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Larval Description and Phylogenetic Placement of the South African Endemic Genera *Coelhydrus* Sharp and *Darwinhydrus* Sharp (Coleoptera: Dytiscidae: Hydroporinae: Hyphydrini)

Yves Alarie
Department of Biology, Laurentian University,
Sudbury, ON P3E 2C6, CANADA
yalarie@laurentian.ca

Mariano C. Michat
Laboratory of Entomology, DBBE-FCEN and IBBEA-CONICET,
University of Buenos Aires, Buenos Aires, ARGENTINA

and

Gilbert L. Challet
30 Celonova Place,
Foothill Ranch, CA 92610, U.S.A.

Abstract

The third instars of *Coelhydrus brevicollis* Sharp and *Darwinhydrus solidus* Sharp are described and illustrated for the first time, with detailed morphometric and chaetotaxic analyses of the cephalic capsule, head appendages, legs, last abdominal segment, and urogomphi. A key for the identification of the third instars of the genera of Hyphydrini known in detail is provided. A parsimony analysis based on 49 informative larval characteristics was conducted using the program TNT. The consensus tree supports a placement of *Coelhydrus* Sharp and *Darwinhydrus* Sharp within the tribe Hyphydrini. Within the Hyphydrini, *Coelhydrus* is postulated to share a monophyletic origin with *Hyphydrus* Illiger and *Desmopachria* Babington. The phylogenetic placement of *Darwinhydrus*, however, remains contentious as our study found it part of an unresolved polytomy with *Andex* Sharp and *Primospes* Sharp.

Key Words: predaceous diving beetles, *Primospes suturalis*, larvae, systematics, Afrotropical

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Coelhydrus Sharp and *Darwinhydrus* Sharp are two monotypic hydroporine genera known to occur only in South Africa (Biström *et al.* 1997; Ribera and Balke 2007). Both genera are currently classified in the tribe Hyphydrini. Hyphydrines constitute a large and diverse group of beetles in warm temperate to tropical regions of the world. Worldwide, Hyphydrini currently contains 371 species distributed among 14 different genera (Nilsson 2015).

Morphology of the larvae of Hyphydrini is not well known. Even for groups where larvae and adults are associated, treatment is so unequal that comprehensive comparisons are difficult (Bertrand 1928, 1930, 1935, 1948, 1963, 1969, 1976; Kurosa 1959; Watts 1963; Barman 1973; De Marzo 1977; Wichard *et al.* 1995; Alarie *et al.* 1997; Nakanishi 2001; Alarie and Watts 2005; Alarie and Challet 2006a, b; Michat and Archangelsky 2007). Recent studies of the larvae of selected hyphydrine suggest that Hyphydrini are monophyletic within the Hydroporinae (Alarie and Challet 2006a, b; Michat and Torres 2008). This hypothesis, however, would benefit from tests with a larger data set that includes more genera.

The recent discovery of the larvae of *Coelhydrus brevicollis* Sharp and *Darwinhydrus solidus* Sharp provided the impetus for this study, which has the following four goals: 1) to describe for the first time the third instar of both *Coelhydrus* and *Darwinhydrus*, 2) to compare the groundplan of larval features of these two genera with those of other associated hyphydrine genera for which the larvae have been described in detail, 3) to provide a key for the identification of the third instar of the known genera of Hyphydrini, and 4) to study the phylogenetic relationships of *Coelhydrus* and *Darwinhydrus* within Hyphydrini based on larval characters.

Material and Methods

Larvae Examined. The descriptions of the third instar and the taxonomic conclusions reported in this paper are based on the examination of larvae co-occuring with adults in a unique South African location on 28 August 2006. Larvae and adults of both species were collected in a salt marsh at the outlet of the Great Fish River approximately 27 km east of Port Alfred on Highway R72. The large pond had both brackish and freshwater in it, which was caused by an upwelling of freshwater coming down the river. Larvae of *Coelhydrus* were more abundantly collected in the more brackish part of the pond, which was characterized by the presence of *Salicornia* L. (Chenopodiaceae) and *Spartina* Schreb. (Poaceae) on the periphery. Larval specimens of *Darwinhydrus*, on the other hand, were generally found in the more freshwater portion of the pond, which was characterized by freshwater plant species. *Darwinhydrus* also was found in more acidic waters of a stream near Tsitsikama Park. *Darwinhydrus* and *Coelhydrus* were the only Hyphydrini at the locality, and both genera included teneral specimens. The distinction of the larvae of these genera, however, is firm as the larvae of *D. solidus* used for this study could be compared upon some drawings provided in Bertrand (1972).

Preparation. Larvae were disarticulated and mounted on standard glass slides with Hoyer's medium. Microscopic examination at magnifications of 80–800X was done using an Olympus BX50 compound microscope equipped with Nomarsky differential interference optics. Figures were prepared through use of a drawing tube attached to the microscope. Drawings were scanned and digitally inked using an Intuos 4 professional pen tablet (Wacom Co., Ltd. Kazo, Saitama, Japan). Voucher specimens are deposited in the larval collection of Y. Alarie (Department of Biology, Laurentian University, Canada).

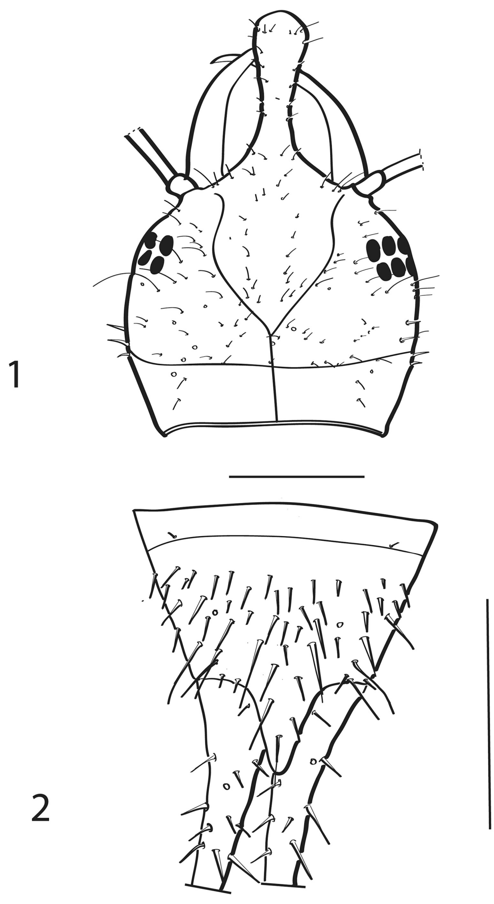
Measurements. All measurements were made with a compound microscope equipped with a micrometer eyepiece. The measured part was adjusted so that it was parallel, as nearly as possible, to the plane of the objectives. We employed, with minimal modifications and additions, the terms used in previous papers dealing with larval morphology of Hyphydrini (Alarie and Challet 2006a, b). The following measurements were taken. Head length (HL): total head length including frontoclypeus, measured medially along epicranial stem. Head width (HW): maximum head width. Length of frontoclypeus (FRL): from apex of nasale to posterior margin of ecdysial suture. Occipital foramen width (OCW): maximum width measured along dorsal margin of occipital foramen. Length of mandible (MN): from laterobasal angle to apex. Width of MN: maximum width measured at base. Length of the antenna (A) and 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). A3' is used as an abbreviation for the apical lateroventral process of the third antennomere. Length of leg (L) including the longest claw 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). Length of trochanter includes only the proximal portion, the length of the distal portion is included in the femoral length. Dorsal length of last abdominal segment (LAS): measured along midline from anterior to posterior margin. Length of urogomphus (U) was derived by adding the lengths of the individual segments; each segment is denoted by the letter U followed by a number (*e.g.*, U1: first urogomphomere). These measurements were used to calculate several ratios, which characterize the body shape.

Chaetotaxic Analysis. Primary (observed in instar I) and secondary (added throughout ontogenetic development) setae and pores were distinguished on the head capsule, head appendages, legs, last abdominal segment, and urogomphus. The setae and pores were coded according to the system proposed by Alarie (1991) and Alarie and Michat (2007a) for the cephalic capsule and head appendages, Alarie *et al.* (1990) for the legs, and Alarie and Harper (1990) for the last abdominal segment and urogomphi. Setae are coded by two capital letters corresponding to the first two letters of the name of the structure on which the seta is located (AN, antenna; CO, coxa; FE, femur; LA, labium; MX, maxilla; TA, tarsus; TI, tibia; TR, trochanter) and a number. Pores are coded in a similar manner except that the number is replaced by a lower-case letter. The position of the sensilla is described by adding the following abbreviations: A, anterior; AD, anterodorsal, AV, anteroventral; D, dorsal; PD, posterodorsal; Pr, proximal; PV, posteroventral.

Color. Description of color is given for all species from ethanol-preserved specimens.

Cladistic Analysis. To examine the phylogenetic signal of the larval characters of *Darwinhydrus* and *Coelhydrus* and to test the relationships of these genera with other Hyphydrini, a cladistic analysis of all species of Hyphydrini with sufficiently detailed larval descriptions (*Hyphydrus* Illiger (seven species), *Microdytes* J. Balfour-Browne (one species), *Desmopachria* Babington (three species), *Andex* Sharp (one species), and *Primospes* Sharp (one species)) was conducted. *Pachydrus* Sharp (tribe Pachydrini), which has sometimes been suggested to be related phylogenetically to the Hyphydrini (Alarie *et al.* 1997), and *Laccornis* Gozis (tribe Laccornini), which is generally recognized as a basal lineage within the subfamily Hydroporinae based on adults

(Roughley and Wolfe 1987; Miller *et al.* 2006), larvae (Alarie and Michat 2007b), and molecules (Miller and Bergsten 2014), were used as outgroups, rooting the tree with *Laccornis*. The analysis was performed using the program TNT (Goloboff *et al.* 2008). All characters were treated as unordered and equally weighted. An exact solution algorithm (implicit enumeration) was implemented to find the most parsimonious trees. Bremer support values were calculated using the commands 'hold 20000', 'sub n' and 'bsupport', where 'n' is the number of extra steps allowed. The process was repeated increasing the length of the suboptimal cladograms by one step, until all Bremer values were obtained (Kitching *et al.* 1998). Jacknife values were calculated with 2000 replicates and P (removal probability) = 36.



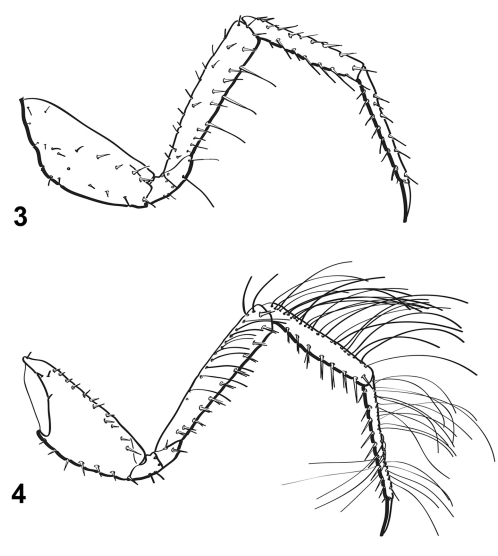
Figs. 1–2. *Coelhydrus brevicollis*, instar III. 1) Head capsule, dorsal view; 2) Abdominal segment VIII and proximal portion of urogomphi, dorsal view. Scale bars = 0.50 mm.

Results

General Morphological Characteristics of Hyphydrini Larvae. Body fusiformate; frontoclypeus elongated, narrow apically; gular sutures fused (visible from instar II), so epicranial plates meet at ventral midline; cardo fused to stipes; primary seta MX1 inserted on stipes; prementum longer than broad, lacking marginal spinulae laterally; without primary pores PAj, ANh, FEa, and ABa, and primary seta TR2; legs of instars II and III with natatory setae; abdominal segments VI–VII sclerotized ventrally; urogomphomere 1 of instars II and III with secondary setae; primary seta UR8 subterminal, proximally articulated on urogomphomere 2 (Uro2'/Uro2 < 0.50).

Description of Larvae of *Coelhydrus* Sharp (Figs. 1–4)

Diagnostic Combination. The third instar of *Coelhydrus* can be distinguished from those of other associated members of genera of Hyphydrini that have been well studied by the following combination of characters: frontoclypeus lacking lateral processes (Fig. 1); HL = 1.00–1.50 mm; ratio HL/LAS = 1.50–1.80; ratio MP/LP > 2.50; ratio A4/A3 < 0.30; ratio LP2/LP1 < 1.30; metathoracic leg > 3.50 times HW; ratio LAS/HW < 0.90; primary setae LA3, LA4, and LA5 articulated distally on prementum; dorsal meso- and metafemoral secondary setae present; procoxa with more



Figs. 3–4. *Coelhydrus brevicollis*, instar III, metathoracic leg. **3)** Anterior surface; **4)** Posterior surface. Scale bar = 0.50 mm.

than 15 secondary setae; femora, tibiae, and tarsi with more than 8, 10, and 10 dorsal natatory setae, respectively; siphon short, clearly constricted at point of insertion of urogomphi, lacking secondary spine-like setae on ventral surface.

Coelhydrus brevicollis Sharp
Instar III

Description. Color: Head capsule predominantly yellow to pale brown; nasale creamy white to pale yellow; head appendages predominantly creamy white to pale yellow except mandible dark brown distally; body predominantly brown, except protergum and abdominal terga VII and VIII predominantly yellow; legs predominantly pale yellow; urogomphi yellow to pale brown, lightly darker distally. **Body:** Subcylindrical, narrowing towards abdominal apex. Measurements and ratios characterizing body shape shown in Table 1. **Head:** Head capsule (Fig. 1) pear-shaped, tapering posteriorly, lacking a neck constriction; ecdysial suture well-developed, coronal suture short; frontoclypeus bluntly rounded, spatulate anteriorly, narrow and elongate, lacking lateral notches; dorsal surface lacking egg bursters (ruptor ovi of Bertrand 1972); epicranial plates meeting ventrally; ocularium present, stemmata not visible ventrally and subdivided into 2 vertical series, stemmata of posterior row more widely spaced; tentorial pits visible medio-ventrally at about midlength. Antenna elongate, slightly shorter than HW; composed of 4 antennomeres, A2 and A3 longest, A4 shortest; A3' relatively elongate, shorter than A4, A3 with a ventroapical spinula. Mandible prominent, falciform, distal half projecting inwards and upwards, apex sharp; mandibular channel present. Maxilla: stipes short and thick, incompletely sclerotized ventrally; cardo fused to stipes; galea and lacinia absent; MP elongate, subequal in length to antenna, composed of 3 palpomeres; MP1 and MP2 longest, subequal in length, MP3 shortest. Labium: prementum subrectangular, much longer than broad, lacking marginal spinulae; LP elongate, distinctly shorter than MP; composed of 2 palpomeres, LP2 subfusiform, slightly longer than LP1. **Thorax:** Terga convex, pronotum slightly shorter than meso- and metanota combined, meso- and metanota subequal; protergite subrectangular to subovate, more developed than meso- and metatergites; meso- and metatergites transverse, with anterotransverse carina; sagittal line well-visible on 3 tergites; sterna membranous; spiracles present on mesothorax. **Legs:** Long (Figs. 3–4), composed of 6 articles (*sensu* Lawrence 1991); L1 shortest, L3 longest; CO robust, elongate, TR divided into 2 parts by an annulus, FE, TI, and TA slender, subcylindrical, PT with 2 long, slender, slightly curved claws; posterior claw shorter than anterior claw on L1 and L2, posterior claw longer than anterior claw on L3; ventral surface of TI and TA lacking elongate spinulae. **Abdomen:** 8-segmented (Fig. 2); segments I–II sclerotized dorsally, membranous ventrally; segments III–V sclerotized dorsally, with a ventral plate, segments VI–VIII completely sclerotized, ring-like; all tergites lacking sagittal line, with anterotransverse carina; spiracles present lateroventrally on segments I–VII; segment VIII (Fig. 2)

Table 1. Measurements and ratios for the third instar of *Coelhydrus brevicollis* (COEB) and *Darwinhydrus solidus* (DARS). l = length; w = width; ** = missing data.

Variable	COEB (*n* = 5)	DARS (*n* = 5)	Variable	COEB (*n* = 5)	DARS (*n* = 5)
HL (mm)	1.41-1.48	1.37-1.42	L1 (mm)	2.65-2.78	2.36-2.44
HW (mm)	0.93-0.99	0.82-0.86	L3 (mm)	3.56-3.79	2.94-3.23
FRL (mm)	1.10-1.18	1.03-1.09	L3/HW	3.62-3.87	3.50-3.79
OCW (mm)	0.69-0.71	0.55-0.60	L3(PC/TA)	0.28-0.32	0.25-0.29
HL/HW	1.42-1.52	1.61-1.66	L3/L1	1.34-1.36	1.25-1.33
HL/LAS	2.41-2.71	1.92-2.04	LAS (mm)	0.55-0.58	0.69-0.72
HW/OCW	1.34-1.43	1.43-1.56	LAS/HW	0.56-0.61	0.81-0.87
A/HW	0.84-0.88	1.06-1.10	U1 (mm)	2.21-2.38	1.65-1.78
A3/A2	0.97-1.05	0.91-1.00	U1+U2'(mm)	**	1.84
A4/A3	0.18-0.22	0.20-0.21	U1+U2 (mm)	**	2.14
A3'/A4	0.66-0.76	0.80-0.93	U1/U2'	**	14.48
MN(l/w)	4.70-4.92	4.06-4.65	U1/LAS	3.88-4.36	2.36-2.49
A/MP	1.02-1.04	1.13-1.15	U1/HW	2.28-2.47	1.96-2.09
MP2/MP1	1.00-1.02	0.87-0.95	U1+U2'/LAS	**	2.66
MP/LP	2.53-2.60	1.78-1.86	U1+U2'/HW	**	2.17
LP2/LP1	1.03-1.17	0.83-0.96	U1+U2/LAS	**	3.09
SpW/FCNW	1.63-1.81	1.57-1.83	U1+U2/HW	**	2.51
FCL/FCNW	9.24-10.81	7.55-9.15			

Table 2. Number of secondary setae on the legs of third instars of selected genera of Hyphydrini: *Andex* (ANDE); *Coelhydrus* (COEL); *Darwinhydrus* (DARW); *Desmopachria* (DESM); *Hyphydrus* (HYPH); *Microdytes* (MICR); *Primospes* (PRIM). CO = coxa; FE = femur; TA = tarsus; TI = tibia; TR = trochanter; Total = total number of secondary setae on segment. Sensillar series: A = anterior; AD = anterodorsal; ADi = anterodistal; AV = anteroventral; D = dorsal; NS = natatory setae; PD = posterodorsal; Pr = proximal; PV = posteroventral; V = ventral; *n* = number of species examined.

Segment	Sensillar series	ANDE (*n* = 1)	COEL (*n* = 1)	DARW (*n* = 1)	DESM (*n* = 1)	HYPH (*n* = 6)	MICR (*n* = 1)	PRIM (*n* = 1)
ProCO	D	9-16	8-10	7-9	3-5	4-11	4-5	8-11
	A	11-14	4-9	4-7	0	3-8	1	5-10
	V	16-27	5-9	6-10	4-6	3-16	2	10-15
	Total	37-57	19-26	17-24	8-10	11-32	7-8	25-36
ProTR	Pr	3-5	2-4	2-3	0	1-3	1	3-4
	ADi	1-3	0	0	0	0	0	0-1
	Total	4-7	2-4	2-3	0	1-3	1	4-5
ProFE	NS (PD)	22-27	14-19	12-15	4-6	17-29	0	22-23
	AD	16-21	9-12	13-15	3-4	5-13	7-8	13-18
	AV	17-24	8-11	13-17	4-6	8-18	0-2	13-16
	PD	0	0	0	0	0	0	1
	PV	14-17	8-10	7-10	3-6	3-9	2-3	6-10
	Total	72-89	41-48	46-52	16-22	35-65	9-11	58-63
ProTI	NS(PD)	25-27	18-22	13-16	4-6	21-35	0-2	21-24
	AD	11-15	6-8	7-10	1	4-9	2-3	7-11
	AV	10-16	6-8	6-10	1-2	2-14	0-2	7-8
	PV	7-10	3-4	6-7	3-4	3-8	2	4-5
	Total	53-67	36-42	33-41	9-12	33-58	5-8	41-45
ProTA	NS(PD)	19-20	13-15	10-11	3-4	13-22	0-1	14-17
	AD	3-5	3-4	3-6	1	1-4	1	2-5
	AV	9-10	5-7	7-10	0-2	4-10	1	6-8
	PV	7-8	4-5	7-9	2-5	4-9	1-3	5-9
	Total	39-43	27-29	29-35	8-11	25-38	4-6	29-36
MesoCO	D	10-13	9-11	7-10	4-6	5-13	2-5	10-14
	A	9-14	4-6	5-9	0	3-8	2	7-10
	V	14-24	7-12	5-13	4-5	4-19	3-4	9-14
	Total	35-48	20-28	18-28	8-11	15-41	7-10	30-37
MesoTR	Pr	4-7	2-4	2-4	1	1-3	1	3-4
	ADi	2-3	0	0	0	0	0	0-1
	Total	6-10	2-4	2-4	1	1-3	1	3-4
MesoFE	NS(PD)	25-32	16-20	15-17	2-5	20-30	0	20-23
	AD	23-28	12-17	15-19	4-5	7-16	8-9	19-23
	AV	18-25	8-12	12-14	3-4	6-16	0	9-13
	PD	0	0	0	0	0	0	1
	PV	17-26	9-10	9-14	5-6	6-13	4-5	8-14
	Total	88-111	46-58	54-59	16-18	43-71	12-14	58-70
MesoTI	NS (PD)	32-36	20-29	16-23	7-8	26-48	1-2	26-31
	AD	19-23	9-13	9-13	2-4	5-13	2-4	11-15
	AV	11-14	4-6	6-8	1-2	4-15	1-2	6-7
	PV	11-16	4-7	6-9	5	4-9	1-2	6-8
	Total	77-89	37-53	38-50	16-18	45-73	8-9	49-57
MesoTA	NS(PD)	31-34	20-27	17-19	4-6	20-29	1-3	22-26
	AD	5-7	4-5	5-8	1	1-5	1-2	3-5
	AV	9-11	6-7	9-11	1-2	2-10	0-1	7-8
	PV	8-13	6-10	10-11	5-6	6-12	2-3	7-10
	Total	54-63	39-45	42-47	11-15	32-51	5-8	39-47
MetaCO	D	10-12	10-12	7-13	4-7	7-19	2-4	12-20
	A	10-14	4-7	6-7	0	4-12	2-3	5-9
	V	13-24	9-13	9-14	3-5	7-25	3-4	13-15
	Total	34-47	24-31	22-31	8-12	20-50	7-11	33-43
MetaTR	Pr	4-7	3-4	3-4	1	1-5	1	3-6
	ADi	2-6	0	0	0	0-1	0	0
	Total	6-13	3-4	3-4	1	1-5	1	3-6

Continued on next page

Table 2. Continued.

Segment	Sensillar series	ANDE (n = 1)	COEL (n = 1)	DARW (n = 1)	DESM (n = 1)	HYPH (n = 6)	MICR (n = 1)	PRIM (n = 1)
MetaFE	NS (PD)	23-29	14-17	12-16	0	12-30	0	19-24
	AD	24-33	16-20	15-24	6-7	9-21	7-10	23-28
	AV	17-23	8-10	12-15	2-4	8-19	0	11-15
	PD	0	0	0	0	0-2	0	1
	PV	19-26	9-11	12-15	8-11	7-17	6-7	10-15
	Total	87-111	48-55	55-63	17-21	38-84	13-16	69-77
MetaTI	NS(PD)	34-40	27-30	17-22	7-9	28-45	1-2	27-31
	AD	21-28	10-15	12-16	3-5	7-17	6	14-18
	AV	11-15	6	7-10	0-4	7-19	0-1	6-9
	PV	11-16	5-7	8-10	4-8	5-13	3-4	7
	Total	79-96	49-57	44-55	19-22	53-84	11-12	58-60
MetaTA	NS(PD)	33-38	24-30	18-21	6-9	23-37	2-3	26-35
	AD	6-10	5-6	6-11	1-2	1-6	1	4-6
	AV	9-11	6-7	9-12	2-3	4-11	2	9-10
	PV	11-13	7-9	11-13	7-9	8-15	3-5	9-12
	Total	62-68	43-50	47-54	18-21	38-64	9-10	49-59

longest and narrowest, projecting backwards into short subconical siphon, well constricted at point of insertion of urogomphi. Urogomphus very long, composed of 2 urogomphomeres; U1 long, much longer than segment VIII; U2 narrower, setiform (length of U2 could not be measured as the structure was broken on every specimen studied). **Chaetotaxy:** Head capsule with numerous secondary setae; lateroventral margin of PA with several secondary spine-like setae; anteroventral margin of nasale with half circle of about 50 lamellae clypeales of different lengths, directed downwards; AN, MX, and LA lacking secondary setae; MN with 1 hair-like secondary seta on basoexternal margin; thoracic and abdominal sclerites I–VIII with numerous secondary setae mainly on posterior half; natatory setae present on dorsal margin of femora, tibiae, and tarsi; secondary leg setation detailed in Table 2 and Figs. 3–4; U with secondary setae (Fig. 2).

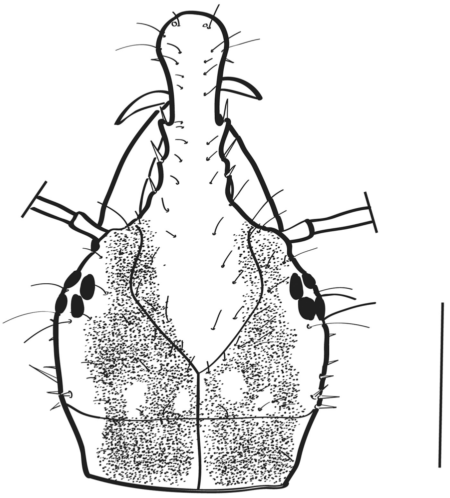
Distribution. Endemic to the Republic of South Africa.

Description of Larvae of *Darwinhydrus* Sharp
(Figs. 5–8)

Diagnostic Combination. The third instar of *Darwinhydrus* can be distinguished from those of other genera of Hyphydrini that have been associated with adults by the following combination of characters: frontoclypeus with short lateral processes, well-visible in dorsal view; HL = 1.00–1.50 mm; ratio HL/LAS = 1.90–2.20; ratio MP/LP = 1.60–1.90; ratio A4/A3 < 0.30; ratio LP2/LP1 < 1.30; metathoracic leg > 3.50 times HW; ratio LAS/HW < 0.90; primary setae LA3, LA4, and LA5 articulated distally on prementum; dorsal meso- and metafemoral secondary setae present; procoxa with more than 15 secondary setae; femora, tibiae, and tarsi with more than 8, 10, and 10 dorsal natatory setae, respectively; siphon moderately elongate, clearly constricted at point of insertion of urogomphi, lacking secondary spine-like setae on ventral surface.

Darwinhydrus solidus Sharp
Instar III

Description. Color: Head capsule predominantly dark brown; narrowly yellow along lateral margin of parietale and frontoclypeus (Fig. 5); head appendages predominantly yellow except apically dark brown mandible; body predominantly dark brown, protergum with a large yellowish macula on each side; siphon yellow to pale brown apically; legs yellow to pale brown, slightly darker over coxae; urogomphus dark brown, pale yellow proximally. **Head:** Head capsule (Fig. 5) pear-shaped, tapering posteriorly, lacking a neck constriction; ecdysial suture well-developed, coronal suture short; frontoclypeus bluntly rounded, spatulate anteriorly, narrow and elongate, with several lateral notches well-visible in dorsal view; dorsal surface lacking egg bursters (ruptor ovi of Bertrand 1972); epicranial plates meeting ventrally; ocularium present, stemmata not visible ventrally and subdivided into 2 vertical series, stemmata of posterior row more widely spaced; tentorial pits visible medio-ventrally at about midlength. Antenna elongate, slightly shorter than HW; composed of 4 antennomeres, A2 and A3 longest, A4 shortest; A3' relatively elongate, subequal in length to A4, A3 with a ventroapical spinula. Mandible prominent, falciform, distal half projecting inwards and upwards, apex sharp; mandibular channel present.



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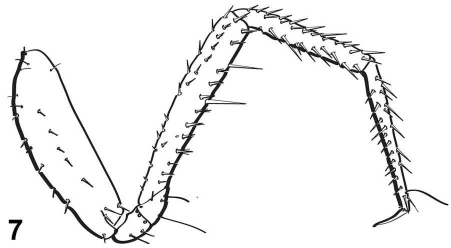
Figs. 5–6. *Darwinhydrus solidus*, instar III. **5)** Head capsule, dorsal view; **6)** Abdominal segment VIII and proximal portion of urogomphi, dorsal view. Scale bars = 0.50 mm.



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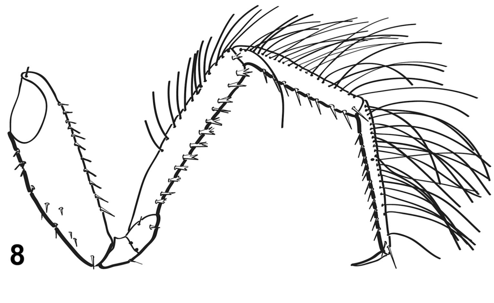

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Figs. 7–8. *Darwinhydrus solidus*, instar III, metathoracic leg. **7)** Anterior surface; **8)** Posterior surface. Scale bar = 0.50 mm.

Maxilla: stipes short and thick, incompletely sclerotized ventrally; cardo fused to stipes; galea and lacinia absent; MP elongate, subequal in length to antenna, composed of 3 palpomeres; MP1 and MP2 longest, subequal in length, MP3 shortest. Labium: prementum subrectangular, much longer than broad, lacking marginal spinulae; LP elongate, distinctly shorter than MP; composed of 2 palpomeres, LP2 subfusiform, slightly shorter than LP1. **Thorax:** Terga convex, pronotum slightly shorter than meso- and metanota combined, meso- and metanota subequal; protergite subrectangular to subovate, more developed than meso- and metatergites; meso- and metatergites transverse, with anterotransverse carina; sagittal line well-visible on 3 tergites; sterna membranous; spiracles present on mesothorax. **Legs:** Long (Figs. 7–8), composed of 6 articles (*sensu* Lawrence 1991); L1 shortest, L3 longest; CO robust, elongate, TR divided into 2 parts by an annulus, FE, TI, and TA slender, subcylindrical, PT with 2 long, slender, slightly curved claws; posterior claw shorter than anterior claw on L1 and L2, posterior claw longer than anterior claw on L3; ventral surface of TI and TA lacking elongate spinulae. **Abdomen:** 8-segmented (Fig. 6); segments I–II sclerotized dorsally, membranous ventrally; segments III–V sclerotized dorsally, with a ventral plate, segments VI–VIII completely sclerotized, ring-like; all tergites lacking sagittal line, with anterotransverse carina; spiracles present lateroventrally on segments I–VII; segment VIII longest and narrowest (Fig. 6), projecting backwards into more or less elongate subconical siphon, well-constricted at point of insertion of urogomphi. Urogomphus very long, composed of 2 urogomphomeres; U1 long, much longer than segment VIII; U2 narrower, setiform, much shorter than U1. **Chaetotaxy:** Head capsule with numerous secondary setae; lateroventral margin of PA with several secondary spine-like setae; anteroventral margin of nasale with half circle of about 60 lamellae clypeales of different lengths, directed downwards; AN, MX, and LA lacking secondary setae; MN with 1 hair-like secondary seta on basoexternal margin; thoracic and abdominal sclerites I–VIII with numerous secondary

Table 3. Characters used for the phylogenetic analysis and the coding of states using selected genera of Hydroporinae as outgroup. 0 indicates plesiomorphic state and number; > 0 indicates progressively more apomorphic states.

(00)	Nasale	0 - broad, subtriangular 1 - narrow, more or less parallel sided
(01)	Apex of nasale	0 - not spatulate 1 - spatulate
(02)	Frontoclypeus	0 - lateral processes lacking, if present then barely visible in dorsal view 1 - with one lateral process well-visible in dorsal view 2 - with several short lateral processes well-visible in dorsal view
(03)	Epicranial plates	0 - separate at the ventral midline 1 - meet on the ventral midline
(04)	Seta FR7	0 - spine-like 1 - hair-like
(05)	Primary seta FR13	0 - absent 1 - present
(06)	Pore FRb	0 - present 1 - absent
(07)	Seta PA3	0 - inserted contiguously to setae PA1 and PA2 1 - inserted far from setae PA1 and PA2
(08)	Pore PAc	0 - not inserted anteriorly to stemmata 1 - inserted anteriorly to stemmata
(09)	Pore PAe	0 - present 1 - absent
(10)	Pore PAj	0 - present 1 - absent
(11)	Secondary spine-like setae on lateral margin of parietals	0 - present 1 - absent
(12)	Antennomere II	0 - longer than antennomere I 1 - subequal in length to antennomere I
(13)	Antennomere III	0 - longer than antennomere I 1 - subequal in length to antennomere I
(14)	Antennomere III	0 - spinula absent 1 - spinula present
(15)	Seta AN3	0 - inserted distally 1 - inserted submedially
(16)	Primary pore ANf	0 - present 1 - absent
(17)	Primary pore ANh	0 - present 1 - absent
(18)	Cardo	0 - not fused to stipes 1 - fused to stipes
(19)	Setae MX4. MX5 and MX6	0 - present 1 - absent
(20)	Setae MX8 and MX9	0 - present 1 - absent
(21)	Pore MXh	0 - inserted on the galea 1 - inserted on the stipes 2 - absent
(22)	Prementum	0 - broader than long 1 - as broad as long 2 - longer than broad
(23)	Labial palpomere 2	0 - narrow, subcylindrical, narrowing to apex 1 - robust, broadest at midlength
(24)	Primary setae LA3, LA4, LA5	0 - articulated distally 1 - articulated proximally
(25)	Seta LA6	0 - inserted distally 1 - inserted submedially
(26)	Primary seta LA10	0 - articulated medially 1 - articulated distally
(27)	Pore LAb	0 - present 1 - absent

Continued on next page

Table 3. Continued.

(28)	Prementum	0 - lacking secondary setae 1 - with one secondary seta 2 - with several secondary setae
(29)	Primary seta TR2	0 - present 1 - absent
(30)	Primary pore FEa	0 - present 1 - absent
(31)	Dorsal mesofemoral natatory setae	0 - absent 1 - present
(32)	Dorsal metafemoral natatory setae	0 - absent 1 - present
(33)	Ventral femoral natatory setae	0 - absent 1 - present
(34)	Seta TI7	0 - short, spine-like 1 - long, hair-like
(35)	Primary pore TIa	0 - present 1 - absent
(36)	Natatory posterodorsal setae on tibia	0 - absent 1 - present
(37)	Dorsal tarsal natatory setae	0 - absent 1 - present
(38)	Ventral surface of abdominal segment II	0 - membranous 1 - sclerotized
(39)	Ventral surface of abdominal segment III	0 - membranous 1 - sclerotized
(40)	Ventral surface of abdominal segments IV–V	0 - membranous 1 - sclerotized
(41)	Ventral surface of abdominal segment VI	0 - membranous 1 - sclerotized
(42)	Siphon	0 - not constricted at point of insertion of urogomphi 1 - strongly constricted at point of insertion of urogomphi
(43)	Setae AB6 and AB7	0 - short 1 - elongate
(44)	Primary pore ABa	0 - present 1 - absent
(45)	Siphon	0 - lacking secondary setae ventrally 1 - with secondary setae ventrally
(46)	Seta UR5	0 - long, hair-like 1 - short, spine-like
(47)	Seta UR8	0 - inserted apically on urogomphomere 2 1 - inserted submedially on urogomphomere 2 2 - inserted proximally on urogomphomere 2
(48)	Urogomphomere I	0 - lacking secondary setae 1 - with secondary setae

setae, mainly on posterior half; natatory setae present on dorsal margin of femora, tibiae, and tarsi; secondary leg setation detailed in Table 2 and Figs. 7–8; U with secondary setae (Fig. 6).

Distribution. Endemic to the Republic of South Africa.

Key to the Third Instars of Hyphydrini Genera

The following key was prepared to separate members of the Hyphydrini genera known in enough detail. However, because Hyphydrini is very speciose and the larvae of several genera are still unknown, the present key is preliminary. We propose it to provide a template upon which future descriptions can be compared.

1. Egg bursters present on frontoclypeus.. Instar I

1′. Egg bursters absent on frontoclypeus 2

2. Spiracles absent on mesothorax and abdominal segments I–VII .... Instar II

2′. Spiracles present on mesothorax and abdominal segments I–VII (Instar III). 3

3. Dorsal metafemoral natatory setae absent; procoxa with less than 15 secondary setae; femur with less than 8 natatory setae; tibia with

less than 10 natatory setae; tarsus with less than 10 natatory setae; ratio A4/A3 > 0.30; smaller species, HL less than 1.00 mm 4

3′. Dorsal metafemoral natatory setae present; procoxa with more than 15 secondary setae; femur with more than 8 natatory setae; tibia with more than 10 natatory setae; tarsus with more than 10 natatory setae; ratio A4/A3 < 0.30; larger species, HL more than 1.00 mm 5

4. Frontoclypeus with several lateral notches well-visible in dorsal view (*e.g.*, Fig. 5); siphon clearly constricted at point of insertion of urogomphus (*e.g.*, Figs. 2, 6); primary setae LA3, LA4, and LA5 articulated distally on prementum; ratio HL/LAS > 1.50; ratio MP/LP > 1.60; ratio LP2/LP1 < 1.30; siphon lacking spine-like setae on ventral surface .... ***Microdytes* Balfour-Browne**

4′. Frontoclypeus lacking lateral notches (*e.g.*, Fig. 1); siphon not constricted at point of insertion of urogomphus; primary setae LA3, LA4, and LA5 articulated proximally on prementum; ratio HL/LAS < 1.20; ratio MP/LP < 1.40; ratio LP2/LP1 > 1.50; siphon with at least 1 ventral spine-like setae on ventral surface .... ***Desmopachria* Babington**

5. Frontoclypeus with several lateral notches well-visible in dorsal view (Fig. 5); ratio MP/LP < 1.90 6

5′. Frontoclypeus lacking lateral notches (Fig. 2); ratio MP/LP > 2.00 7

6. Smaller species, HL < 1.50 mm; ratio HL/LAS < 2.20 .... ***Darwinhydrus* Sharp**

6′. Larger species, HL > 1.80 mm; ratio HL/LAS > 2.40 .... ***Andex* Sharp**

7. Siphon elongated, not constricted at point of insertion of urogomphus; ratio HL/LAS < 1.20; siphon with several spine-like setae on ventral surface; metathoracic leg < 3.30 times HW .... ***Hyphydrus* Illiger**

7′. Siphon short, clearly constricted at point of insertion of urogomphus (Fig. 2); ratio HL/LAS > 2.40; siphon lacking spine-like setae on ventral surface; metathoracic leg > 3.50 times HW 8

8. Ratio MP/LP < 2.20; ratio LAS/HW > 1.20; larger species, HL > 1.60 mm .... ***Primospes* Sharp**

8′. Ratio MP/LP > 2.40; ratio LAS/HW < 0.90; smaller species, HL < 1.50 mm .... ***Coelhydrus* Sharp**

Cladistic Analysis

Forty-nine characters (44 binary and five multistate) were coded for larvae of seven genera of Hyphydrini and two outgroups. The characters used and their states are listed in Table 3. The analysis of the data matrix (Table 4) with TNT resulted in three most parsimonious cladograms of 71 steps (CI = 0.76; RI = 0.61), which differed in the relative position of the genera *Andex*, *Darwinhydrus*, and *Primospes*. The strict consensus tree was calculated (Fig. 9), in which both *Coelhydrus* and *Darwinhydrus* are part of a relatively well-supported clade Hyphydrini except *Microdytes*. Character state changes are mapped on one of the most parsimonious trees (Fig. 10).

Discussion

Larvae of *Coelhydrus* and *Darwinhydrus* were described and documented in detail in this contribution.

Table 4. Character matrix of 49 characters of selected genera of Hyphydrini, using Laccornini and Pachydrini as outgroups. Columns correspond to the character numbers listed in Table 3.

	Character									
Genera	**00000 01234**	**00000 56789**	**11111 01234**	**11111 56789**	**22222 01234**	**22222 56789**	**33333 01234**	**33333 56789**	**44444 01234**	**4444 5678**
Laccornis	00000	00000	00000	00000	00000	00000	00000	00000	00000	0000
Pachydrus	11110	10000	00110	11111	02100	00111	10011	00000	01001	0011
Andex	11210	11111	10001	11110	11200	00021	11101	11100	11111	0121
Coelhydrus	11010	11111	10001	11110	11210	00001	11101	01101	11111	0??1
Darwinhydrus	11210	11111	10001	11110	11200	00101	11101	01101	11111	0??1
Desmopachria	10011	11111	11001	11111	11211	11101	11001	01111	11011	1121
Hyphydrus	11011	11111	11001	01110	11210	00011	11101	01100	11011	1121
Microdytes	00211	11011	10001	01110	11200	00001	10000	01111	11111	0021
Primospes	11010	11111	10001	11110	11200	00001	11101	01101	11111	0?21

Missing data coded with ‘?’.

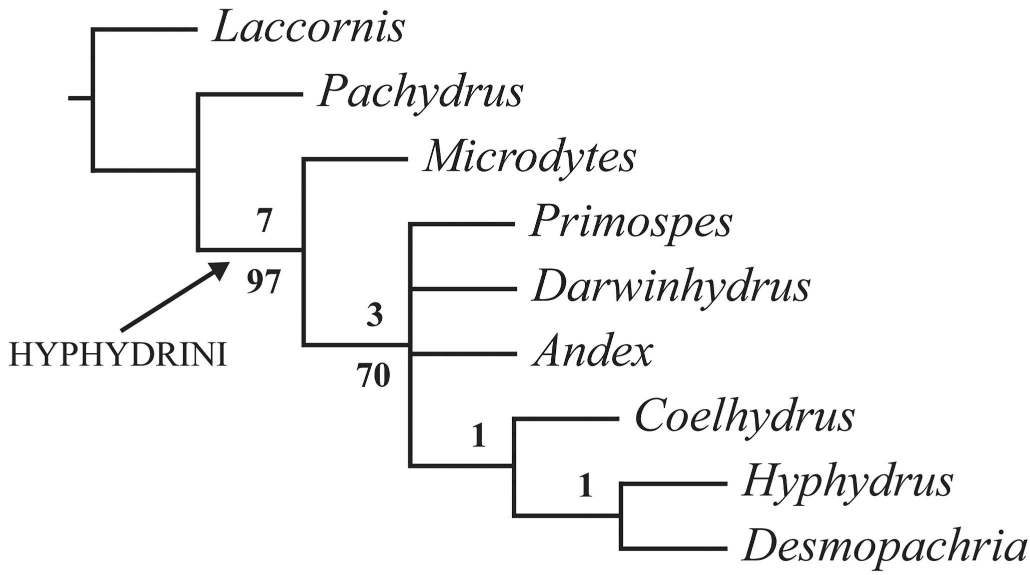

Fig. 9. Strict consensus cladogram obtained from the cladistic analysis of nine terminal taxa of Hydroporinae, with Bremer support values indicated above branches and Jackknife values higher than 50 indicated below branches.

As the larvae of five other genera of Hyphydrini have also been described with much detail (Alarie *et al.* 1997; Alarie and Watts 2005; Alarie and Challet 2006a, b; Michat and Archangelsky 2007), it seems worthwhile to attempt a comparison of the genera in this article.

Placement of *Coelhydrus* and *Darwinhydrus* within the tribe Hyphydrini seems well supported based on larval morphology (Jackknife support value = 97; Bremer support value = 7). Larvae of both these genera share with other members of the Hyphydrini several character states as per comparison with the outgroups studied (Fig. 10): pore FRb absent (character 06); pore PAc inserted anteriorly to stemmata (character 08); pore PAe absent (character 09); pore PAj absent (character 10); presence of a ventroapical spinula on antennomere 3 (character 14); setae MX8 and MX9 present

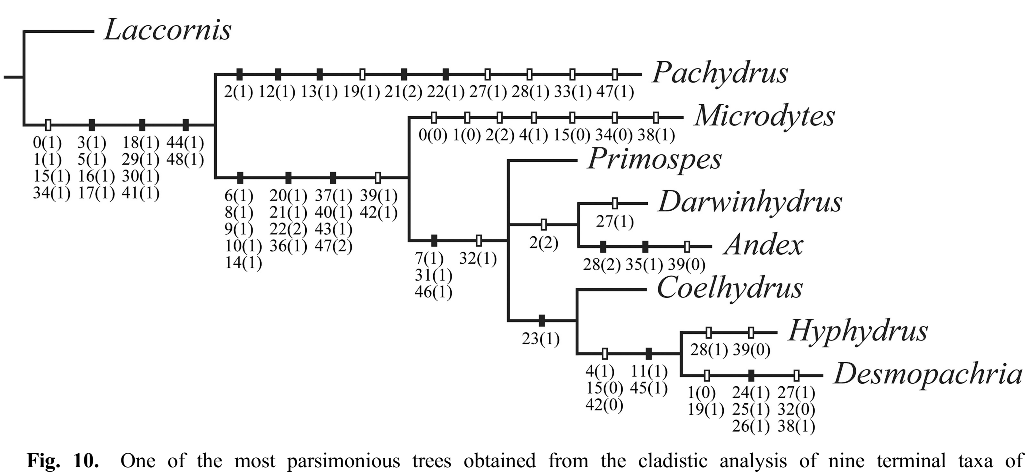

Fig. 10. One of the most parsimonious trees obtained from the cladistic analysis of nine terminal taxa of Hydroporinae, with character changes mapped for each clade (using ACCTRAN optimization). Solid rectangles indicate unique character state transformations; open rectangles indicate homoplasious character state transformations. Numbers outside parentheses are characters listed in Table 3; numbers within parentheses are character states as given in Table 3.

(character 20); pore MXh inserted on stipes (character 21); prementum longer than broad (character 22); natatory posterodorsal setae present on tibiae (character 36); natatory setae present on tarsi (character 37); ventral surface of abdominal segments IV–V sclerotized (character 40); and, setae AB6 and AB7 elongate (character 43). Based on the actual knowledge of the larval morphology of the Hyphydrini, it is postulated that seta UR8 would likely be inserted proximally on urogomphomere 2 (character 47) in both *Coelhydrus* and *Darwinhydrus* like for any other Hyphydrini. Owing to damage to the urogomphi on the specimens studied, however, the exact position of seta UR8 in these two taxa could not be assessed.

A high jackknife value (70) also indicates strong support for the monophyly of *Primospes* + *Andex* + *Coelhydrus* + *Hyphydrus* + *Desmopachria*. All five genera are characterized by seta PA3 positioned apart from both setae PA1 and PA2 (character 07) as well as by the presence of natatory setae along the dorsal margin of the mesofemora (character 31).

Coelhydrus is here postulated to be sister to *Hyphydrus* + *Desmopachria*, owing to the shared presence in these taxa of a robust and broad labial palpomere 2 (character 23). Such results contradict former studies of members of Hyphydrini (Ribera and Balke 2007; Ribera *et al.* 2008), which postulated a monophyletic origin of the five endemic hyphydrine genera of the Cape Region. Whereas inclusion of *Coelhydrus* among a clade comprised of *Hyphydrus* and *Desmopachria* seems supported based on larval morphology, the relationship of *Darwinhydrus* remains unresolved. Indeed, in the context of this study, *Darwinhydrus* stands as part of a polytomy including *Primospes* and *Andex*. It is noteworthy, however, that both *Andex* and *Darwinhydrus* share a similar frontoclypeus characterized by the presence of several lateral processes (character 02). Such character state may be suggestive of a closer phylogenetic relationship between these taxa in accordance with recent studies based on molecular analyses (Ribera and Balke 2007; Ribera *et al.* 2008). This hypothesis, based on the larval characters, requires a larger data set including more characters, taxa, and/or life stages.

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