

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

YVES ALARIE¹ AND MARIANO C. MICHA²

Department of Biology, Laurentian University, Sudbury, ON, Canada P3E 2C6

Ann. Entomol. Soc. Am. 100(5): 655–665 (2007)

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 Hydroporini 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 ($\approx 2,000$ 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, Hydrovatini, Hyphyrini, Vatellini, Hydroporini, Hygrotini, 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 (Hydrovatini) 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 hydroporines except Laccornini. Miller (2001) supported the hypothesis of Wolfe (1985, 1988) of a basal position of Laccornini and Methlini. He suggested, however,

that Hydrovatini is more derived within Hydroporinae and that it should be placed close to Hygrotini.

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 2006a, 2006b; 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 Hyphyrini (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 copelatooides* (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-

¹ Corresponding author, e-mail: yalarie@laurentian.ca.

² Laboratorio de Entomología, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Av. Int. Güiraldes s/n, Ciudad Universitaria, 1428 Buenos Aires, Argentina.

Table 1. Taxa of Hydroporinae and Laccophilinae coded for phylogenetic analysis

Taxa	Scientific name	Source
Hydroporinae		
Bidessini	<i>Liodesus affinis</i> (Say)	Alarie and Harper (1990), Alarie et al. (1990a), Alarie (1991b)
Hydroporini	<i>Antiporus unceifer</i> Sharp	Alarie and Watts (2004)
	<i>Canthyporus kenyensis</i> Billardo and Sanfilippo	Shaverdo and Alarie (2006)
	<i>Deronectes latus</i> (Stephens)	Alarie et al. (1999)
	<i>Heterosternuta wickhami</i> (Zaitzev)	Alarie (1991a, 1991b)
	<i>Hydrocolus paugus</i> (Fall)	Alarie (1991a)
	<i>Hydroporus columbianus</i> Fall	Alarie (1991a)
	<i>Laccornellus lugubris</i> (Aubé) ^a	This study
	<i>Nebrioporus rotundatus</i> (LeConte)	Alarie and Nilsson (1997), Alarie and Watts (2004)
	<i>Neoporus undulatus</i> (Say)	Alarie (1991a)
	<i>Oreodytes laevis</i> (Kirby)	Alarie (1997)
	<i>Oreodytes scitulus</i> (LeConte)	Alarie (1997)
	<i>Scarodytes halensis</i> (F.)	Alarie et al. (1999)
	<i>Stictotarsus griseostriatus</i> (De Geer)	Alarie and Nilsson (1997), Alarie and Watts (2004)
	<i>Stictonectes canariensis</i> Machado	Alarie and Nilsson (1997)
Hydrovatini	<i>Hydrovatus caraiibus</i> Sharp	Michat (2006)
Hygrotini	<i>Herophydrus musicus</i> (Klug)	Alarie et al. (2001)
	<i>Hygrotus masculinus</i> (Crotch)	Alarie et al. (1990b)
	<i>Hygrotus sayi</i> J. Balfour-Browne	Alarie et al. (1990b)
Hyphydrini	<i>Andex insignis</i> Sharp	Alarie and Challet (2006b)
	<i>Desmopachria convexa</i> (Aubé)	Alarie et al. (1997)
	<i>Hyphydrus ovatus</i> (Linnaeus)	Alarie et al. (1997)
	<i>Microdytes uenoi</i> Sat	Alarie et al. (1997)
	<i>Pachydrus globosus</i> (Aubé) ^b	Alarie et al. (1997)
	<i>Primospes suturalis</i> Sharp ^b	Alarie and Challet (2006a)
Laccornini	<i>Laccornis latens</i> (Fall)	Alarie (1989)
Methlini	<i>Celina parallela</i> (Babington)	Michat et al. (2007)
Vatellini	<i>Vatellus haagi</i> Wehncke	Michat and Torres (2005)
Laccophilinae		
Laccophilini	<i>Laccophilus maculosus</i> Say	Alarie et al. (2000)

^a Instar I only.
^b Instar III only.

lowing 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,

Table 2. Characters used for the phylogenetic analysis and the coding of states by using *Laccophilus maculosus* (Laccophilinae) as outgroup

Character		States	Character		States
01	Nasale	0 - absent 1 - broad, subtriangular 2 - narrow, more or less parallel sided	31	Seta LA12	0 - absent 1 - inserted medially 2 - inserted distally
02	Frontoclypeus	0 - lateral processes lacking, if present barely visible in dorsal view 1 - with one or several lateral processes well visible in dorsal view	32	Natatory setae on legs (instars II and III)	0 - present 1 - absent
03	Seta FR7	0 - setiform 1 - spiniform	33	Secondary setae on TI	0 - present 1 - absent
04	Egg bursters	0 - posterior to stemmata 1 - at level of stemmata	34	Secondary setae on TA	0 - present 1 - absent
05	Occipital suture (instar I)	0 - absent 1 - present	35	Seta TR2	0 - present 1 - absent
06	Occipital suture (instars II-III)	0 - absent 1 - present	36	Pore FEa	0 - present 1 - absent
07	Secondary spine-like setae on lateral margin of parietal	0 - present 1 - absent	37	Seta TI7	0 - short, spiniform 1 - elongate, setiform
08	Pore PAc	0 - absent 1 - located posteriorly, proximad to seta PA8 2 - located anteriorly, proximad to seta PA21	38	Ventral surface of abdominal segment VI	0 - membranous 1 - sclerotized
09	Pore PAD	0 - present 1 - absent	39	Secondary ventral setae on siphon	0 - absent 1 - present
10	Pore PAe	0 - present 1 - absent	40	Seta AB2	0 - present 1 - pore-like 2 - absent
11	Pore PAj	0 - present 1 - absent	41	Seta AB10	0 - setiform 1 - spiniform
12	Antennomere 1	0 - subequal in length to antennomere 2 1 - shorter than antennomere 2	42	Pore ABa	0 - present 1 - absent
13	Secondary setae on antennomere 2	0 - absent 1 - present	43	Secondary setae on urogomphomere 1	0 - present 1 - absent
14	Ventral spinula on antennomere 3	0 - absent 1 - present	44	Setae UR2, UR3 and UR4	0 - not contiguous, apart from each other 1 - contiguous 2 - only UR2 and UR3 contiguous 3 - only UR3 and UR4 contiguous
15	Pore ANf	0 - present 1 - absent	45	Seta UR5	0 - elongate, setiform 1 - short, spiniform
16	Pore ANh	0 - present 1 - absent	46	Seta UR8	0 - inserted on urogomphomere 1 1 - inserted subapically on urogomphomere 2 2 - inserted medially on urogomphomere 2 3 - inserted proximally on urogomphomere 2 4 - inserted apically on urogomphomere 2 5 - absent
17	Cardo	0 - not fused to stipes 1 - fused to stipes	47	Seta AB15	0 - inserted along the posteroventral margin 1 - inserted at mid-distance on the ventral surface 2 - absent
18	Galea	0 - elongate, finger-like 1 - short, finger-like 2 - absent	48	Seta AB7	0 - minute, setiform 1 - elongate, spiniform
19	Seta MX8	0 - present 1 - absent	49	Pore FRb	0 - present 1 - absent
20	Seta MX9	0 - present 1 - absent	50	Antennomere 3	0 - longer than antennomere 1 1 - subequal in length to antennomere 1
21	Pore MXh	0 - present 1 - absent	51	Prementum	0 - lacking dorsal secondary setae 1 - with dorsal secondary setae
22	Seta MX5	0 - present 1 - absent	52	Setae LA4 and LA5	0 - articulated distally 1 - articulated proximally
23	Palpifer	0 - seta MX10 present 1 - seta MX10 absent	53	Setae FE5 and FE6	0 - inserted distally 1 - inserted proximally
24	Prementum	0 - broader to as long as broad 1 - longer than broad	54	Pore TIa	0 - present 1 - absent
25	Lateral spinulae on prementum (instar I)	0 - absent 1 - present	55	Secondary setae on maxillary palpomere 1	0 - absent 1 - present
26	Seta LA2	0 - present 1 - absent	56	Seta MX4	0 - present 1 - absent
27	Seta LA3	0 - present 1 - absent	57	Spiracular openings (instar III)	0 - present 1 - absent
28	Seta LA5	0 - spiniform 1 - setiform			
29	Seta LA8	0 - inserted subdistally 1 - inserted proximally			
30	Seta LA10	0 - absent 1 - inserted medially 2 - inserted distally			

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

Table 3. Matrix of 57 morphological characters of larvae of selected genera of the dytiscid subfamilies Hydroporinae and Laccophilinae

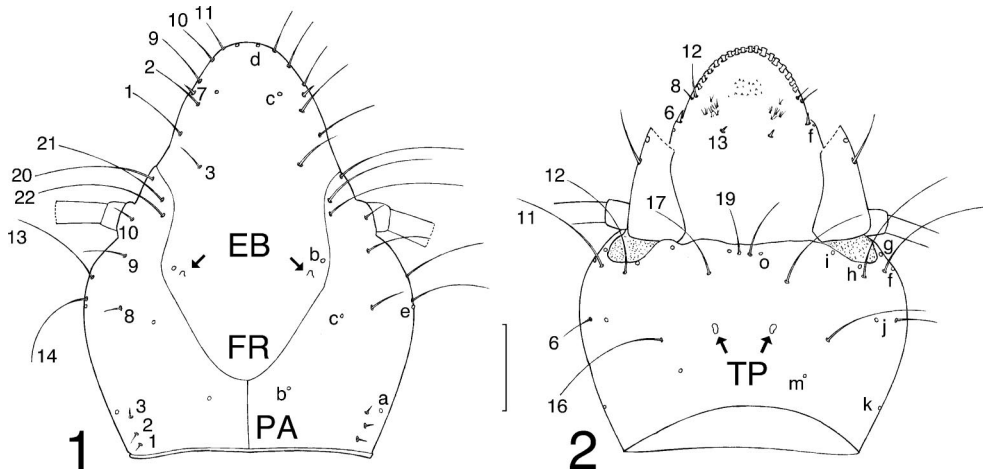
Taxon	Characters											
	01–05	06–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–57
<i>Laccophilus maculosus</i>	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00
<i>Liodesmus affinis</i>	10010	11111	01001	01211	11100	00101	11001	10000	11130	12000	00000	00
<i>Antiporus uncifer</i>	21110	10110	01001	01211	11101	00101	10001	00000	01000	12000	00000	00
<i>Canthyporus kenyensis</i>	10110	11111	01000	00211	10101	10101	11110	10100	11110	52000	00000	00
<i>Deronectes latus</i>	10110	10111	01010	00211	11100	00101	11000	00000	01020	12000	00000	00
<i>Heterosternuta wickhami</i>	10110	10111	01010	00211	11101	00101	11001	00000	01020	12000	00000	01
<i>Hydrocolus paugus</i>	10110	10111	01110	00211	11101	00101	11010	00000	01120	12000	00000	00
<i>Hydroporus columbianus</i>	10110	10111	01010	00211	11101	00101	11010	00000	01120	12000	00000	00
<i>Oreodytes laevis</i>	10111	10111	01011	01211	11100	00101	11000	00000	01000	12000	00000	00
<i>Oreodytes scitulus</i>	10111	10111	01011	01211	11100	00101	11010	00000	01000	12000	00000	00
<i>Scarodytes halensis</i>	10111	10111	01011	01211	11101	00101	10000	00000	01020	12000	00000	00
<i>Stictonectes canariensis</i>	10110	10110	01011	00211	10100	00101	11000	00000	01120	12000	00000	00
<i>Hydrocolus caraiibus</i>	20000	01111	11000	11100	00100	11111	11111	10110	11110	52000	00000	00
<i>Herophydrus musicus</i>	10110	10110	01001	00211	10101	00101	11001	00000	01120	22000	00000	00
<i>Hygrotus masculinus</i>	10110	10110	01001	00211	10101	00101	10001	00000	01020	22000	00000	00
<i>Hygrotus sayi</i>	10110	10110	01001	00211	10101	00101	11001	00000	01120	22000	00000	00
<i>Desmopachria convexa</i>	20010	10211	11011	11211	10110	00112	20001	11111	11011	32110	01000	00
<i>Hyphydrus ovatus</i>	20010	10211	11011	11211	10110	00111	20001	11111	11031	32110	00000	00
<i>Microdytes uenoi</i>	11010	10211	11011	11211	10110	00111	20001	10101	11011	32110	00000	00
<i>Pachydrus globosus</i>	21???	10???	?0000	11211	1?10?	00111	20001	1110?	??0??	32?11	00100	00
<i>Laccornis latens</i>	10110	10100	01000	00100	00100	00101	11110	10000	00110	42000	00000	00
<i>Celina parallela</i>	10111	10100	01000	00100	00000	01101	11000	00010	01110	42?00	00000	00
<i>Vatellus haagi</i>	21010	10110	10111	01211	11101	10000	00001	01002	11000	42100	00001	10
<i>Andex insignis</i>	21110	10211	11011	11211	10110	00011	20001	11101	11031	32110	10010	00
<i>Primospes suturalis</i>	20???	10???	?1011	11211	1?11?	00111	20001	1?10?	?10??	32?10	00000	00
<i>Laccornellus lugubris</i>	10110	??110	01?00	00100	00101	10101	1???0	100?0	10?10	51100	?000?	0?
<i>Neoporus undulatus</i>	10110	10111	01010	00211	11101	00101	10001	00000	01020	12000	00000	01
<i>Nebiroporus rotundatus</i>	10111	10111	01011	01211	11101	00101	10000	00000	01000	12000	00000	00
<i>Stictotarsus griseostriatus</i>	10111	10111	01011	01211	11101	00101	10000	00000	01000	12000	00000	00

Note that the 57 columns correspond to the character number (see Table 2); ?, missing data.

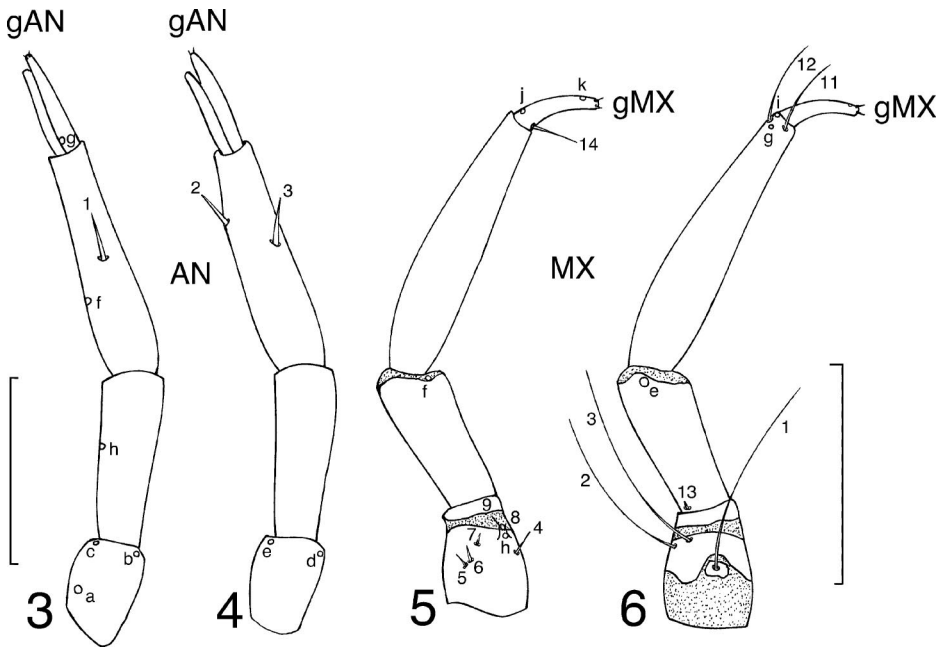
(characters). Although these features do not assist with the resolution of the relationships among taxa, they still represent important facies by which terminal clades (i.e., genera) can be clearly recognized.

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



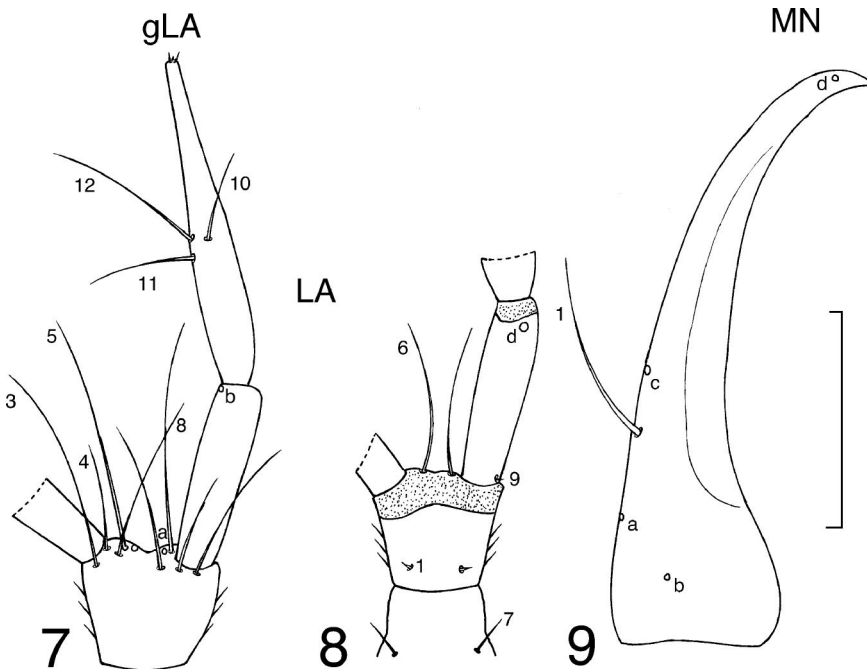
Figs. 1–2. *L. lugubris*, head capsule, instar I. (1) Dorsal aspect. (2) Ventral aspect. EB, egg bursters; FR, frontoclypeus; PA, parietale; TP, tentorial pits. Numbers and lowercase letters refer to primary setae and pores, respectively. Color pattern and stemmata not represented. Scale bar = 0.10 mm.



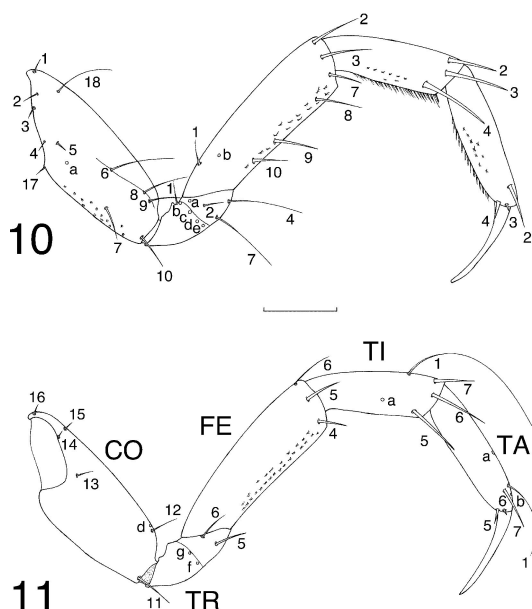
Figs. 3–6. *L. lugubris*, antenna and maxilla, instar I. (3–4) Antenna. (3) Dorsal aspect. (4) Ventral aspect. (5–6) maxilla. (5) Dorsal aspect. (6) Ventral aspect. AN, antenna; gAN, antenna group; MX, maxilla; gMX, maxilla group. Numbers and lowercase letters refer to primary setae and pores, respectively.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

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. 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.



Figs. 10–11. *L. lugubris*, metathoracic legs, instar I. (10) Anterior surface. (11) Posterior surface. CO, coxa; FE, femur; PT, pretarsus; TR, trochanter; TI, tibia; TA, tarsus. Numbers and lowercase letters refer to primary setae and pores, respectively; pores TAc, TAd, TAE, TAF, and setae PT1 and PT2 not represented. Scale bar = 0.10 mm.

assess branch support. The consistency index (CI) (Kluge and Farris 1969) and retention index (RI) (Farris 1989) are given.

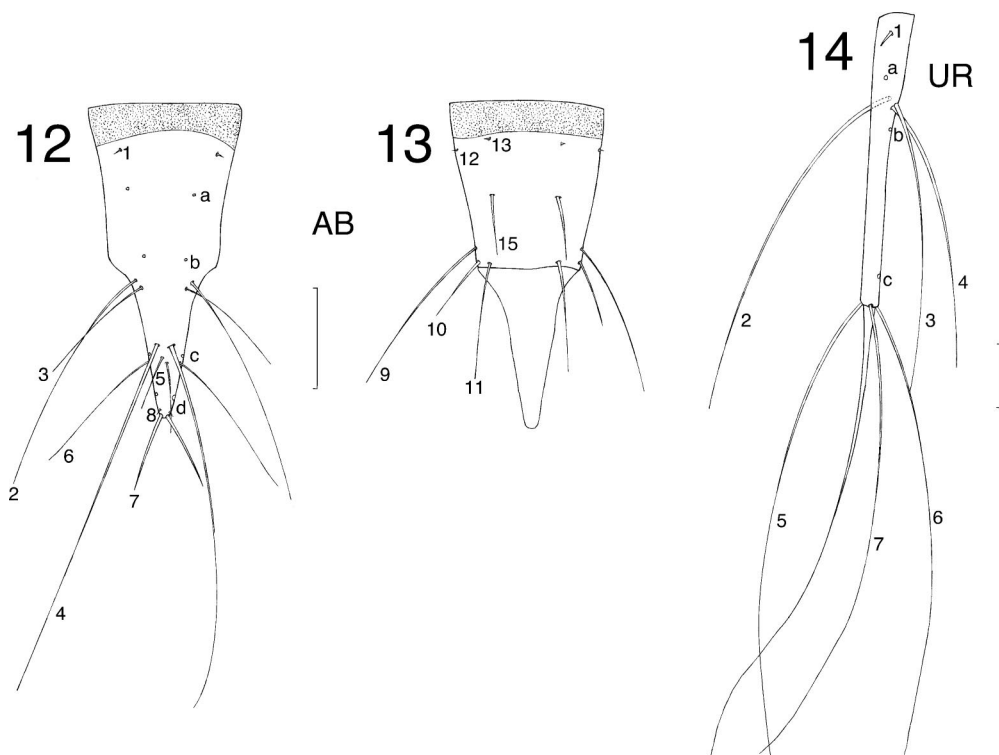
Description of First Instar of *L. lugubris* (Figs. 1–14)

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/



Figs. 12–14. *L. lugubris*, abdominal segment eight and urogomphus, instar I. (12–13) Abdominal segment eight. (12) Dorsal aspect. (13) Ventral aspect. (14) Urogomphus, dorsal aspect. AB, abdominal segment eight; UR, urogomphus. Numbers and lowercase letters refer to primary setae and pores, respectively. Scale bars = 0.10 mm.

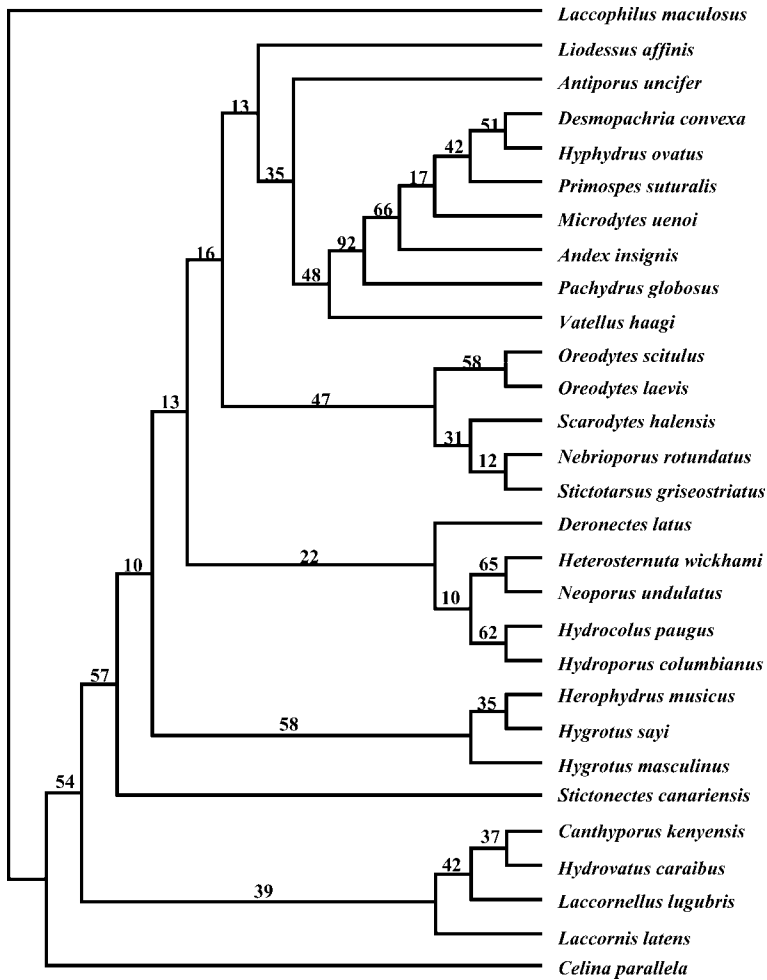


Fig. 15. Bootstrap consensus tree of 100 most parsimonious trees reconstructed (48 informative characters of larvae of 29 taxa).

HW = 1.16–1.23), maximum width at stemmata; ecdysial suture well developed, coronal suture short, 0.18–0.19 times HL; HW/OcW = 1.34; frontoclypeus bluntly rounded, with minute lateral branches; dorsal surface with egg bursters (*ruptorovi* 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 = 0.80–0.81); antennomeres 2 and 3 longest ($A_2/A_3 = 0.73$ –0.76), antennomere 4 shortest, subequal to antennomere 1 in length; lateral elongation of antennomere 3 (A_3') shorter than antennomere 4 ($A_3'/A_4 = 0.78$); 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; palpus three-segmented, shorter than antenna (length of antenna/length of maxillary palpus = 1.27); palpomere 1 0.60–0.61 time as long as palpomere 2. *Labium* (Figs. 7–8). Prementum subquadrangular, ≈ 0.90 times longer than broad, with marginal spinulae; labial palpus two-segmented, about as long as maxillary palpus (length of maxillary palpus/length of labial palpus = 0.95); palpomere 2 subfusiform, 1.13 times length of palpomere 1. *Chaetotaxy*. All primary setae and pores of generalized hydroporine larva present, except pores PAd and MXd and seta LA2. Seta FR2 articulated anteriorly, close to the seta FR7; 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

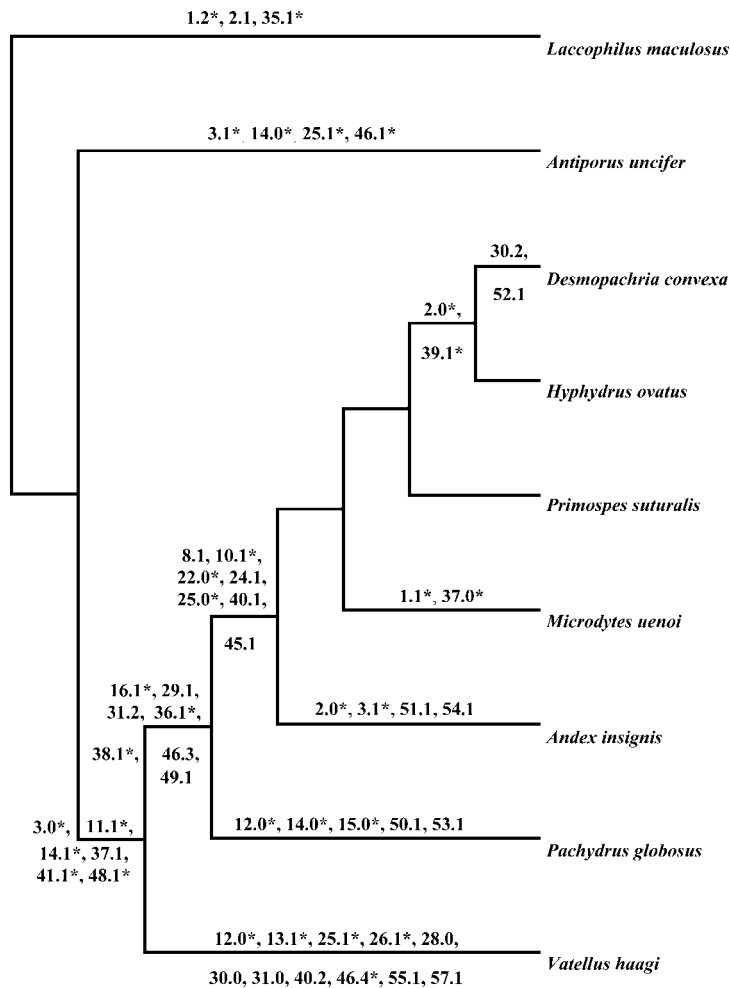


Fig. 16. Part of one of the 100 most parsimonious trees obtained through a heuristic search with random-addition sequence replicates after removal of nine uninformative characters (tree length = 132; CI = 0.46; RI = 0.73) with character changes mapped for the clade *Antiporus* + (*Hyphydrini* + *Vatellus*) (character codes as in Table 2). *, homoplasious character-state transformations.

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 TI4/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 fully sclerotized; terga 1–7 with an anterodorsal transverse carina; spiracular openings lacking; 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;

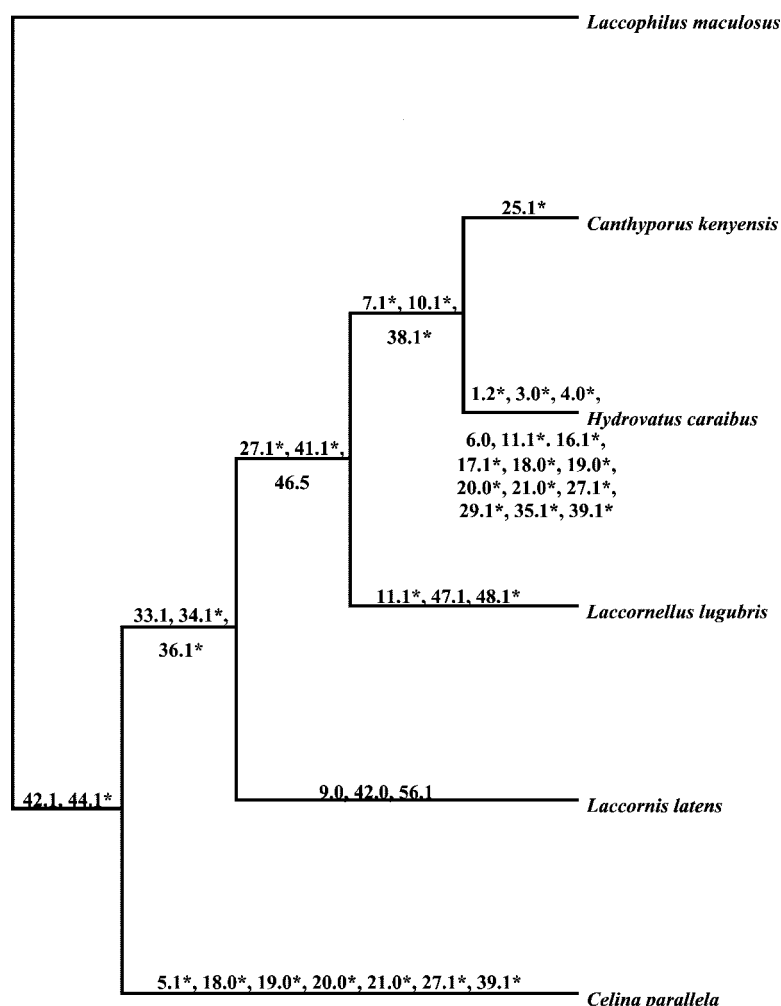


Fig. 17. Part of one of the 100 most parsimonious trees obtained through a heuristic search with random-addition sequence replicates after removal of nine uninformative characters (tree length = 132; CI = 0.46; RI = 0.73) with character changes mapped for ancestral lineages (character codes as in Table 2). *, homoplasious character-state transformations.

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. *Chaetotaxy*. 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).

Distribution. Uruguay and east central Argentina (Roughley and Wolfe 1987). Benetti and Régil Cueto (2003) cited this species from southern Brazil.

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 Hydroporinae 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 Hygrotini (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 the following publications for character analyses: Methlini (Michat et al. 2007), Hyphydrini (Alarie et al. 1997; Alarie and Challet 2006a, 2006b), Hygrotini (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 *Stictonectes* are more closely related to some other taxa than they are to other members of Hydroporini studied (*Hydroporus*, *Hydrocolus*, *Neoporus*, *Heterosternuta*, *Deronectes*, *Stictotarsus*, *Nebrioporus*, *Scarodytes*, and *Oreodytes*). This is similar to a suggestion made by Miller (2001) and Miller et al. (2006) 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 trochantera (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 *Sternopriscus* 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 ridge 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* + (*Hydrovatus* + *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 *Hydrovatus*. 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 homoplasy. However, larvae of *Laccornis*, *Laccornel-*

lus, *Hydrovatus*, 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 *Hydrovatus* 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 *Hydrovatus* near the base of the Hydroporinae contradicts the opinion that *Hydrovatini* is derived within the Hydroporinae (Miller 2001, Michat 2006, Miller et al. 2006). It is worth stressing that *Hydrovatus* 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.

Acknowledgments

Financial support was provided by the Natural Sciences and Engineering Research Council of Canada in the form of an operating research grant to Y. A. Field and laboratory work by M.C.M. was supported by a postgraduate scholarship and grant PIP 02541/00 from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

References Cited

- Alarie, Y. 1989. The larvae of *Laccornis* Des Gozis 1914 (Coleoptera: Adepaga: Dytiscidae) with description of *L. latens* (Fall 1937) and redescription of *L. conoideus* (Leconte 1850). *Coleopt. Bull.* 43: 365–378.
- Alarie, Y. 1991a. Description of larvae of 17 Nearctic species of *Hydroporus* Clairville (Coleoptera: Dytiscidae: Hydroporinae) with an analysis of their phylogenetic relationships. *Can. Entomol.* 123: 627–704.
- Alarie, Y. 1991b. Primary setae and pores on the cephalic capsule and head appendages of larval Hydroporinae (Coleoptera: Dytiscidae). *Can. J. Zool.* 69: 2255–2265.
- Alarie, Y. 1997. Taxonomic revision and phylogenetic analysis of the genus *Oreodytes* Seidlitz (Coleoptera: Dytiscidae: Hydroporinae) based on larval morphology. *Can. Entomol.* 129: 399–503.
- Alarie, Y., R. G. Beutel, and C.H.S. Watts. 2004. Larval morphology of three species of *Hygrobiidae* (Coleoptera: Adepaga: Dytiscoidea) with phylogenetic considerations. *Eur. J. Entomol.* 101: 293–311.
- Alarie, Y., and G.L. Challet. 2006a. Description of the larvae of *Primospes suturalis* Sharp (Coleoptera: Dytiscidae, Hydroporinae) with implications for the phylogeny of the Hyphydrini. *Aquat. Insects* 28: 23–30.

- Alarie, Y., and G. L. Challet. 2006b. Larval description and phylogenetic placement of the South African endemic genus *Andex* Sharp (Coleoptera: Adephegidae: Dytiscidae). *Ann. Entomol. Soc. Am.* 99: 743–754.
- Alarie, Y., J.G.M. Cuppen, L. Hendrich, and A. N. Nilsson. 2001. Description of larvae of *Herophydrus musicus* (Klug) and analysis of relationships with members of the genus *Hygrotus* Stephens (Coleoptera: Dytiscidae, Hydroporinae). *Aquat. Insects* 23: 193–207.
- Alarie, Y., and P.-P. Harper. 1990. Primary setae and pores on the last abdominal segment and the urogomphi of larval Hydroporinae (Coleoptera: Adephegidae: Dytiscidae), with notes on other dytiscid larvae. *Can. J. Zool.* 68: 368–374.
- Alarie, Y., P.-P. Harper, and A. Maire. 1990a. Primary setae and pores on legs of larvae of Nearctic Hydroporinae (Coleoptera: Dytiscidae). *Quaest. Entomol.* 26: 199–210.
- Alarie, Y., P.-P. Harper, and R. E. Roughley. 1990b. Description of the larvae of eleven Nearctic species of *Hygrotus* Stephens (Coleoptera: Dytiscidae: Hydroporinae) with an analysis of their phyletic relationships. *Can. Entomol.* 122: 985–1035.
- Alarie, Y., and M. C. Michat. 2007. Primary setae and pores on the maxilla of larvae of the subfamily Hydroporinae (Coleoptera: Adephegidae: Dytiscidae): ground plan pattern reconsidered. *Coleopt. Bull.* (in press).
- Alarie, Y., and A. N. Nilsson. 1997. Larvae of *Stictonectes* Brinck: generic characteristics, description of *S. canariensis* Machado, and analysis of phylogenetic relationships with other genera of the tribe Hydroporini (Coleoptera: Dytiscidae). *Coleopt. Bull.* 51: 120–139.
- Alarie, Y., and C.H.S. Watts. 2004. Larvae of the genus *Antiporus* (Coleoptera: Dytiscidae) and phylogenetic implications. *Invert. Syst.* 18: 523–546.
- Alarie, Y., and C.H.S. Watts. 2005. Descriptions of larvae of four species of the *Hyphydrus lyratus* species-group (Coleoptera: Dytiscidae: Hydroporinae). *Aust. J. Entomol.* 44: 244–251.
- Alarie, Y., L.-J. Wang, A. N. Nilsson, and P. J. Spangler. 1997. Larval morphology of four genera of the tribe Hyphydrini Sharp (Coleoptera: Dytiscidae: Hydroporinae) with an analysis of their phylogenetic relationships. *Ann. Entomol. Soc. Am.* 90: 709–735.
- Alarie, Y., A. N. Nilsson, and L. Hendrich. 1999. Larval morphology of the Palaearctic genera *Deronectes* Sharp and *Scarodytes* Gozis (Coleoptera: Dytiscidae: Hydroporinae) with implications for the phylogeny of the *Deronectes*-group of genera. *Entomol. Scand.* 30: 173–195.
- Alarie, Y., A. N. Nilsson, L. Hendrich, and C.H.S. Watts. 2000. Larval morphology of four genera of Laccophilinae (Coleoptera: Adephegidae: Dytiscidae) with an analysis of their phylogenetic relationships. *Insect Syst. Evol.* 31: 121–164.
- Bertrand, H. 1972. Larves et nymphes des Coléoptères aquatiques du globe. F. Paillart, France.
- Benetti, C. J., and J. A. Régil Cueto. 2003. Primera cita de la tribu Hydroporini Aubé, género *Laccornellus* Roughley & Wolfe (Coleoptera, Dytiscidae, Hydroporinae) para Brasil. *Bol. S.E.A.* 32: 157–159.
- Farris, J. S. 1989. The retention index and the rescaled consistency index. *Cladistics* 5: 417–419.
- Kluge, A. G., and J. S. Farris. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* 30: 1–32.
- Larson, D. J., Y. Alarie, and R. E. Roughley. 2000. Predaceous diving beetles (Coleoptera: Dytiscidae) of the Nearctic region, with emphasis on the fauna of Canada and Alaska. NRC Research Press, Ottawa, ON, Canada.
- Loudon, C., B. A. Best, and M.A.R. Koehl. 1994. When does motion relative to neighboring surfaces alter the flow through arrays of hairs? *J. Exp. Biol.* 193: 233–254.
- Maddison, W. P., and D. R. Maddison. 2000. *MacClade*. Analysis of phylogeny and character evolution. Sinauer, Sunderland, MA.
- Michat, M. C. 2006. The phylogenetic position of *Hydrovatus* Motschulsky: evidence from larval morphology of *H. caraiibus* Sharp (Coleoptera: Dytiscidae: Hydroporinae). *Insect Syst. Evol.* 37: 419–432.
- Michat, M. C., and P.L.M. Torres. 2005. Larval morphology of *Macrocatellus haagi* (Wehncke) and phylogeny of Hydroporinae (Coleoptera: Dytiscidae). *Insect Syst. Evol.* 36: 199–217.
- Michat, M. C., Y. Alarie, P.L.M. Torres, and Y. S. Megna. 2007. Larval morphology of *Celina* Aubé and the phylogeny of basal members of Hydroporinae. *Invert. Syst.* 21: 239–254.
- Miller, K. B. 2001. On the phylogeny of the Dytiscidae (Insecta: Coleoptera) with emphasis on the morphology of the female reproductive system. *Insect Syst. Evol.* 32: 45–92.
- Miller, K. B., G. W. Wolfe, and O. Biström. 2006. The phylogeny of the Hydroporinae and classification of the genus *Peschetius* Guignot, 1942. (Coleoptera: Dytiscidae). *Insect Syst. Evol.* 37: 257–279.
- Nilsson, A. N. 1988. A review of primary setae and pores on legs of larval Dytiscidae (Coleoptera). *Can. J. Zool.* 66: 2283–2294.
- Nilsson, A. N. 2001. Dytiscidae (Coleoptera). World catalogue of insects 3. Apollo Books, Stenstrup, Denmark.
- Roughley, R. E., and G. W. Wolfe. 1987. *Laccornellus* (Coleoptera: Dytiscidae), a new hydroporine genus from austral South America. *Can. J. Zool.* 65: 1346–1353.
- Shaverdo, H. V., and Y. Alarie. 2006. Description of the larva of *Canthyporus kenyensis* Bilardo & Sanfilippo (Coleoptera: Dytiscidae: Hydroporinae) with implication for the phylogeny of the Hydroporini. *Aquat. Insects* 28: 113–130.
- Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4.0b10. Sinauer, Sunderland, MA.
- Vogel, S. 1994. Life in moving fluids. The physical biology of flow. Willard Grant Press, Boston, MA.
- Wolfe, G. W. 1985. A phylogenetic analysis of plesiotypic hydroporine lineages with an emphasis on *Laccornis* Des Gozis (Coleoptera: Dytiscidae). *Proc. Acad. Nat. Sci. Phil.* 137: 132–155.
- Wolfe, G. W. 1988. A phylogenetic investigation of *Hydrovatus*, Methilini and other plesiotypic Hydroporines (Coleoptera: Dytiscidae). *Psyche* 95: 327–344.

Received 31 August 2006; accepted 24 May 2007.