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LARVAL MORPHOLOGY OF *AGABETES* CROTCH (COLEOPTERA: ADEPHAGA: DYTISCIDAE): THE HYPOTHESIS OF SISTER-GROUP RELATIONSHIP WITH THE SUBFAMILY LACCOPHILINAE REVISITED

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Abstract

Description of all larval instars of *Agabetes acuductus* (Harris) is presented including a detailed chaetotaxic and porotaxic analysis of the cephalic capsule, head appendages, legs, last abdominal segment and urogomphi. A parsimony analysis based on the 43 informative larval characteristics was conducted with PAUP. Boostrap values indicate strong support for the monophyly of the clade containing the genus *Agabetes* Crotch and members of the subfamily Laccophilinae. This is supported by five synapomorphies: (i) egg bursters positioned posteriorly, contiguous to the coronal suture, (ii) presence of two lamellae clypeales in instar I, (iii) absence of the primary setae LA10 and LA12, (iv) absence of a ventroapical spinula on antennomere 3, and (v) abdominal venter 6 sclerotized.

The genus name *Agabetes* was proposed by Crotch (1873) to receive the single Nearctic species *Colymbetes acuductus* Harris. More recently, a second species, *A. svetlanae* Nilsson (1989), was described from Iran. The taxonomic placement of the genus *Agabetes* has been the matter of much debate. Traditionally, *Agabetes* has been treated as a member of the subfamily Colymbetinae either as a distinct tribe, the Agabetini (Branden 1885; Pederzani 1995) or as member of the tribe Copelatini (Zimmermann 1920; Gueorguiev 1968). In a comparative study of the Hydradephaga ovipositor, Burmeister (1976, 1990) established several synapomorphies between *Agabetes* and the genus *Laccophilus* Leach (subfamily Laccophilinae) and postulated that the genus *Agabetes* (i) represents a separate subfamily (Agabetinae), (ii) it should occupy a more basal phylogenetic position within the Dytiscidae, and (iii) it is the sister-group to the subfamily Laccophilinae. The isolated position of *Agabetes* and its relationship with members of the Laccophilinae was also postulated by Nilsson (1989) who positioned it in a separate tribe (Agabetini) within the Laccophilinae.

The elevation of the genus *Agabetes* to subfamily level, its placement as one of the more primitive subfamilies of the Dytiscidae, and its sister-group relationship to the Laccophilinae merit further investigation and, in particular, need to be broadly tested in a large-scale comparative study including more taxa and larger character sets and using modern cladistic methods. The recent

publication of preliminary larval phylogenetic reconstructions in the dytiscid subfamilies Laccophilinae (Alarie *et al.* 2000), Colymbetinae (Alarie *et al.* 1998; Alarie *et al.* 2001) and Hydroporinae (Alarie *et al.* 1999) provide a basic framework to evaluate the taxonomic status of the genus.

The mature larva of *Agabetes acuductus* was described by Spangler and Gordon (1973). However, as this description did not include detailed chaetotaxic analysis, it can hardly be used in the context of recent studies of larval Dytiscidae. The recent discovery of first and second instars of *Agabetes acuductus* provided the impetus to explore the uncertain cladistic relationship of *Agabetes* with members of the Laccophilinae. Inclusion of the characters found in the first instar is of the utmost interest because those character states are deemed to represent a very conservative expression of the phenotype and as such are phylogenetically very important.

The objectives of our study were: (*i*) description of all instars of *Agabetes acuductus* with an emphasis on the chaetotaxy and the porotaxy of the cephalic capsule, head appendages, legs, last abdominal segment, and urogomphi and (*ii*) phylogenetic comparison of the genus *Agabetes* with the genera of Laccophilinae and Colymbetinae for which larvae have been described in detail. The purpose of this comparison was to reevaluate the hypothesis of sister-group relationship of *Agabetes* with members of the subfamily Laccophilinae.

Materials and Methods

The methods, terminology, and format of this paper follow those of recent studies on larvae of the Laccophilinae (Alarie *et al.* 2000) and of the Colymbetinae (Alarie and Larson 1998; Alarie *et al.* 1998, 2001). Specimens representative of each of the three instars were disarticulated and mounted on standard glass slides with Hoyer's medium. Examination at magnifications of 80–800× was done using an Olympus BX50 compound microscope equipped with Nomarsky differential interference optics. Voucher specimens are deposited in the research larval collection of Y.A. (Laurentian University, Department of Biology, Sudbury, Ontario, Canada), and in the National Museum of Natural History, Smithsonian Institution, Washington.

Measurements. The part to be measured was adjusted so that it was, as nearly as possible, parallel to the plane of the objectives. Abbreviations and definitions of some terms used in descriptions are:

HL: total head length including the frontoclypeus measured medially along the epicranial stem.

HW: maximum head width measured posterior to the stemmata.

FCL: length of frontoclypeus measured from apex of the frontoclypeus to the back of the ecdysial suture.

OcW: maximum occipital foramen width measured along the dorsal margin.

Length of antenna: derived by adding the length of each individual antennomere; antennomeres are indicated by the capital letter A and a number corresponding to the segment considered (*e.g.*, **A1** for antennomere 1); **A3′** is the lateral elongation of antennomere 3 (= sensorial appendage).

Length of maxillary and labial palpus: derived by adding the length of each individual palpomere, excluding the length of palpifer.

Length of maxillary galea: maximal length measured from apex of the galea to margin of the maxillary stipes.

Length of legs: derived by adding the length of each individual segment including the longest claw; the length of each segment was taken at the longest

point except for the trochanter, which includes only the proximal portion (the length of distal portion being included in the femoral length).

LLAS: dorsal length of last abdominal segment; includes the whole sclerite measured from the anterior margin of the prescutum to the apex of the siphon. Siphon refers to the dorsal prolongation of the 8th abdominal segment (= last abdominal segment); the length of the siphon was determined by measuring the difference between the dorsal and ventral lengths of the segment.

Length of urogomphus: maximum length measured along the outer margin.

Chaetotaxic Analysis. Primary and secondary setae and pores were distinguished on the cephalic capsule, head appendages, legs, last abdominal segment, and urogomphi. The setae and pores are coded according to the systems proposed by Alarie (1995) for the legs, the last abdominal segment and urogomphi, and Alarie (1998) for the cephalic capsule and head appendages. The count of number of secondary setae present on anteroventral surface (AV) of femur was more difficult owing to the presence of two additional primary setae within *Agabetes*. In order to solve that problem, we applied the following rule:

Number of AV secondary setae of femur = total number of setae over anteroventral surface − [number of primary setae (= 6, excluding seta FR1) + 2]

Color. Description of color is based on ethanol-preserved specimens.

Pectens: refers to comb-like spinula [= ''écailles pectinées'' of Bertrand (1928)] on the ventral margin of femur.

Material Examined. Descriptions of the larval stages and taxonomic conclusions reported in this paper are based on examinations of three first-, eight second-, and 16 third instar larvae collected in association with adults at the following locality: U.S.A. Maryland, Worcester Co. 10 km SW Pokomoke City, Hickory Point, 38°01′N, 75°38′W, 19.v.1997, coll. W.E. Steiner; Talbot Co. 3 km SE Easton, Seth Forest, 38°45′N, 76°02′W, 05.v.1997, 10.v.1997. 08.vi.1997. colrs. W.E. Steiner and J.M. Swearingen; Talbot Co. Wittman, 38°48′N, 76°17′W, 25.v.1997, colrs. W.E. Steiner and J.M. Swearingen. The identification is firm as *A. acuductus* is the only species of *Agabetes* found in the Nearctic.

Parsimony Analysis. To test the monophyletic relationships of the genus *Agabetes* with members of the Laccophilinae, a phylogenetic analysis of all species of Colymbetinae and Laccophilinae with sufficiently detailed larval descriptions (Alarie 1995, 1998; Alarie and Larson 1998; Alarie *et al.* 1998, 2000, 2001), was made, using *Copelatus glyphicus* (Say) as the outgroup. The genus *Copelatus* is deemed to represent the sister-group of other Dytiscidae (Ruhnau and Brancucci 1984). The characters used (Table 1) as well as the distribution of character states among the terminal taxa (Table 2) are listed.

The parsimony analysis was based on the 43 informative characters (within the matrix shown in Table 2). All characters were treated as non-additive (unordered characters) and equally weighted and were analyzed using the heuristic search algorithms of PAUP 4.0b4a (Swofford 1999). The data were bootstrapped with 100 replicates to assess branch support. The consistency index (CI) (Kluge and Farris 1969) and retention index (RI) (Farris 1989) were given. MacClade 4.0 (Maddison and Maddison 2000) was used to examine the effects of different characters on the phylogeny.

Larvae of *Agabetes acuductus* (Harris)
(Figs. 1–17)

Diagnostic Combination. Head capsule not constricted posteriorly, with temporal spines (instar II and III); instar I with two spatulate lamellae clypeales

Table 1. Characters used for the phylogenetic analysis and the coding of states; 0 indicates plesiomorphic state and number > 0 indicates progressively more apomorphic states.

	Character	States
01	Egg bursters (Instar I)	0-at about level of pore FRb 1-posterior to pore FRb, contiguous to coronal suture
02	Egg burters (Instar I)	0-spine-like 1-blade-like
03	Frontoclypeus (Instar I)	0-rounded posteriorly 1-truncate posteriorly
04	Frontal suture (Instar I)	0-concave, not/more or less sinuate 1-deeply sinuate
05	Lamellae clypeales (Instar I)	0-> 4 1-two
06	Frontoclypeus	0-adnasalia delineated 1-adnasalia not delineated
07	Pore FRe	0-present 1-lacking
08	Labial palpomere 2 (Instar III)	0-<1.70 times length of palpomere 1 1->2.20 times length of palpomere 1
09	Spinulose epipharyngeal band	0-lacking 1-present
10	Setae LA10 and LA12	0-present 1-lacking
11	Pore ANf	0-present 1-lacking
12	Head appendages (Instar II and III)	0-lacking or with a few secondary setae 1-with several secondary setae
13	Seta MX5	0-present 1-lacking
14	Prementum	0-additional pore present 1-additional pore lacking

Table 1. Continued.

15	Occipital suture	0-present 1-lacking
16	Antennomere III, ventroapical spinula	0-spine-like 1-hole-like 2-lacking
17	Antennomere III, additional ventroapical pores (Instar I)	0-absent 1-present
18	Antennomere IV	0-pore ANg inserted basally 1-pore ANg inserted medially
19	Antennomere III, lateral projection (A3′)	0-elongate, ≥ 0.30 times length of A4 1-short, < 0.20 times length of A4
20	Mandible, additional setae (Instar I)	0-absent 1-present
21	Maxilla, galea	0-longer than palpifer 1-shorter or subequal to palpifer
22	Maxillary stipes, additional setae (Instar I)	0-present 1-absent
23	Mandible	0-lacking a mandibular channel 1-with a mandibular channel
24	Legs, natatory setae (Instar II and III)	0-lacking 1-present
25	Seta CO7, meso-and metacoxa	0-articulated distally 1-articulated proximally
26	Coxa, pectens	0-lacking 1-present
27	Trochantera, (Instar II and III)	0-with a few (<10) secondary setae 1-with several (>20) secondary setae
28	Femur, pectens	0-lacking 1-present
29	Seta FE5, metafemur (Instar I)	0-short, spine-like 1-elongate, hair-like

Table 1. Continued.

30	Seta FE5, mesofemur (Instar I)	0-short, spine-like 1-elongate, hair-like
31	Seta FE6, metafemur (Instar I)	0-short 1-elongate
32	Seta FE1	0-proximal 1-distal
33	Femur, dorsal margin (Instar I)	0-lacking additional setae 1-with additional setae
34	Tibia, ventral additional setae (Instar I)	0-absent 1-present
35	Seta TI2, pro- and mesofemur	0-present 1-absent
36	Seta TI6, metatibia	0-short, spine-like 1-elongate, hair-like
37	Seta TI7	0-short 1-elongate
38	Protibia	0-seta TI6 present 1-seta TI6 absent
39	Tibia (Instar I)	0-lacking additional natatory setae 1-with additional natatory setae
40	Pretarsal claws	0-marginal spinulae absent 1-marginal spinulae present
41	Pro- and mesotibiae	0-normal shape 1-chelate
42	Pronotum	0-lacking a neck constriction 1-with a neck constriction
43	Abdominal venter 6	0-membranous 1-sclerotized
44	Siphon	0-lacking a cresent shape setal pattern 1-with a cresent shape setal pattern

Table 1. Continued.

45	Abdominal segment 8 (Instar I)	0-additional setae absent 1-additional setae present
46	Setae AB8 and AB14	0-spine-like 1-lanceolate
47	Urogomphus	0-two-segmented 1-one-segmented
48	Urogomphus	0-short, < 0.60 HW 1-elongate > 0.70 HW
49	Urogomphus, additional setae (Instar I)	0-absent 1-present
50	Urogomphomere 1 (Instar II and III)	0-lacking secondary setae 1-with secondary setae
51	Urogomphomere 1 (Instar II and III)	0-lacking a subbasal suture 1-with a subbasal suture
52	Temporal secondary spines	0-present 1-absent
53	Antennomere 1 (Instar III)	0-longer than broad 1-shorter than broad
54	Abdominal venter 4-5	0-membranous 1-sclerotized

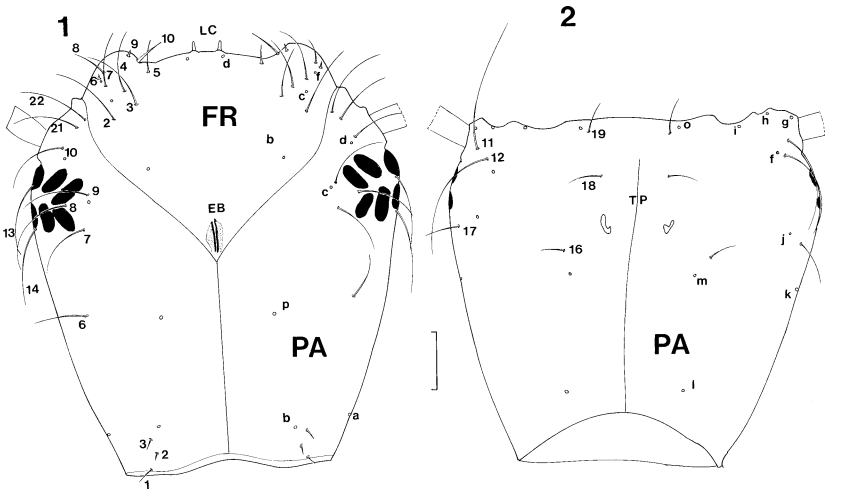
Table 2. Matrix of 54 morphological characters of larvae of selected species of the subfamily Dytiscidae.

Taxon	Characters										
	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–54
Copelatus sp.	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	0000
Agabetes acuductus	11001	01111	00001	20000	11100	00100	00000	00000	00100	01000	0000
Hydrotrupes palpalis	00000	01010	00011	00010	10000	00000	01000	00000	00000	10100	0000
Agabus anthracinus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus arcticus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus bifarius	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus bjorkmanae	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus confinis	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus discolor	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus elongatus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus falli	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus phaeopterus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus roguus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus semipunctatus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus seriatus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus strigulosus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus subfuscatus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabus wasastjernae	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Ilybius angustior	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Ilybius biguttulus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Illybius picipes	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Illybius subaeneus	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Ilybius vittiger	00000	01000	00011	00010	10100	00000	01000	00000	00000	00100	0000
Agabinus glabrellus	00000	01000	00011	00010	11100	00000	01000	00000	00000	00100	0000
Rhantus (R.) binotatus	00000	00000	00010	01110	01110	00011	10010	11001	00000	01101	0000

Table 2. Continued.

Rhantus (R.) consimilis	00000	00000	00010	01110	01110	00011	10010	11001	00000	01101	0000
Rhantus (R.) suturellus	00000	00000	00010	01110	01110	00011	10010	11001	00000	01101	0000
Rhantus (R.) wallisi	00000	00000	00010	01110	01110	00011	10010	11001	00000	01101	0000
Rhantus (N.) grapii	00000	00000	00010	21110	01110	00011	10110	11001	00000	01101	0000
Colymbetes dolabratus	00000	00000	00010	01110	01110	00000	10000	11001	00000	01101	0000
Colymbetes paykulli	00000	00000	00010	01110	01110	00000	10000	11001	00000	01101	0000
Colymbetes sculptilis	00000	00000	00010	01110	01110	00000	10000	11001	00000	01101	0000
Neoscutopterus hornii	00000	00000	00010	21111	01110	00010	10110	11001	00001	01111	0000
Allomatus nannup	00000	01000	01010	11010	01110	01010	10110	10110	00001	01111	0000
Batrachomatus daemeli	00000	01000	01010	11110	01110	01010	10000	10110	00001	01111	0000
Matus bicarinatus	00000	11000	01010	10000	01110	00010	10001	10000	10001	01101	0000
Neptosternus meridianus	10011	01001	10101	20000	11111	00011	00000	10000	01100	00101	0000
Neptosternus hydaticoides	?????	0?001	10101	20000	11111	000??	?0??0	??0?0	0110?	001?1	0000
Africophilus montalentii	?????	0?011	00011	20000	1?100	00000	00000	00000	0010?	00000	0111
Australphilus montanus	?????	0?001	00111	20000	11111	000??	?0??0	??0?0	0110?	001?1	0000
Australphilus saltus	?????	0?001	00111	20000	11111	000??	?0??0	??0?0	0110?	001?1	0000
Laccophilus maculosus	10101	01011	00011	20000	11111	10110	00000	10000	00110	00101	1000
Laccophilus minutus	10101	01011	00011	20000	11111	10110	00000	10000	00110	00101	1000
Laccophilus hyalinus	10101	01011	00011	20000	11111	10110	00000	10000	00110	00101	1000

NOTE: The 54 columns correspond to the character number (Table 2); state 0 is the state observed in the outgroup; ?, missing data.



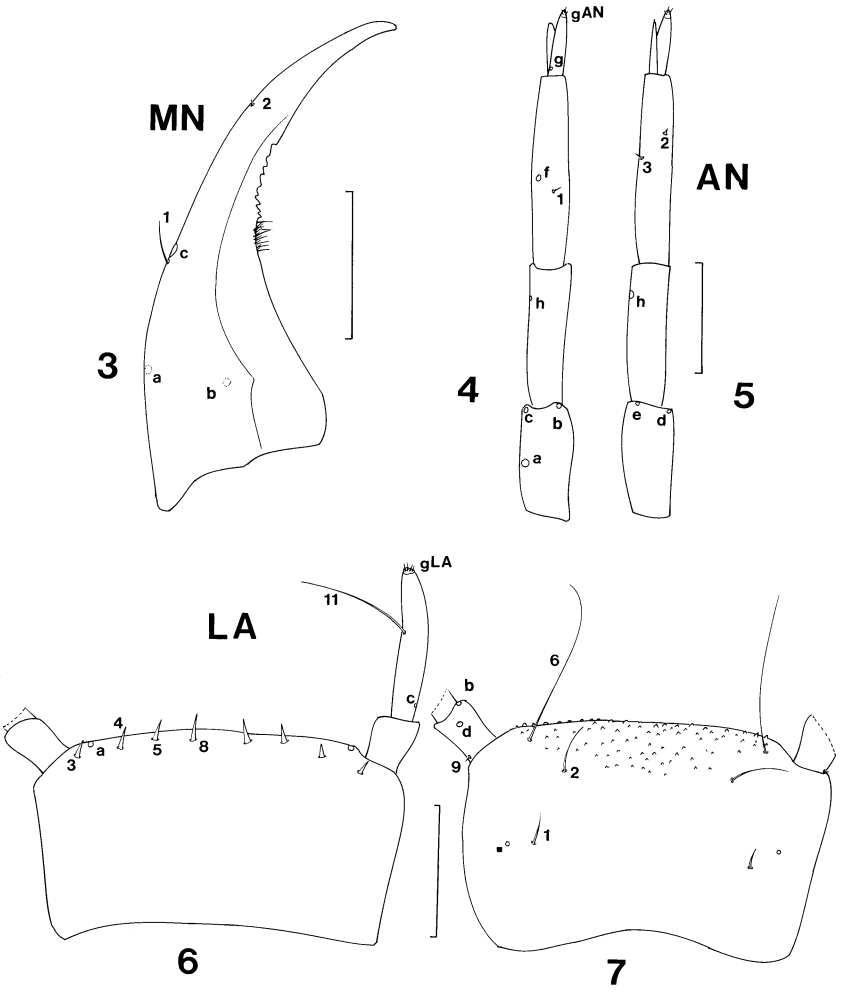
Figs. 1–2. *Agabetes acuductus* (Harris), head capsule, Instar I. **1)** Dorsal aspect; **2)** ventral aspect; **EB** = egg bursters, **LC** = *lamellae clypeales*, **FR** = frontoclypeus, **PA** = parietale, **TP** = tentorial pits; numbers and lowercase letters refer to primary setae and pores, respectively. Scale bar = 0.10 mm.

(Fig. 1); egg bursters blade-like, contiguous, proximad to coronal suture (Fig. 1); spinulose epipharyngeal band present; primary pore ANf present (Fig. 4); labial palpomere 2 > 2.20 times as long as labial palpomere 1 (Fig. 6); mandible toothed on inner margin (Fig. 3); pronotum lacking a neck constriction; legs lacking natatory setae; pecten present on posteroventral margin of femur (Fig. 10); abdominal segments 4–5 membranous ventrally; last abdominal segment subconical, not constricted at point of insertion of urogomphi (Fig. 12); siphon narrow, lacking a crescent shaped setal pattern comprised of numerous secondary spines; urogomphus one-segmented, short (<0.50 times HW), lacking a subbasal suture and secondary setae (Figs. 14–15).

Description. Instar I (Figs. 1–15) (measurements, $n = 2$).

Color. Body uniformly dark brown; head capsule predominantly brown (bright orange when alive), narrowly testaceous along anterior frontoclypeal margin.

Head (Figs. 1–8). HL = 0.71–0.73 mm; HW = 0.62–0.63 mm; FCL = 0.37 mm. *Cephalic capsule* (Figs. 1–2) obovate, longer than broad (HL/HW = 1.15–1.16), lacking a constriction posteriorly, HW/OcW = 2.11–2.12; ecdysial suture well developed; coronal suture about 0.50 times HL, occipital suture lacking; frontoclypeus triangular in outline, 0.51–0.53 times HL, anterior margin slightly convex mesally, extending below level of lateral lobes [= adnasalia]; dorsal surface of frontoclypeus with two contiguous blade-like egg bursters (ruptor ovi of Bertrand 1972) located posteriorly at junction of frontal and coronal sutures; apical margin of frontoclypeus with 2 finger-like lamellae clypeales; spinulose epipharyngeal band [''area o banda spinulosa del palato'' of De Marzo (1979)] present; gular suture fused, so epicranial plates meeting on ventral mid-line; ocularium present, stemmata visible ventrally and subdivided into 2 vertical series; tentorial pits visible ventrally on each side of



Figs. 3–7. *Agabetes acuductus* (Harris), head appendages, Instar I. **3)** Mandible, ventral aspect; **(4–5)** antenna, **4)** dorsal aspect, **5)** ventral aspect; **(6–7)** labium, **6)** dorsal aspect, **7)** ventral aspect; **AN** = antenna; **gAn** = antennal group; **gLA** = labial group; **LA** = labium; **MN** = mandible; numbers and lowercase letters refer to primary setae and pores, respectively; square = additional seta. Scale bar = 0.10 mm.

middle at about midlength; occipital foramen indented ventrally. *Antenna* (Figs. 4–5) Four-segmented, shorter than HW (length of antenna/HW = 0.72–0.74); A4 < A1 < A2 < A3, A2/A3 = 0.72–0.75; lateral elongation of antennomere 3 long, A3′/A4 = 0.78–0.81; antennomere 3 lacking a ventroapical spinula. *Mandible* (Fig. 3) Falciform, 2.90 times as long as broad, ≈0.50 times HL; mandibular channel present, inner margin of mesal groove toothed (best seen at 400×), pubescent proximally. *Maxilla* (Fig. 8) Stipes trapezoidal; cardo and galea present, lacinia lacking; galea short, 0.56–0.61 times length of pal-

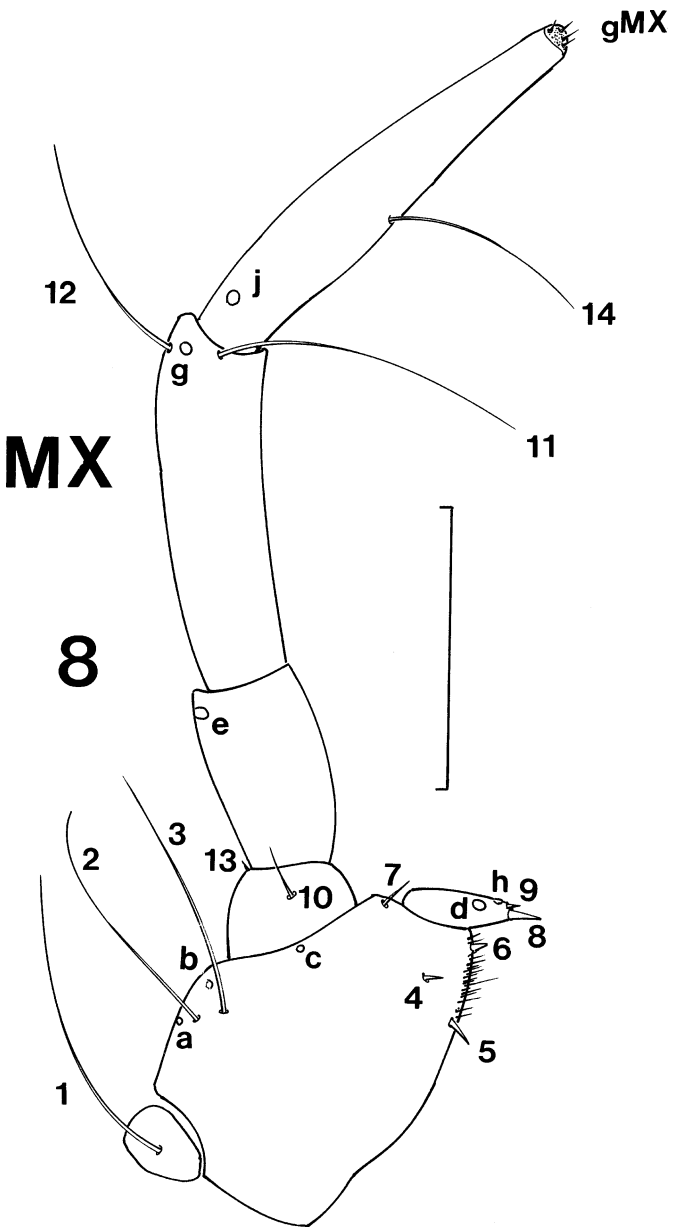
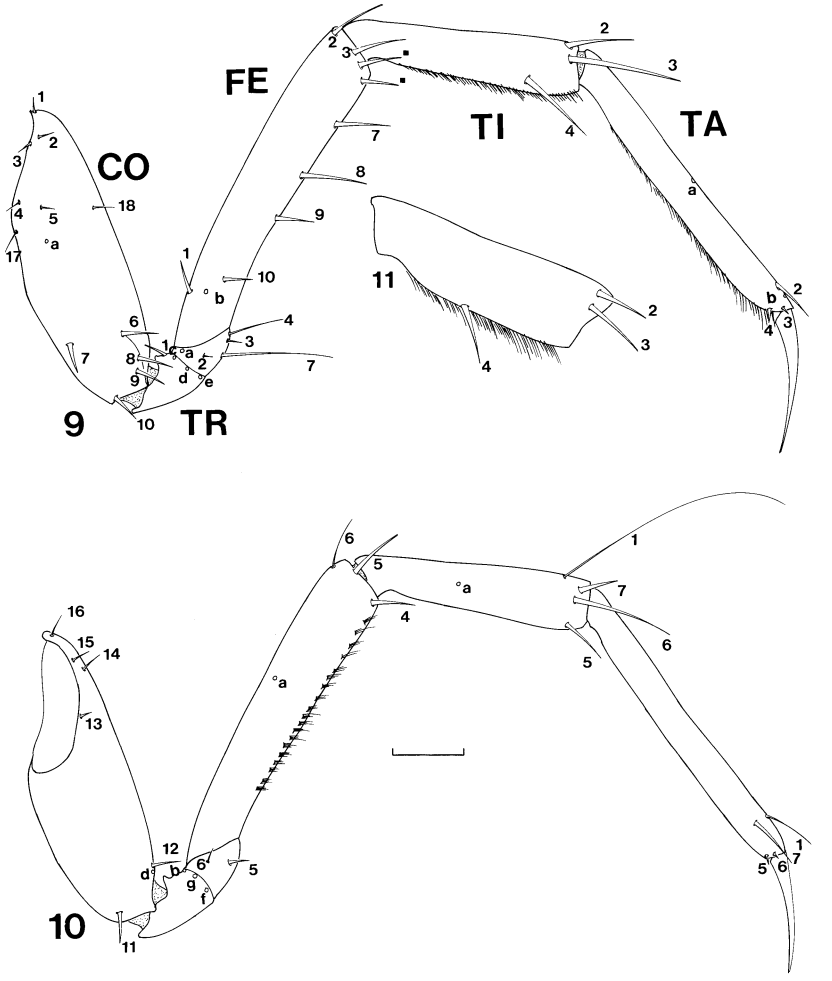
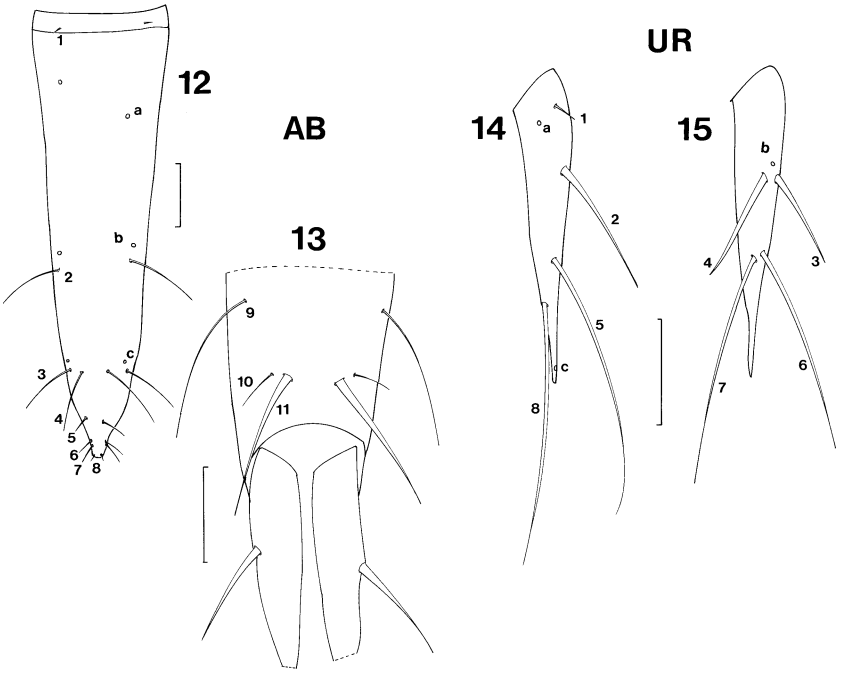


Fig. 8. *Agabetes acuductus* (Harris), ventral aspect of maxilla, Instar I; **MX** = maxilla; **gMX** = maxilla group; numbers and lowercase letters refer to primary setae and pores, respectively. Scale bar = 0.10 mm.



Figs. 9–11. *Agabetes acuductus* (Harris), Instar I, leg. **(9–10)** Metathoracic leg, **9)** anterior surface, **10)** posterior surface; **11)** protibia, anterior surface; **CO** = coxa, **FE** = femur, **TA** = tarsus, **TI** = tibia, **TR** = trochanter, numbers and lowercase letters refer to primary setae and pores, respectively; filled square = additional setae; pretarsus not represented. Scale bar = 0.10 mm.

pomere 1; palpifer similar to a palpomere, 0.37–0.43 times length of palpomere 1; palpus 3-segmented, shorter than antenna (length of antenna/length of maxillary paplus = 1.22–1.27); palpomere 1 < 2 < 3; length of palpomere 3/length of palpomere 2 = 1.22–1.25. *Labium* (Figs. 6–7) Prementum subrectangular, broader than long, densely covered with spinulae ventrally; palpus 2-segmented, shorter than maxillary palpus (length of maxillary palpus/length of labial palpus = 2.53–2.54), palpomere 2 3.33–3.93 as long as palpomere 1. *Chaetotaxy and Porotaxy* All primary setae and pores of generalized colymbetine larva present, except pores FRe, ANi and setae LA10, LA12.

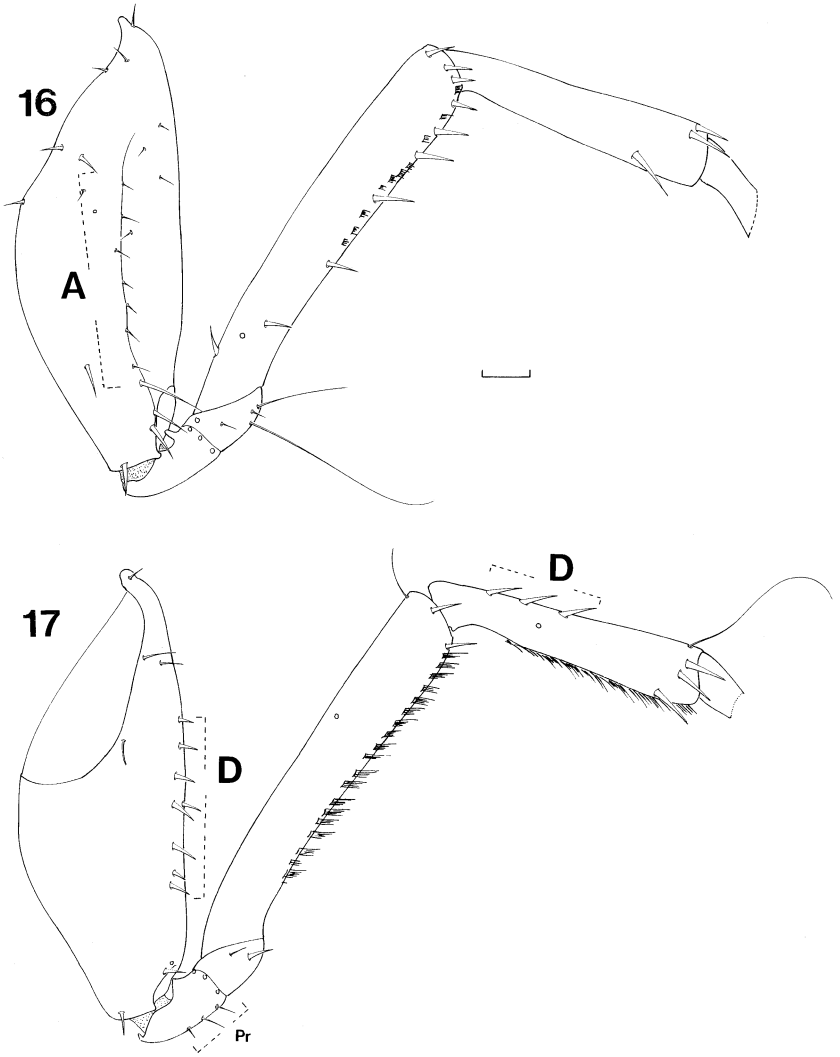


Figs. 12–15. *Agabetes acuductus* (Harris), Instar I. **(12–13)** Abdominal segment 8, **12)** dorsal surface, **13)** distal portion of ventral surface; **(14–15)** urogomphus, **14)** dorsal aspect, **15)** ventral aspect; protibia, anterior surface; **AB** = abdominal segment 8; **UR** = urogomphus; numbers and lowercase letters refer to primary setae and pores, respectively. Scale bar = 0.10 mm.

Thorax. Pronotum elliptical dorsally; length of pronotum about twice that of mesonotum; metanotum subequal to mesonotum in length, both slightly wider than pronotum; pronotum lacking transverse carina, meso- and metathoracic terga with an anterotransverse carina; maximum body width at level thoracic and abdominal segments 1 and 2; thoracic venter membranous; spiracular openings absent.

Legs (Figs. 9–11). Five-segmented; metathoracic legs longest, ≈1.30 times length of prothoracic legs, and 3.30 times HW; meta[femur > coxa ≈ tarsus > tibia > trochanter]; tarsus with two claws, posterior claw shorter than anterior claw; anterior metathoracic claw 0.48–0.50 times as long as metatarsus; pectens present on posteroventral margin of femur; spine-like spinulae strongly developed on tibia and tarsus. *Chaetotaxy and Porotaxy* All primary setae and pores of generalized colymbetine present; seta CO7 inserted proximally on procoxa; 2 additional setae are observed on anteroventral margin of femur; seta TI4 inserted ventrally and more proximally on protibia; metabial seta TI3 0.43–0.48 times length of metatibia; metatibial seta TI4 0.40–0.46 times length of metatibia.

Abdomen (Figs. 12–13). LLAS = 0.68–0.69 mm. Eight-segmented, dorsally sclerotized; segments 1–6 membranous ventrally, segment 7 with a ventral plate well demarcated from reminder of sclerite, segment 8 completely



Figs. 16–17. *Agabetes acuductus* (Harris) Instar III, metathoracic legs (excluding tarsus). **12)** Anterior face; **13)** posterior face. Sensillar series: **A** = anterior; **D** = dorsal; Pr = proximal. Scale bar = 0.10 mm.

sclerotized; all terga with an anterodorsal transverse carina; spiracular openings absent; segment 8 subcylindrical, longer than HW, LLAS/HW = 1.09–1.10. *Siphon* Short, 0.29–0.30 times as long as LLAS, bluntly rounded, constricted apically. *Chaetotaxy and Porotaxy* All primary setae and pores of generalized colymbetinae larva present except primary seta AB15. Seta AB11 0.23–0.24 times LLAS.

Urogomphus (Figs. 14–15). One-segmented, length = 0.30–0.31 mm,

0.44–0.46 times as long as LLAS, 0.48–0.50 times as long as HW. *Chaetotaxy and Porotaxy* All primary setae and pores of generalized colymbetine larva present.

Instar II. As instar I except as follows (measurements, $n = 4$).

Color. Cephalic capsule paler.

Head. HL = 0.98–1.05 mm (mean = 1.01 mm); HW = 0.92–0.97 mm (mean = 0.95 mm); FCL = 0.46–0.49 mm (mean = 0.47 mm). *Cephalic capsule* HL/HW = 1.06–1.08, HW/OcW = 1.63–1.82; frontoclypeus, 0.60–0.66 times HL, lacking egg-bursters, with 21–23 finger-like to spatulate lamellae clypeales; parietals with 9–11 lateral and 5–9 ventral spines. *Antenna* Length of antenna/HW = 0.61–0.64; A4 < A1 < A2 < A3; A2/A3 = 0.77–0.85; A3′/A4 = 0.68–0.76. *Mandible* 2.84–3.01 times as long as broad. *Maxilla* Galea 0.43–0.46 times length of palpomere 1; palpifer 0.36–0.44 times length of palpomere 1; palpomere 1 < 2 ≈ 3; length of antenna/length of maxillary paplus = 1.19–1.23; length of palpomere 3/length of palpomere 2 = 1.03–1.09. *Labium* Length of maxillary palpus/length of labial palpus = 2.55–2.74; palpomere 2 2.59–2.67 times as long as palpomere 1. *Chaetotaxy and Porotaxy* Cephalic capsule with several secondary setae; head appendages lacking secondary setae.

Thorax. Pronotum elliptical to subtrapezoidal dorsally; all terga with a posterotransverse carina; secondary setation on each tergum.

Legs. Metathoracic legs ≈1.30 times length of prothoracic legs, and 3.00 times HW; meta[coxa ≈ femur ≈ tibia > tarsus > trochanter]; anterior metathoracic claw 0.34–0.42 times as long as metatarsus; pectens present on posteroventral margins of femur; spine-like spinulae strongly developed on ventral margin of tibia and tarsus. *Chaetotaxy and Porotaxy* Position and number of secondary setae as expressed in Table 1; natatory setae lacking.

Abdomen. LLAS = 1.23–1.29 mm (mean = 1.28 mm). Segment 6 narrowly membranous ventrally; segments 7 and 8 completely sclerotized; LLAS/HW = 1.29–1.37. *Siphon* 0.13–0.15 times as long as LLAS, broadly rounded, lacking a constriction apically. *Chaetotaxy and Porotaxy* Secondary tergal setation present. *Urogomphus* Length of urogomphus = 0.24–0.26 mm (mean = 0.25 mm), 0.19–0.21 times as long as LLAS, 0.25–0.27 times as long as HW. *Chaetotaxy and Porotaxy* Urogomphus lacking secondary setae.

Instar III (Figs. 16–17; see also Spangler and Gordon 1973). As instar II except as follows (measurements, $n = 2$).

Head. HL = 1.26–1.37 mm; HW = 1.24–1.27 mm; FCL = 0.56–0.59 mm. *Cephalic capsule* HL/HW = 1.02–1.07 lateral margin faintly constricted posteriorly, HW/OcW = 1.69–1.75; frontoclypeus 0.44–0.45 times HL, with ≈42–50 finger-like to spatulate lamellae clypeales; parietals with 28–30 lateral and 30–32 ventral spines. *Antenna* Length of antenna/HW = 0.54–0.59; A4 < A1 ≈ A2 ≈ A3; A2/A3 = 0.89–0.98; A3′/A4 = 0.85–0.91. *Mandible* 3.08–3.23 times as long as broad. *Maxilla* Galea 0.36–0.37 times length of palpomere 1; palpifer 0.24–0.29 times length of palpomere 1; palpomere 1 < 2 ≈ 3; length of antenna/length of maxillary palpus = 1.15–1.18; length of palpomere 3/length of palpomere 2 = 0.88–0.89. *Labium* Length of maxillary palpus/length of labial palpus = 2.76–2.86; palpomere 2.25–2.28 times as long as palpomere 1. *Chaetotaxy and Porotaxy* Mandible one secondary seta (best seen at a magnification of 400×).

Thorax. Mesopleural region with spiracular opening on each side.

Legs (Figs. 16, 17). Metathoracic legs ≈ 1.30 times length of prothoracic legs, and ≈ 2.90 times HW; meta[coxa ≈ femur > tarsus ≈ tibia > trochanter];

Table 3. Number of secondary setae on the legs of the second- and third-instar of *Agabetes acuductus* (Harris). A = anterior, CO = coxa, D = dorsal, FE = femur, Pr = proximal, TA = tarsus, TI = tibia, TR = trochanter, V = ventral, *n* = number of specimens studied, Range = total number of secondary setae on segment.

Segment	Sensillar series	Second instar (*n* = 4)	Third instar (*n* = 2)
ProCO	D	4–6	9–11
	A	0–1	6–11
	Range	4–6	16–21
ProTR	Pr	1	2–3
ProFE	AV	0	0–1
	Range	0	0–1
ProTI	Range	0	0
ProTA	Range	0	0
MesoCO	D	4–5	8–9
	A	0–1	8–11
	Range	4–6	16–20
MesoTR	Pr	1	2–5
MesoFE	AV	0	0–1
	Range	0	0–1
MesoTI	Range	0	0
MesoTA	Range	0	0
MetaCO	D	3–4	6–11
	A	0	10–15
	Range	3–4	17–24
MetaTR	Pr	1–2	3–5
MetaFE	AV	0	0–1
	Range	0	0–1
MetaTI	AD	2–3	3–4
	Range	2–3	3–4
MetaTA	Range	0	0

anterior metathoracic claw 0.28–0.31 times as long as metatarsus; pectens present on posteroventral margin of femur; spine-like spinulae strongly developped on ventral margin of tibia and tarsus. *Chaetotaxy and Porotaxy* Position and number of secondary setae as expressed in Table 3.

Abdomen. LLAS = 1.80–1.95 mm. Segment 5 almost completely sclerotized ventrally; segments 6, 7, 8 fully sclerotized; terga 1–7 with posterodorsal transverse carina; LLAS/HW = 1.45–1.54; segments 1–7 each with pair of spiracular openings. *Siphon* 0.06–0.10 times as long as LLAS. *Chaetotaxy and Porotaxy* Secondary tergal setation present.

Urogomphus. Length of urogomphus = 0.22–0.20 mm, 0.11–0.12 times as long as LLAS, 0.16–0.17 times as long as HW.

Distribution. *Agabetes acuductus* ranges from southern Québec and southern Ontario south to Florida and west to Wisconsin and Illinois.

Biology. *Agabetes acuductus* is found predominantly in heavily shaded, deciduous woodland pools, where they are often taken in dense litter of fallen leaves. Some specimens were taken in wet leaf pack well above the water line (Larson *et al.* 2000). Adults have flight wings but flight muscles may not be functional (see Young 1954). However, teneral specimens having been commonly taken at UV lights near vernal pool sites in Maryland (May to July), it is possible that newly emerged adults of *Agabetes*, like many water beetles,

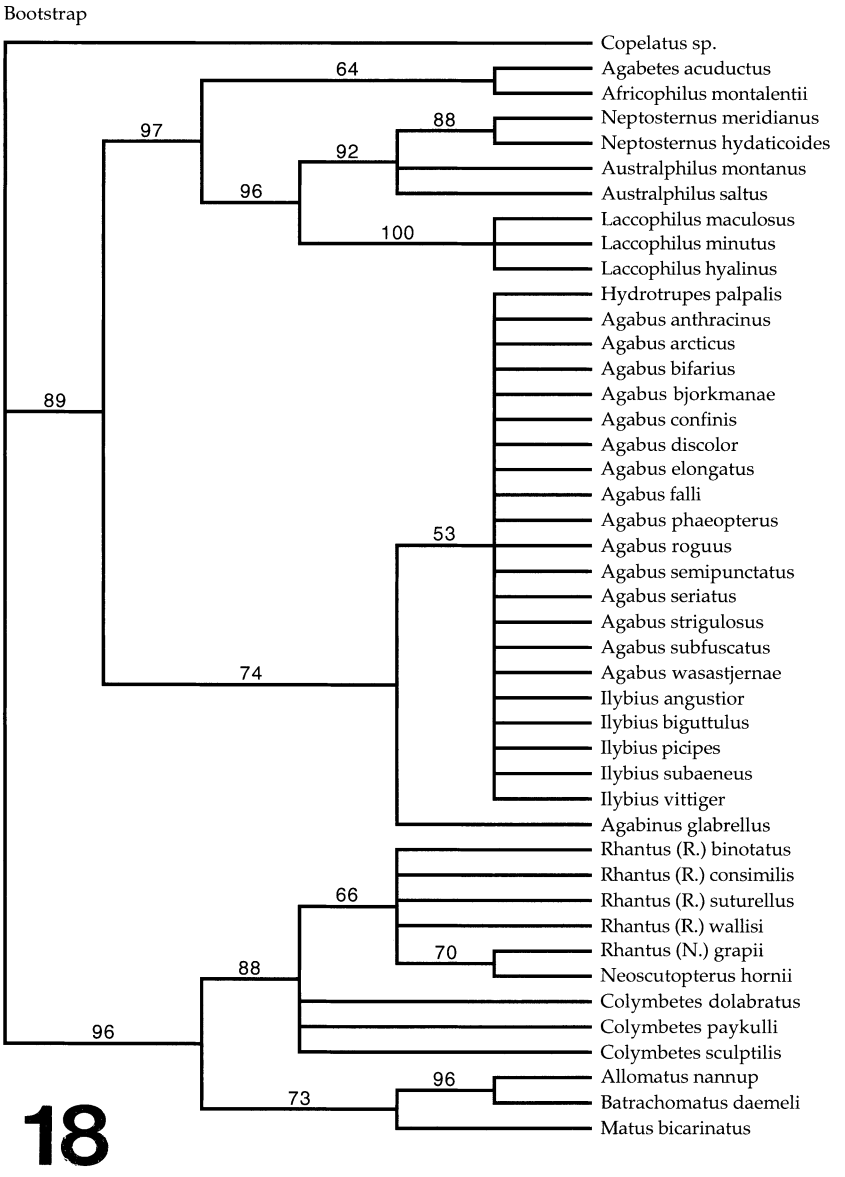


Fig. 18. Bootstrap consensus tree of 12 most parsimonious trees reconstructed for 44 species of Dytiscidae based on larval characters. CI = 0.63, RI = 0.91.

have a dispersal flight early in life, and then after they find a suitable habitat, may lose the ability to fly as muscles become atrophied.

Remarks. Larvae of *Agabetes acuductus* are characterized by two unique features among the Dytiscidae: the presence of two contiguous blade-like egg bursters (Fig. 1) inserted posteriorly on the frontoclypeus and the very short length of the labial palpomere 1 (palpomere 2 > 2.20 times length of palpomere 1) (Fig. 6).

Discussion

Analysis of the data matrix results in 12 most parsimonious trees with a tree length of 70 steps. The consensus tree is given in Figure 18 (CI = 0.63 and RI = 0.91) with bootstrap support values at each node.

The subfamily Laccophilinae is treated as a monophyletic group in current classifications based both on larval (Alarie *et al.* 2000) and adult morphology (Burmeister 1990). Bootstrap value indicates support for the monophyly of *Agabetes* with the Laccophilinae (Fig. 18) with respect to the chosen outgroup. This monophyly is defended by five synapomorphies: the egg bursters (ruptor ovi) located posteriorly, contiguous to the coronal suture (character 01, Fig. 1); the presence of two lamellae clypeales on the frontoclypeus (character 05, Fig. 1); the absence of the setae LA10 and LA12 on labial palpomere 2 (character 10, Fig. 6); the absence of a ventroapical spinula on antennomere 3 (homoplastic) (character 16, Fig. 5); the abdominal venter 6 sclerotized (character 43). Both *Agabetes* and *Africophilus* Guignot are postulated to occupy a basal position within that clade which make them good candidates as sister to other members of the clade. However, discovery of first instar of *Africophilus* would clarify their taxonomic placement.

Quite surprisingly, our data also support the placement of members of the tribe Agabini as sister to *Agabetes* + Laccophilinae although the bootstrap value indicates that node as one of the weaker of the tree. Until a formal analysis of all genera within the subfamily Colymbetinae can be performed, we do not feel that a formal inclusion of the Agabini within that clade is supported by this analysis and this was not our intent.

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Literature Cited

Alarie, Y. 1995. Primary setae and pores on the legs, the last abdominal segment, and the urogomphi of larvae of Nearctic Colymbetinae (Coleoptera: Adephaga: Dytiscidae) with an analysis of their phylogenetic relationships. The Canadian Entomologist 127:913–943.

Alarie, Y. 1998. Primary setae and pores on the cephalic capsule and head appendages of larvae of Nearctic Colymbetinae (Coleoptera: Adephaga: Dytiscidae) with an analysis of their phylogenetic relationships. The Canadian Entomologist 130:803–824.

Alarie, Y., and D. J. Larson. 1998. Larvae of *Agabinus* Crotch: generic characteristics,

description of *A. glabrellus* (Motschulsky), and comparison with other genera of the subfamily Colymbetinae(Coleoptera: Adephaga: Dytiscidae). The Coleopterists Bulletin 52:339–350.

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. Entomologica Scandinavica 30:173–195.

Alarie, Y., A. N. Nilsson, L. Hendrich, C. H. S. Watts, and M. Balke. 2000. Larval morphology of four genera of Laccophilinae (Coleoptera: Adephaga: Dytiscidae) with an analysis of their phylogenetic relationships. Insects Systematics and Evolution 31:121–164.

Alarie, Y., P. J. Spangler, and P. D. Perkins. 1998. Study of the larvae of *Hydrotrupes palpalis* (Coleoptera: Adephaga: Dytiscidae) with implications for the phylogeny of the Colymbetinae. The Coleopterists Bulletin 52:313–332.

Alarie, Y., C. H. S. Watts, and A. N. Nilsson. 2001. Larval morphology of the tribe Matini (Coleoptera: Dytiscidae: Colymbetinae): descriptions *of Batrachomatus daemeli*, *Matus bicarinatus*, and *Allomatus nannup* and phylogenetic relationships. The Canadian Entomologist 133:165–196.

Bertrand, H. 1972. Larves et nymphes des coléoptères aquatiques du globe. F. Paillart, France. 804 pp.

Branden, C. Van den. 1885. Catalogue des Coléoptères carnassiers aquatiques (Haliplidae, Amphizoidae, Pelobiidae et Dytiscidae. Annales de la Société Entomologique de Belgique 29:5–114.

Burmeister, E.-G. 1976. Der Ovipositor der Hydradephaga Coleoptera und seine phylogenetische Bedeutung unter besonderer Berücksichtigung der Dytiscidae. Zoomorphologie 85:165–257.

Burmeister, E.-G. 1990. The systematic position of the genus *Agabetes* Crotch within Dytiscidae (Coleoptera, Adephaga). Quaestiones Entomologicae 26:221–238.

Crotch, G. R. 1873. Revision of the Dytiscidae from the United States. Transactions of the American Entomological Society 4:383–424.

DeMarzo, L. 1979. Studi sulle larve dei Coleotteri Dytiscidi. X. Anatomia e funzionamento dell'apparato succhiante cibariofaringeo in alcune forme larvali delle subff. Dytiscinae, Colymbetinae, Laccophilinae e Hydroporinae. Entomologica (Bari): 15:5–72.

Farris, J. S. 1989. The retention index and the rescaled consistency index. Cladistics 5: 417–419.

Gueorguiev, V. 1968. Essai de classification des Coléoptères Dytiscidae. I. Tribus Copelatini. Bull. Inst. Zool. Mus. 28:5–45.

Kluge, A. G., and J. S. Farris. 1969. Quantitative phyletics and the evolution of anurans. Systematic Zoology 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, Ontario, Canada. 982 pp.

Maddison, W. P., and D. R. Maddison. 2000. MacClade. Analysis of Phylogeny and Character Evolution. Sinauer Associates, Inc. Sunderland, Massachusetts.

Nilsson, A. N. 1989. On the genus *Agabetes* Crotch (Coleoptera, Dytiscidae), with a new species from Iran. Annales Entomologici Fennici 55:35–40.

Pederzani, F. 1995. Keys to identification of the genera and subgenera of adult Dytiscidae (sensu lato) of the world (Coleoptera: Dytiscidae). Atti dell'Accademia Roveretana degli Agiati, a 244, ser. vii. 4:5–83.

Ruhnau, S., and M. Brancucci. 1984. Studies on the genus *Lancetes.* 2. Analysis of its phylogenetic position using preimaginal characters (Coleoptera, Dytiscidae). Entomologica Basiliensia 9:80–107.

Spangler, P. J., and R. D. Gordon. 1973. Descriptions of the larvae of some predacious water beetles (Coleoptera: Dytiscidae). Proceedings of the Biological Society of Washington 86:261–278.

Swofford, D. L., 1999. PAUP*. Phylogenetic analysis using parsimony (* and other methods), version 4. Sinauer, Sunderland, MA.

Young, F. N. 1954. The water beetles of Florida. University of Florida Studies in Biology, Science Series 5:ix + 1–238.

Zimmermann, A. 1920. Dytiscidae, Haliplidae, Hygrobiidae, Amphizoidae. Coleopterorum Catalogus 71:1–325.

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