Phylogenetic Analysis of Hydroporinae (Coleoptera: Dytiscidae) Based on Larval Morphology, with Description of First Instar of *Laccornellus lugubris*

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ABSTRACT

The first instar of *Laccornellus lugubris* (Aubé) is described. A parsimony analysis of selected taxa of the subfamily Hydroporinae based on 48 informative larval characters was conducted using the program PAUP®. The 100 most parsimonious trees support a placement of *Laccornellus* Roughley & Wolfe outside the tribe Hydoropini as part of a clade made up of *Hydrovatus* Motschulsky + *Canthyporus* Zimmermann, which is supported by the absence of the primary seta UR8 and of the primary seta LA2, and the spine-like condition of the primary seta AB10. Larvae of *Laccornellus* evolved one unique character state within the Hydroporinae, which is the presence of the primary seta AB15 on ventral surface of last abdominal segment.

KEY WORDS

Coleoptera, Dytiscidae, *Laccornellus*, larvae, phylogeny

The Dytiscid subfamily Hydroporinae (Coleoptera) is a large (=2,000 species), heterogeneous grouping of minute- to moderate-sized beetles (1.00–6.00 mm in length). Whereas the group is difficult to characterize on the basis of adult features, larvae are readily recognized by the shape of the frontoclypeus, which is produced as a triangular lobe above the mandibles (=nasale) (Larson et al. 2000).

The subfamily Hydroporinae is composed of nine tribes worldwide (Nilsson 2001) (Laccornini, Methlini, Bidessini, Hydrovatinia, Hyphrydrini, Vatellini, Hydroporini, Hygrotonini, and Carabhydrini). Whereas this subfamily is likely a monophyletic group (Miller 2001), evidence is still equivocal in regard to the phylogenetic relationships of the hydroporine lineages, particularly at the most basal levels. Wolfe (1985, 1988) who studied the phylogeny of ancestral members of the Hydroporinae based on adult characters postulated the following: 1) Laccornini is the sister group of the remainder of the subfamily; 2) Methlini and *Hydrovatus* Motschulsky (Hydrovatinia) are closely related phylogenetically; and 3) the clade made up of Methlini, *Hydrovatus*, *Canthyporus* Zimmermann, and *Laccornellus* Roughley & Wolfe may be the sister group to all other hydroporine except Laccornini. Miller (2001) supported the hypothesis of Wolfe (1985, 1988) of a basal position of Laccornini and Methlini. He suggested, however, that Hydrovatinia is more derived within Hydroporinae and that it should be placed close to Hygrotoni.

Larval morphology is of great interest in the study of phylogenetic relationships among Holometabola. As different expressions of the same genotype, larval characters help to complement adult characters that have been traditionally the primary basis for classification. The larval groundplan of Hydroporinae is well known with detailed descriptions available for several genera (e.g., Alarie and Watts 2005; Michat and Torres 2005; Alarie and Challet 2005; Michat 2006; Shaverdo and Alarie 2006; Michat et al. 2007). Whereas the hypothesis of a basal placement of Laccornini received support from larval morphology (Alarie and Harper 1990, Alarie 1991b), recent studies of larvae of some ancestral hydroporine lineages suggested that 1) *Celina* Aubé (Methlini) is sister to other Hydroporinae (Michat et al. 2007), 2) *Canthyporus* does not belong to the Hydroporini (Shaverdo and Alarie 2006), and 3) *Hydrovatus* is more closely related to members of the tribe Hyphrydrini (Michat 2006).

This article aims at describing the larval morphology of the South American endemic genus *Laccornellus*. The genus name *Laccornellus* was proposed by Roughley and Wolfe (1987) to receive the South American species *Laccornellus copelatoides* (Sharp) and *Laccornellus lugubris* (Aubé) placed inappropriately in the genus *Laccornis* Gozis. Although this genus is presently included in the tribe Hydroporini (Nilsson 2001), Wolfe (1985, 1988) argued in favor of a more basal placement within the Hydroporinae.

The recent discovery of first-instar specimens of *L. lugubris* provided the impetus for this study, al-

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The larva of this putatively basal hydroporine to be described for the first time. Inclusion of the character states found in the first instar is of the utmost interest, because they are deemed to represent a very conservative expression of the phenotype and as such they are phylogenetically very important. The objectives of this article are 1) to provide a description of the first instar of *L. lugubris* and 2) to determine whether larval characters confirm the placement of the genus *Laccornellus* within the subfamily Hydroporinae in the same position as based on adult morphology.

**Materials and Methods**

**Larvae Examined.** The description of the larval stages and the taxonomic conclusions reported in this article are based on the examination of three instar I specimens collected in association with adults. Specimens were collected and stored in 70% ethanol. The association is firm because *L. lugubris* was the only *Laccornellus* adult collected at that locality. Voucher larval specimens are deposited in the research larval collection of M.C.M. (Laboratory of Entomology, Buenos Aires University, Buenos Aires, Argentina).

Larvae and adults of *L. lugubris* were collected at the following locality: Argentina, Buenos Aires City, June and July 2001, M. C. Michat coll.

**Preparation.** Specimens were cleared in lactic acid, dissected, and mounted on glass slides with either Hoyer’s or polyvinyl-lacto-glycerol. Examination at magnifications of 80–1,000× was done using an Olympus BX50 compound microscope (Olympus, Tokyo, Japan) equipped with Nomarski differential interference optics. Figures were prepared with a drawing tube attached to the microscope.

**Measurements.** All measurements were made with a compound microscope equipped with a micrometer eyepiece. The part to be measured was adjusted so that it was, as accurately as possible, parallel to the plane of the objectives. The characters and terms used in the morphometry follow recent studies on the larval morphology of the Hydroporinae (Alarie and Watts 2004, 2005; Michat 2006; Michat et al. 2007).

**Chaetotaxic Analysis.** Primary (observed in instar I) setae and pores were coded according to the system proposed by Alarie (1991b) and Alarie and Michat (2007) for the cephalic capsule and head appendages, Nilsson (1988) and Alarie et al. (1990a) for the legs, and Alarie and Harper (1990) for the last abdominal segment and urogomphi.

**Color.** Description of color is given from ethanol-preserved specimens.

**Cladistic Analysis.** To examine the phylogenetic signal of the larval characters of *L. lugubris* and to test the relationship of the species with other Hydroporinae,
cladistic analyses were conducted rooting the cladogram with the genus *Laccophilus* Leach (Laccophilinae), which has been suggested as the sister-group of the Hydroporinae (Wolfe 1985). The taxa examined for cladistic analysis are presented in Table 1.

The characters used (Table 2) and the distribution of character states among the terminal taxa (Table 3) are listed. Apart from selecting synapomorphies, which usefully group taxa into broad categories, we have included single autapomorphies.
Although these features do not assist with the resolution of the relationships among taxa, they still represent important facies by which terminal clades (i.e., genera) can be clearly recognized.

PAUP* version 4.0b10b (Swofford 2002) and MacClade 4.0 (Maddison and Maddison 2000) software packages were used for parsimony searches, character editing, and cladogram examination. The analyses included only informative characters. All characters were treated as unordered and equally weighted except characters 19–21, which were ordered. All of these characters are related to presence or absence of a galea within the Hydroporinae. Because most Adephaga are characterized by the presence of a galea (Alarie et al. 2004), it is postulated that

Figs. 1–2. L. lugubris, head capsule, instar I. (1) Dorsal aspect. (2) Ventral aspect. EB, egg bursters; FR, frontoclypeus; PA, parietale; TP, tentorial pits. Numbers and lowercase letters refer to primary setae and pores, respectively. Color pattern and stemmata not represented. Scale bar = 0.10 mm.
absence or reduction of a galea evolved posteriorly within the subfamily Hydroporinae. A heuristic search strategy was used to find minimum-length trees. Searches were conducted with 100 random-addition replicates (tree bisection-reconnection [TBR]). The data were bootstrapped with 1,000 replicates to

Figs. 3–6. *L. lugubris*, antenna and maxilla, instar I. (3–4) Antenna. (3) Dorsal aspect. (4) Ventral aspect. (5–6) maxilla. (5) Dorsal aspect. (6) Ventral aspect. AN, antenna; gAN, antenna group; MX, maxilla; gMX, maxilla group. Numbers and lowercase letters refer to primary setae and pores, respectively. Scale bars = 0.10 mm.

Figs. 7–9. *L. lugubris*, labium and mandible, instar I. (7–8) Labium. (7) Dorsal aspect. (8) Ventral aspect. (9) Mandible, dorsal aspect. LA, labium; gLA, labium group; MN, mandible; Numbers and lowercase letters refer to primary setae and pores, respectively. Scale bar = 0.10 mm.
assess branch support. The consistency index (CI) (Kluge and Farris 1969) and retention index (RI) (Farris 1989) are given.

**Description of First Instar of *L. lugubris***

(Figs. 1–14)

**Diagnosis**

First instars of *L. lugubris* can be distinguished from those of other Hydroporinae by the following combination of characters: occipital suture lacking (Fig. 1); nasale short, subtriangular, slightly sinuate laterally, with minute lateral branches (Fig. 1); cardo not fused to stipes (Fig. 6); galea present (Fig. 5); pores PAd (Fig. 1) and FEa (Fig. 11) absent; setae LA2 (Fig. 8) and UR8 (Fig. 14) absent; seta TR2 present (Fig. 10); seta AB15 present (Fig. 13).

**First Instar, Description.**

*Color.* Head capsule predominantly light brown, areas around stemmata and anterior portion of frontoclypeus pale yellow; head appendages creamy white to pale yellow; thoracic and abdominal sclerites dark brown, siphon lightly paler; legs light brown; urogomphi creamy white to pale yellow.

*Head* (Figs. 1–9). HL = 0.46–0.51 mm; HW = 0.40–0.41 mm; FCL = 0.38–0.42 mm. *Cephalic capsule* (Figs. 1–2). Pear-shaped, tapering posteriorly, lacking a neck constriction; longer than broad (HL/
HW/H11005 = 1.16–1.23), maximum width at stellmata; ecdysial suture well developed, coronal suture short, 0.18–0.19 times HL; HW/OcW/H11005 = 1.34; frontoclypeus bluntly rounded, with minute lateral branches; dorsal surface with egg bursters (ruptor ovi of Bertrand 1972); ventroapical margin of frontoclypeus with ~17 spatulate setae (lamellae clypeales of Bertrand 1972); epicranial plates meeting ventrally; ocularium present, stemmata not visible ventrally and subdivided into two vertical series, stemmata of the posterior row more widely spaced; tentorial pits visible medio-ventrally at about mid-length. Antenna (Figs. 3–4). Four-segmented, shorter than HW (length of antenna/HW/H11005 = 0.80–0.81); antennomeres 2 and 3 longest (A2/A3/H11005 = 0.73–0.76), antennomere 4 shortest, subequal to antennomere 1 in length; lateral elongation of antennomere 3 (A3') shorter than antennomere 4 (A3'/A4/H11005 = 0.78); antennomere 3 lacking a ventroapical spinula. Mandible (Fig. 9). Falciform, curved inward and upward apically, ~0.56 times as long as HL, narrow and elongate. Maxilla (Figs. 5–6). Stipes short and thick, incompletely sclerotized ventrally; cardo and galea present, lacinia absent; stipes short and thick, incompletely sclerotized ventrally; cardo and galea present, lacinia absent; palpomere 1 0.60–0.61 times as long as palpomere 2. Labium (Figs. 7–8). Prementum subquadrangular, ~0.90 times longer than broad, with marginal spinulae; labial palpomere two-segmented, about as long as maxillary palpomere 1 (length of maxillary palpomere 1/H11005 = 0.95); maxillary palpomere 2 subfusiform, 1.13 times length of maxillary palpomere 1. Chaetotaxy. All primary setae and pores of generalized hydroporine larva present, except pores Pa d and MXd and seta LA2. Seta FR2 articulated anteriorly, close to the seta FR2; seta MX1 articulated on maxillary cardo. Thorax. Pronotum trapezoidal dorsally, ovate laterally, widest at posterior margin; length of pronotum ~2 times length of mesonotum; metanotum subequal to mesonotum in length, both slightly wider than...
pronotum; pronotum without transverse carina; both meso- and metathoracic terga with anterotransverse carina; ventral surface of thoracic segments membranous; spiracular openings lacking.

Legs (Figs. 10–11). Five-segmented; metathoracic legs longest, 1.22 times length of prothoracic legs, 2.90 times as long as HW; trochanter shortest, ~0.40 times length of coxa; coxa and femur longest, subequal in length; tarsus slightly longer than tibia; tarsus with two claws, posterior claw slightly shorter than anterior claw on pro- and mesothoracic legs, subequal or slightly longer on metathoracic leg; posterior metathoracic claw ~0.70 times as long as length of metatarsus; ventral marginal spinulae present on tibiae and tarsi; surface of legs mostly covered with minute, slender spinulae in transverse rows. Chaetotaxy. All primary setae and pores of generalized hydroporine larva present except pore FEa. Length of longest between setae FE8 and FE9/width of metafemur = 0.74–0.77; length of seta TH/width of metatibia = 1.63–1.74; length of seta TA2/width of metatarsus = 1.19–1.35.

Abdomen (Figs. 12–13). Eight-segmented; segments 1–8 dorsally sclerotized, segments 1–6 membranous ventrally, segment 7 sclerotized ventrally, ventral plate demarcated from rest of sclerite; segment 8 short, LLAS = 0.30–0.32 mm; LLAS/HW = 0.74–0.77, constricted posterior to insertion of urogomphi; siphon acute apically, ~0.50 times LLAS. Chaetotaxy. All primary setae and pores of generalized hydroporine larva present; setae AB3, AB4, AB6, AB7, AB9 elongate; setae AB4, AB7, AB10 spine-like. Seta AB15 present. Length of seta AB11/LLAS = 0.42.

Urogomphi (Fig. 14). Two-segmented, longer than LLAS; total length of urogomphus = 1.20–1.22 mm;
length of urogomphus/HW = 2.93–3.00; length of Uro1 = 0.44–0.48 mm; length of Uro1/HW = 1.07–1.19; Uro1 = 0.58–0.60 times as long as Uro2. Chae- totaxy. All primary setae and pores of generalized hydroporine larva present except seta UR8 absent. Basal articulation of primary setae UR2, UR3, and UR4 contiguous; setae UR2–UR7 elongate.

Habitat. Larvae were collected in rain pools (30 m in length, 1 m in width, 15 cm in depth) with soil bottom and sparse vegetation (predominantly grasses).


Cladistic Analysis

Heuristic searches with random-addition sequence replicates found minimal length topologies of 132 steps from our data matrix (Table 3). TBR swapping of minimal-length trees from 100 random-addition replicates yielded to 100 trees (CI = 0.46; RI = 0.73). In all trees, the genus Laccornellus stands out as member of a clade comprised of Hydrovatus and Canthyporus. The bootstrap consensus tree (Fig. 15) supports a sister-group relationship of Laccornis (Laccornellus (Hydrovatus (Canthyporus)) with all other Hydro- porinae minus Celina.

Discussion

The results of this analysis suggest several significant conclusions with respect to classification of the tribes of Hydroporinae. Bootstrap values (Fig. 15) indicate support in favor of a sister group relationship of Celina (Methlini) with other Hydroporinae (bootstrap value = 54) and of Vatellus (Vatellini) with members of the Hyphydrini (bootstrap value = 48); similarly, a monophyletic origin of members of the Hyphydrini...
(bootstrap value = 92) as well as of those of the Hygototimi (bootstrap value = 54) is well supported. These results are in accordance with several recent independent studies dealing with the larval morphology of these tribes [see also the following publications for character analyses: Methlini (Michat et al. 2007), Hyphrydrini (Alarie et al. 1997; Alarie and Challet 2006a, 2006b), Hygototimi (Alarie et al. 1990b, 2001), and Vatellini (Michat and Torres 2005)].

An interesting outcome of the current study is the suggestion that the tribe Hydroporini is polyphyletic as it is shown that Laccornellus, Canthyporus, Antiporus, and to a lesser extent Stictometes are more closely related to some other taxa than they are to other members of Hydroporini studied (Hydroporus, Hydrocolus, Neoporus, Heterosternuta, Derometes, Stictotarsus, Nebrioporus, Scarodytes, and Oreodytes). This is similar to a suggestion made by Miller (2001) and Miller et al. (2006b) based on adult morphology.

The sister-group relationship of the Australian endemic genus Antiporus with a clade made up of the Vatellini + Hyphydrini is noteworthy knowing that this genus was related to Nebrioporus Régimbart, Scarodytes Gozis, and Stictotarsus Zimmermann in previous studies of the larval morphology of the Hydroporini (Alarie et al. 1999, Alarie and Watts 2004). Larvae of Antiporus share with those of the Vatellini and Hyphydrini (Fig. 16) 1) a narrow and more or less parallel sided nasale (character 01.2), 2) the presence of elongated frontoclypeal lateral processes (character 02.1), and 3) the absence of the primary seta TR2 on trochanter (character 35.1). A closer phylogenetic relationship between Antiporus and selected members of the Hyphydrini has been proposed recently based on adult morphology (Miller et al. 2006). A study of the larval morphology of the Australian endemic genera Necterosoma Macleay and Sternoptiricus Sharp would be particularly interesting because the adults of these genera are closely related to Antiporus (Miller et al. 2006), and they have evolved many characters that are unknown within northern hemisphere Hydroporini (e.g., the presence of a posterior ligula on the ventral edge of the elytron) (Alarie and Watts 2004). The resolution of the Vatellini as sister group to Hyphydrini is in disagreement with the results of Miller et al. (2006).

In our analysis the clade Laccornis + [Laccornellus + (Hydrocatus + Canthyporus)] is supported among several of the most parsimonious topologies as sister to other Hydroporinae minus Celina (Methlini). This reinforces the hypothesis that all of these taxa occupy a relatively basal position within the subfamily Hydroporinae (Wolfe 1985, 1988) (Fig. 15). This arrangement is in agreement with the results of Miller et al. (2006) based on adult morphology except for the relative position of Hydrocatus. However, it is worth stressing that basal relationships among these taxa are poorly supported as indicated by relatively low bootstrap values and weaker character support in general. Indeed, it is difficult to find synapomorphies that are not confounded by what seem to be secondary loss or homoplasys. However, larvae of Laccornis, Laccornellus, Hydrocatus, and Canthyporus share the absence of secondary setae on tibiae (character 33.1) and tarsi (character 34.1) (not observed in Laccornellus owing to absence of instars II and III) and of the primary pore FEa on femora (character 36.1) (Fig. 17). Hair-bearing appendages may have a direct functional relationship to swimming ability (Loudon et al. 1994, Vogel 1994). Absence or reduced number of secondary setae on the legs of these ancestral lineages may suggest that Hydroporinae swimming propensity evolved secondarily.

In this study, larvae of Laccornellus, Canthyporus, and Hydrocatus are postulated to have lost the primary seta UR8 on the urogomphus (character 46.5), a unique character state within the family Dytiscidae. This character state allied to the absence of the primary seta LA2 (character 26.1, homoplastic in Vatellini) and the spine-like condition of the primary seta AB10 (character 41.1, homoplastic in Vatellini, Hyphydrini, and Bidessini) suggest a monophyletic origin for these taxa. However, placement of Hydrocatus near the base of the Hydroporinae contradicts the opinion that Hydrocatus is derived within the Hydroporinae (Miller 2001, Michat 2006, Miller et al. 2006). It is worth stressing that Hydrocatus is unique within this clade being characterized by 14 homoplastic character states (Fig. 17), which could pose some doubt about its correct placement within the tree.

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