length including the frontoclypeus, measured medially along the epicranial stem); maximum head width (HW); length of frontoclypeus (from anterior margin to the joint of frontal and coronal sutures) (FRL); occipital foramen width (maximum width measured along dorsal margin of occipital foramen) (OCW); coronal suture length (COL); length of mandible (MNL) (measured from laterobasal angle to apex); width of mandible (MNW) (maximum width measured at base); length of maxillary palpalifer (PPF); length of galea (GA). Length of antenna (A), maxillary (MP) and labial (LP) palpi were derived by adding the lengths of the individual segments; each segment is denoted by the corresponding letter(s) followed by a number (e.g., A1, first antennomere). The maxillary palpus was considered as being composed of three segments united to the stipes through a palpalifer (Archangelsky and Michat, 2007). Length of leg, including the longest claw (CL), was derived by adding the lengths of the individual segments; each leg is denoted by the letter L followed by a number (e.g., L1, prothoracic leg); the length of trochanter includes only the proximal portion, considered from the base to the beginning of the femur; the leg was considered as being composed of six segments (Lawrence, 1991). Length of terminal hooks of abdominal segment X, separated in medial hook (MH) and lateral hook (LH). These measurements were used to calculate several ratios that characterize body shape.

**Chaetotaxic analysis**

Primary setae and pores were distinguished on the cephalic capsule, head appendages and legs. Sensilla were coded by two capital letters, in most cases corresponding to the first two letters of the name of the structure on which they are located, and a number (setae) or a lower case letter (pores). The following abbreviations were used: AN, antenna; CO, coxa; FE, femur; FR, frontoclypeus; LA, labium; MN, mandible; MX, maxilla; PA, parietal; PT, pretarsus; TA, tarsus; TI, tibia; TR, trochanter. Setae and pores present in the first-instar larva of *E. sulcatus* were labeled by comparison with previous papers dealing with the primary chaetotaxy of members of the family Gyrinidae (Archangelsky and Michat, 2007; Michat et al., 2010). Homologies were recognized using the criterion of similarity of position (Wiley, 1981). Setae located at the apices of the maxillary and labial palpi were extremely difficult to distinguish due to their position and small size. Accordingly, they are not well represented in the drawings.

**Results**

**Description of the first-instar larva of Enhydrus sulcatus**

(Wiedemann, 1821)

**Diagnosis**

Larvae of *Enhydrus* can be distinguished from those of other known gyrinid genera by the following combination of characters: cephalic capsule constricted at level of occipital region (Figs. 2, 4 and 5); occipital suture present (Figs. 2 and 4); coronal suture short; medial lobe of FR with four very inconspicuous teeth (Figs. 2 and 4); lacinia not dentate on posterior margin, indented apically (Figs. 9 and 10); claws lacking basoventral spinulae (Figs. 13 and 14); tracheal gills lacking spinulae (Fig. 3); terminal hooks subequal in length (Figs. 3 and 15); seta FR3 short, hair-like (Fig. 4); seta PA6 short, hair-like (Fig. 4); MN with additional setae (Fig. 8); cardo with some very short additional setae (Fig. 10); pore MXg proximal on MP2 (Fig. 10); pore MXj medial on MP3 (Fig. 9); MP1, MP2 and LP1 with minute pore-like additional structures (Figs. 9–12); pore LAc medial on LP2 (Fig. 11); seta CO12 medial (Fig. 14); additional setae on CO present (Figs. 13 and 14); seta TR2 absent (Fig. 13); seta TR5 short (Fig. 14); abdominal segment X with ventral spinulae (Fig. 15).

**Description**

Color (Figs. 1–3). Cephalic capsule and mandibles brown, antennae, maxillae and labium testaceous to light brown; thoracic sclerites light brown, rest of thorax and legs testaceous; abdomen testaceous except terminal hooks brown.

Body (Fig. 1). Elongate, parallel sided, head and pronotum strongly sclerotized, rest of thorax and abdomen soft. Measurements and ratios that characterize the body shape are shown in Table 1.

Head (Fig. 2). Cephalic capsule (Figs. 4 and 5). Subrectangular, longer than broad, parallel-sided with distinct narrow neck; occipital foramen slightly emarginate both dorsally and ventrally; occipital suture present; coronal suture short; frontal sutures U-shaped, extending to anterior bases; posterior tentorial pits visible ventromedially; area near occipital suture with reticulation; FR elongate, anterior margin with three weakly delimited lobes; medial lobe slightly produced anteriorly, with four very inconspicuous teeth; lateral lobes weakly developed, not projected beyond medial lobe; PA with egg bursters formed by a single cuneiform spine on each posterolateral surface, and six stern mata at each side, four dorsal and two ventral (not shown in Figs. 4 and 5 because they could not be recognized after the clearing process). Antenna (Figs. 6 and 7). Moderately long, slender, shorter than HW, composed of four antennomeres: A1 shortest, A2, A3 and A4 longest, subequal in length; A3 with two minute structures (probably spinulae) on ventrodorsal surface, and two subapical flat plates on inner margin, distal one interpreted as the sensorium (A3∗) which does
not protrude; A4 with a subapical flat sensorial plate on inner margin, accompanied by two minute structures (probably spinulae or pores). Mandible (Fig. 8). Relatively elongate, curved, broad basally, distal half projected inward, apex sharp; inner margin more or less toothed on distal third; retinaculum absent, although one of the teeth may be interpreted as that structure; mandibular channel present. Maxilla (Figs. 9 and 10). Well developed, prominent; cardo strongly developed, subquadrate, bearing a group of minute spinulae on dorsal surface; stipes short, broad, subtrapezoidal, bearing a lacinia and GA on distal inner margin and a PPF on distal outer margin; lacinia well developed, slender, indented apically; GA elongate, two-segmented, basal segment shorter, globose, distal segment longer, narrow, apically pointed; PPF short, palpomere-like, projected apicodorsally in a subtriangular process; MP long, composed of three palpomeres separated by oblique joints; MP1 and MP2 shortest, subequal in length, MP3 longest. Labium (Figs. 11 and 12). Well developed, prominent; prementum divided longitudinally into two subcylindrical halves fused basally, each half bearing minute spinulae on dorsal surface and projected apicodorsally in a subtriangular process; LP long, composed of two palpomeres separated by oblique joints; LP1 slightly longer than LP2.

Thorax (Fig. 1). Long, narrow, subcylindrical; pronotum longest and broadest, meso- and metanotum subequal; protergite well developed, covering almost whole segment dorsally, anterior and posterior margins truncate, lateral margins rounded; membrane between pronotum and mesonotum with a single narrow transverse sclerite; both sclerites with sagittal line, lacking anterior transverse carina; meso- and metaterga lacking sclerites;
ventral surface membranous except for a single large subrectangular sclerite covering anterior half of prothorax, and small sclerites on the regions of articulation of coxae; spiracles absent. Legs (Figs. 13 and 14). Long, slender, composed of six segments; L3 longest, L1 shortest; CO elongate, robust, TR short, lacking anulus, FE, TI and TA slender, subcylindrical, PT with two long, slender, slightly curved claws, posterior claw shorter than anterior claw; spinulae absent.

Abdomen (Fig. 1). Ten-segmented, long, narrow, subcylindrical, entirely membranous; segments I–VIII similar in shape and size (segment VIII somewhat smaller), bearing a tracheal gill on posterolateral angle; segment IX visibly narrower, bearing two tracheal gills on posterolateral angle; tracheal gills slender, almost devoid of spinulae (exception: 2–3 at tip), those of segment IX longer than the others; segment X (Figs. 3, 15) smallest and narrowest, pygopod-like, arising on posterodorsal surface of segment IX, not carrying gills, bearing ventral spinulae and four strongly sclerotized terminal hooks which are subequal in length (Figs. 3, 15–17).

Chaetotaxy. Frontoclypeus (Fig. 4). Medial lobe of anterior margin with two spine-like setae (FR10, FR11), one short hair-like seta (FR5), and one pore (FRd); lateral lobe of anterior margin with two minute spine-like setae (FR6, FR9), two short hair-like setae (FR7, FR8), and one pore (FRe); lateral margin with two short hair-like setae (FR1, FR2) and two pores (FRa, FRc) on distal third and one short hair-like seta (FR3) on basal third; central portion with one short hair-like seta (FR4); surface with numerous short hair-like additional setae. Parietal (Figs. 4 and 5). Dorsal surface with one short hair-like seta (PA10) posterior to antennal base, a longitudinal row of four short hair-like setae (PA5, PA7, PA8, PA9) close to frontoclypeal margin, two short hair-like setae (PA4, PA6) on basal third, close to egg bursters, and three short spine-like setae (PA1, PA2, PA3) and two pores (PAa, PAb) on neck region; ventral surface with three short hair-like setae (PA17, PA18, PA19) and one pore (PAo) on anteromedial region, four short hair-like setae (PA11, PA12, PA13, PA14) and four pores (PAf, PaG, PaH, PAi) on anterolateral angle, one short hair-like seta (PA16) and one pore (PAk) at mid length, and one long hair-like seta (PA15) and one pore (PAm) on basal third; dorsal and ventral surface with numerous short hair-like additional setae (except on neck region). Antenna (Figs. 6 and 7). A1 with three pores (A1a, Anb, Anc) on dorsal surface and two pores (A1d, A1e) on ventral surface; A2 with one minute pore (A1i) on ventromedial region; A3 with one pore (A1f) on dorsomedial region, one short hair-like seta (A1g) on dorsodistal portion and one short hair-like seta (A1h) on ventrodistal portion; A4 with one pore (A1g) on dorsodistal portion and two minute spine-like setae (A1n, A1s) at apex. Mandible (Fig. 8). Dorsal surface with one pore (MNa) on basal fourth, two pores (Mnb, Mnc) at mid length, one short hair-like seta (MN1) on distal third, and two short hair-like setae (MN2 and one additional seta) near tip; dorsal surface with numerous minute additional setae. Maxilla (Figs. 9 and 10). Cardo with one short hair-like seta (MX1) and some very short additional setae on ventral surface; stipes with one short hair-like seta (MX3) on dorsoexternal margin, one short hair-like seta (MX2) and two pores (MXa, MXb) on ventroexternal margin, one very short seta (MX4) ventrally near base of lacinia, and two short curved spine-like setae (MX5, MX6) dorsally at base of lacinia; proximal segment of GA with one short hair-like seta (MX7) on anteroventral margin and one short hair-like additional seta on dorsal margin; distal segment of GA with one short hair-like seta (MX8) on dorsoproximal surface, two pores (MXd, MXh) on ventroproximal surface, and one short hair-like seta (MX9) and one minute additional setae near apex; PPF with one short hair-like seta (MX10) on ventral margin; MP1 with one pore (MXe) and one minute seta (MX13) on ventroproximal portion, and one pore (MXf) on dorsodistal portion; MP2 with one pore (MXg) on ventroproximal portion and two short hair-like setae (MX11, MX12) on dorsodistal and ventrodistal portions respectively; MP3 with one pore (MXj) on dorsoexternal margin and one short hair-like seta (MX14) near apex; palpomeres with several minute pore-like additional structures both on dorsal and ventral surface. Labium (Figs. 11 and 12). Prementum with three short hair-like setae (LA3, LA4, LA5) and one pore (Laa) on dorsodistal surface, one short hair-like seta (LA2) on ventrodistal surface, and one minute seta (LA1) on ventroproximal surface; LP1 with one minute seta (LAb) on ventroproximal portion and one pore (LAb) on ventrointernal margin; LP2 with one pore (LAc) on dorsomedial portion and one short hair-like seta (LA12) near apex; palpomeres with several minute pore-like additional structures both on dorsal and ventral surface. Thorax. Surface of thoracic terga with several
hair-like setae. Legs (Figs. 13 and 14). Anterior surface of CO with six very short spine-like setae (CO1, CO2, CO3, CO4, CO5, CO17) and one very short hair-like seta (CO18) on proximal portion, one long hair-like seta (CO6), one short hair-like seta (CO7) and one pore (COa) on medial portion, and three short hair-like setae (CO8, CO9, CO10) on distal portion; posterior surface of CO with four very short spine-like setae (CO13, CO14, CO15, CO16) on proximal portion, one short hair-like seta (CO12) on medial portion, and one short spine-like seta (CO11) and one pore (COD) on distal portion; dorsal surface of CO with several short hair-like additional setae that obscure the recognition of setae CO7 and CO18, which are therefore tentatively assigned; anterior surface of TR with one short hair-like seta (TR1) on dorsal margin, one long (TR4) and one short (TR3) hair-like setae on ventrodistal margin, five pores (TRA, TRb, TRc, TRd, TRE) on central portion, and one additional pore on ventral margin; posterior surface of TR with one short hair-like seta (TR7) on ventral margin, and two short hair-like setae (TR5, TR6) and two pores (TRf, TRg) on distal margin; anterior surface of FE with one short spine-like seta (FE1) and one pore (FEB) on proximal portion, and two short spine-like setae (FE2, FE3) on distal portion; posterior surface of FE with two short spine-like setae (FE4, FE5) and one short hair-like seta (FE6) on distal portion; anterior surface of TI with one short hair-like seta (TI1) on proximal portion and three short spine-like setae (TI2, TI3, TI4) on distal portion; posterior surface of TI with one pore (TIa) on central portion, and two short spine-like setae (TI5, TI6) and one long hair-like seta (TI7) on distal portion; anterior surface of TA with three short spine-like setae (TA2, TA3, TA4), one minute seta (TA7), and three pores (TAa, TAc, TAd) on distal
portions; posterior surface of TA with three short spine-like setae (TA1, TA5, TA6) and three pores (TAa, TAc, TAf) on distal portion; anterior surface of PT with one long spine-like seta (PT1) on basoventral portion; posterior surface of PT with one short spine-like seta (PT2) on basoventral portion. Abdomen. Segments I–IX with some short hair-like setae on dorsal and ventral surfaces; tracheal gills with one short spine-like seta near base, few short hair-like setae along their length, and one long hair-like seta at tip; segment X (Fig. 15) with two short spine-like setae, two long spine-like setae, and four pores on ventral surface; terminal hooks (Figs. 16 and 17) with two pores at distal third of ventral margin.

Discussion

The so far unknown larva of the genus Enhydrus is documented in this contribution which represents an additional step in the study of the larval morphology of the Gyrinidae. However, as the larvae of several gyrinid genera are still unknown or remain insufficiently documented, in particular some key genera such as Spanglerogyrus Folkerts, 1979 and Heterogyrus Legros, 1953, the phylogenetic value of some of the characters mentioned below should be taken with caution.

The larvae of Enhydrus bear egg bursters on the parietal, one additional sensorial plate on the third antennomere, a well-developed cardo and lacinia, a completely divided prementum, lateral abdominal tracheal gills, and four terminal hooks on the pygopod. These characters were considered as potential autapomorphies of the Gyrinidae by previous authors (Beutel and Roughley, 1994, 2005; Archangelsky and Michat, 2007; Michat and Gustafson, 2016). In particular, the presence of well developed, spiniform egg bursters on the posterolateral surface of the parietal in Enhydrus larvae reinforces the hypothesis that this structure is extended within the family.

The Dineutini are probably the tribe with better known larval morphology, due to detailed descriptions (including primary chaetotaxy) of the genera Andogyrus (Arndt et al., 1993; Archangelsky and Michat, 2007) and Macrogyrus (Michat and Gustafson, 2016). The larvae of Dineutus were also partially examined for chaetotaxy, although the chaetotaxy pattern of this genus was not formally described (Archangelsky and Michat, 2007).
Considering the description of *Enhydrus* provided herein, only the larvae of the genus *Porrorhynchus* remain unknown. The first instar of *Enhydrus* shares with those of the other known Dineutini genera the presence of numerous very short to minute additional setae on the dorsal and external surfaces of the mandible (Fig. 8), the presence of additional setae on the cardo (Fig. 10), the presence of numerous minute pore-like additional structures on the ultimate maxillary and labial palpomeres (Figs. 9–12), the submedian position of the primary seta CO12 on the coxa (Fig. 14), and the absence of the primary seta TR2 on the trochanter (Fig. 13). Beutel and Roughley (1994) mentioned an extremely long and slender cariso as a possible autapomorphy of Dineutini. According to Michat and Gustafson (2016), however, this character is not so clear in the first instar, although it is more evident in the third instar. As only first instars of *Enhydrus* were available for study, the state of this character remains to be tested when later instars are discovered.

Larvae of *Enhydrus* can be distinguished from those of the other known genera of Dineutini by the following combination of characters: neck constriction present (=*Andogyrus* and *Dineutus*, absent in *Macrogyrus*) (Figs. 2, 4 and 5); posterior margin of lacinia not dentate (=*Dineutus*, dentate in *Andogyrus* and *Macrogyrus*) (Figs. 9 and 10); first and second maxillary palpomeres and first labial palpomere bearing minute pore-like additional structures (absent in *Andogyrus, Dineutus* and *Macrogyrus*) (Figs. 9–12); labial pore LAc submedian (=*Andogyrus* and *Dineutus*, distal in *Macrogyrus*) (Fig. 11); additional setae on coxa present (=*Andogyrus*, absent in *Dineutus* and *Macrogyrus*) (Figs. 13 and 14); tracheal gills not plumose (plumose in *Andogyrus, Dineutus* and *Macrogyrus*); abdominal segment X with ventral spinulae (absent in *Andogyrus, Dineutus* and *Macrogyrus*) (Fig. 15). Although the presence of additional setae on the cardo is common to all known dineutine genera, *Enhydrus* has several of these setae whereas the other three genera have only one seta (accidentally overlooked in Archangelsky and Michat, 2007). On the other hand, the larvae of all known dineutine genera bear pore-like additional structures on their palpi, in *Enhydrus* these structures are present in all maxillary and labial palpomeres, whereas in the other three genera they are restricted to the ultimate palpomeres (Michat and Gustafson, 2016).

**Conflicts of interest**

The authors declare no conflicts of interest.

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**References**


