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Larval morphology of Agabinae (Coleoptera: Dytiscidae): descriptions of three species of the subgenus *Agabus* s. str. Leach, 1817 with phylogenetic considerations

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ABSTRACT
The larvae of three species of *Agabus* s. str. Leach, 1817, *A. bifarius* (Kirby, 1837), instar III, *A. disintegratus* (Crotch, 1873), instars I–III, and *A. falli* (Zimmermann, 1934), instars I–III, are described including for the first time a detailed chaetotaxy analysis of the cephalic capsule, head appendages, legs, last abdominal segment and urogomphi. Larvae of *A. falli* show several unique morphological features that differentiate them from those of the other species of Agabini studied. A provisional parsimony analysis based on 55 larval characteristics of 17 species in seven genera of the subfamily Agabinae was conducted using the program TNT. As a result of this analysis, Agabini was recovered as polyphyletic while Platynectini and Hydrotrupini are deemed monophyletic.

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Agabinae; Agabini; Hydrotrupini; Platynectini; chaetotaxy; Nearctics

Introduction

The dytiscid genus *Agabus* Leach, 1817 is classified in the tribe Agabini of the subfamily Agabinae (Miller and Bergsten 2016). At the present time, this primarily Holarctic group contains 174 described species (Nilsson and Hájek 2019). Whereas represented by a few lotic species, most members of this group are found in shallow lentic habitats, including temporary situations such as vernal snowmelt pools (Miller and Bergsten 2016). The species included within *Agabus* have experienced some rearrangements over the past several years. Historically, *Agabus* included species that are now placed in *Platambus* Thomson, 1859, *Ilybius* Erichson, 1832 and *Ilybiosoma* Crotch, 1873. More recently Nilsson (2000) reorganised the group into three subgenera: *Acatodes* Thomson, 1859, *Gaurodytes* Thomson, 1859, and *Agabus* s. str. As pointed out by Miller and Bergsten (2016), however, even with this restructuring, *Agabus* appears to be paraphyletic with respect to certain groups of *Platambus*.

Larval morphology is important in the study of phylogenetic relationships amongst Holometabola. As different expressions of the same genotype, larval characters help to complement adult characters that have been traditionally the primary basis for

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classification. Recently, comparisons of information-rich larval traits have greatly expanded the set of characters used to infer phylogenetic relationships among the Agabinae (Michat and Archangelsky 2009; Alarie, Michat, Jia, and Hájek 2019; Benetti, Michat, Alarie, and Hamada 2019; Hájek, Alarie, Štastný, and Vondráček 2019; Okada, Alarie, and Michat 2019) including for the first time a phylogeny based on larval characters (Okada et al. 2019). These results and ideas, however, merit further investigation and, in particular, need to be more broadly tested in the study including more taxa and larger character sets.

This article is part of a long-term study that aims to characterise the larval morphology of genera included in the subfamily Agabinae. More specifically, it aims to describe the larvae of three species grouped in the subgenus *Agabus* s. str., namely *A. bifarius* (Kirby, 1837), *A. disintegratus* (Crotch, 1873), and *A. falli* (Zimmermann, 1934) in the context of modern works on Agabinae larvae, which incorporate detailed morphometric and chaetotaxic analyses (cf. supra). Although the larvae of some species of *Agabus* s. str. have been described in the past (*A. aeruginosus* Aubé, 1836–1838 (Matta 1986); *A. antennatus* Leech, 1939 (Watts 1970) as *A. clavatus* LeConte, 1859); *A. bifarius* (Kirby, 1837) (Watts 1970); *A. disintegratus* (Crotch, 1873) (Barman 1972; Barman, Wright, and Mashke 2000); *A. labiatus* (Brahm, 1791) (Bertrand 1928; Galewski 1980a, 1983a, 1986); *A. punctatus* Melsheimer, 1844 (Matta 1986; Barman, Nichols, and Sizer 1996); *A. serricornis* (Paykull, 1799) (Nilsson 1979; Galewski 1980b); *A. undulatus* (Schrank, 1776) (Bertrand 1928; Galewski 1980a, 1983a, 1986); *A. uliginosus* (Linnaeus, 1761) (Galewski 1968, 1983b), all these descriptions remain superficial and do not allow for a thorough comparison. Moreover, as the larvae of several Agabinae genera were previously described in detail (see Okada et al. 2019), this paper opens up our understanding of the larval morphology of the genus *Agabus* by comparing the larvae of the species studied with those of other genera.

Material and methods

Source of material

Descriptions of larvae are based on several specimens either found in association with adults or reared *ex ovo* (Alarie, Harper, and Maire 1989). The localities from which the specimens were obtained are provided under the individual species descriptions.

Preparation and observation

Larvae were disarticulated and mounted on standard glass slides in Hoyer's medium. Microscopic examination at magnifications of 80–800× 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). Contrary to previous studies, the colouration of larvae is not provided. As the specimens had lain in alcohol for more than 20 years before being described and, since the colouration is particularly evanescent in alcohol, we felt there can be no assurance that the colour description would be accurate.

Material depository

The specimens included in this study are deposited in the larval collection of Y. Alarie (Department of Biology, Laurentian University, Canada).

Methods

The methods and terms used herein follow those employed in Alarie et al. (2019), Hájek et al. (2019), and Okada et al. (2019). The reader is referred to those papers for a complete list and additional explanations of the terms used here. The count of number of secondary setae present on femur was more difficult owing to the presence of a variable number of additional setae. Accordingly, additional setae were included in the count of secondary setae.

Phylogenetic analysis

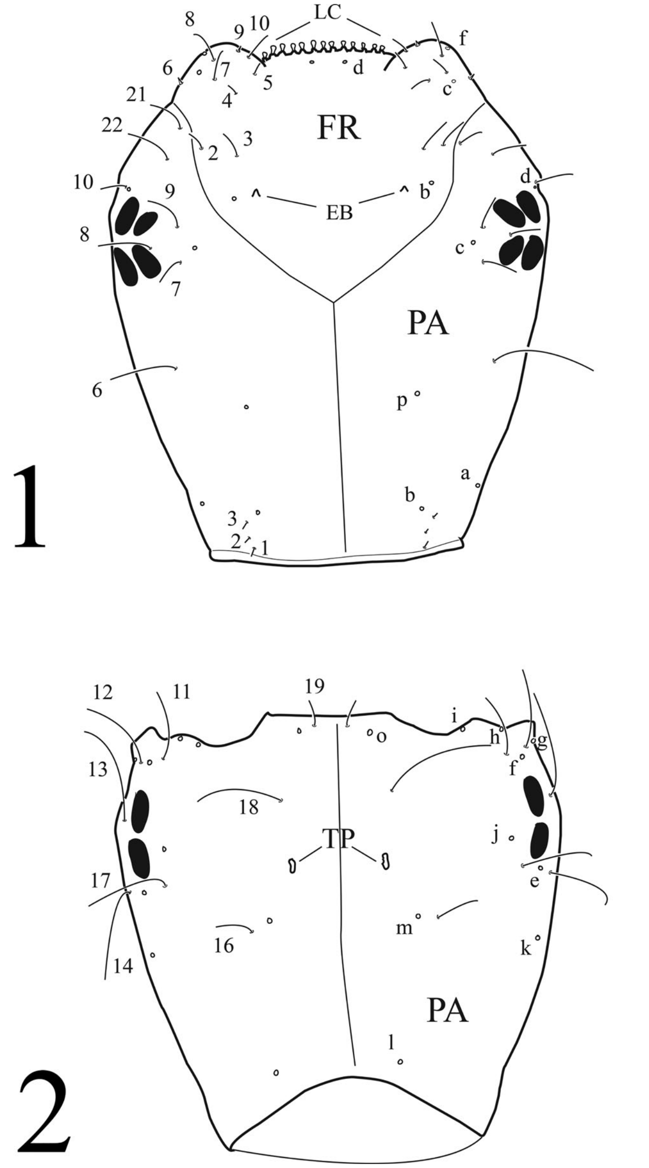
The phylogenetic relationships of *Agabus* s. str. and other genera of the subfamily Agabinae that have been described in much detail as larvae were analysed cladistically using the program TNT (Goloboff, Farris, and Nixon 2008). A total of 17 Agabinae species in seven genera (*Agabus*, *Agabinus* Crotch, 1873, *Hydrotrupes* Sharp, 1882, *Ilybius*, *Ilybiosoma*, *Platynectes* Régimbart, 1879, and *Platambus*) were included. The remaining two genera of the subfamily (*Andonectes* Guéorguiev 1971 and *Hydronebrius* Jakovlev, 1897) were not included because their larvae are either unknown or imperfectly described. Members of two other dytiscid subfamilies (*Meridiorhantus* Balke, Hájek, and Hendrich 2017 (Colymbetinae), and *Matus* Aubé 1836–1838 (Matinae) were used as outgroups. All characters were treated as equally weighted. Multistate characters were treated as non-additive. 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, Forey, Humphries, and Williams 1998). Jackknife values were calculated with 1,000 replicates and P (removal probability) = 36.

Results

Larvae of the three species of *Agabus* s. str. described in this paper (description of instars I and II based on *Agabus disintegratus* and *A. falli* only as they are unknown for *A. bifarius*) share the combination of characters given below.

Description of instar I

(Figures 1–14, 17–28).



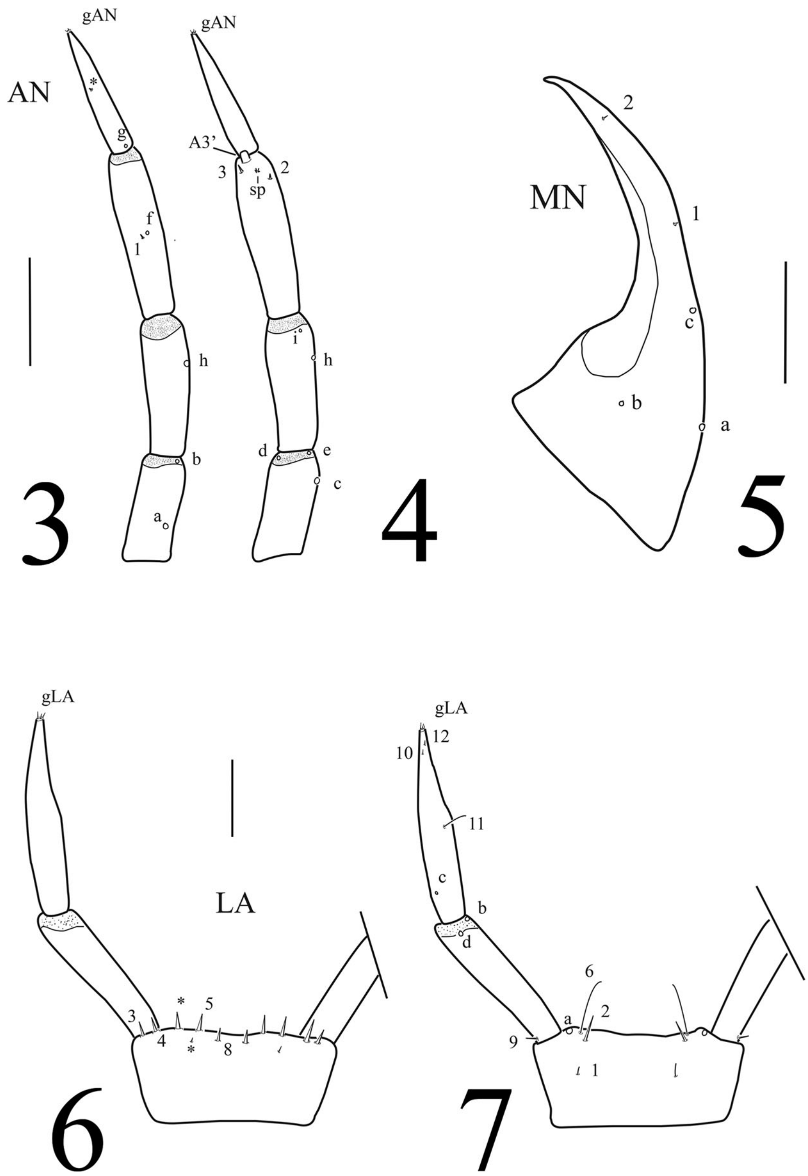
Figures 1–2. *Agabus* (s. str.) *disintegratus* (Crotch, 1873), head capsule, instar I: (1) dorsal aspect; (2) ventral aspect. EB: egg bursters; FR: frontoclypeus; LC: lamellae clypeales; PA: parietale; TP: tentorial pits. Numbers and lowercase letters refer to primary setae and pores, respectively. Scale bar = 0.1 mm.

Body

Subcylindrical narrowing towards abdominal apex. Measurements and ratios that characterise the body shape are shown in Table 1.

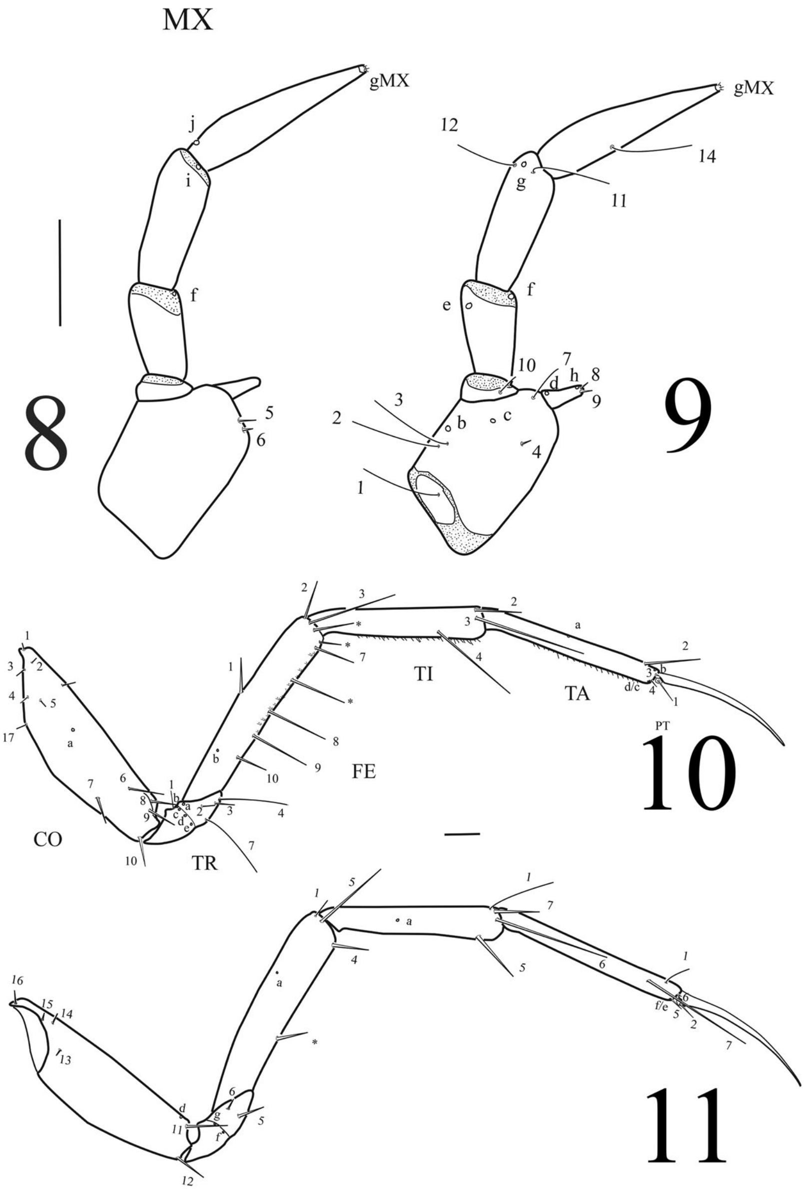
Head

(Figures 1–2, 17–18).



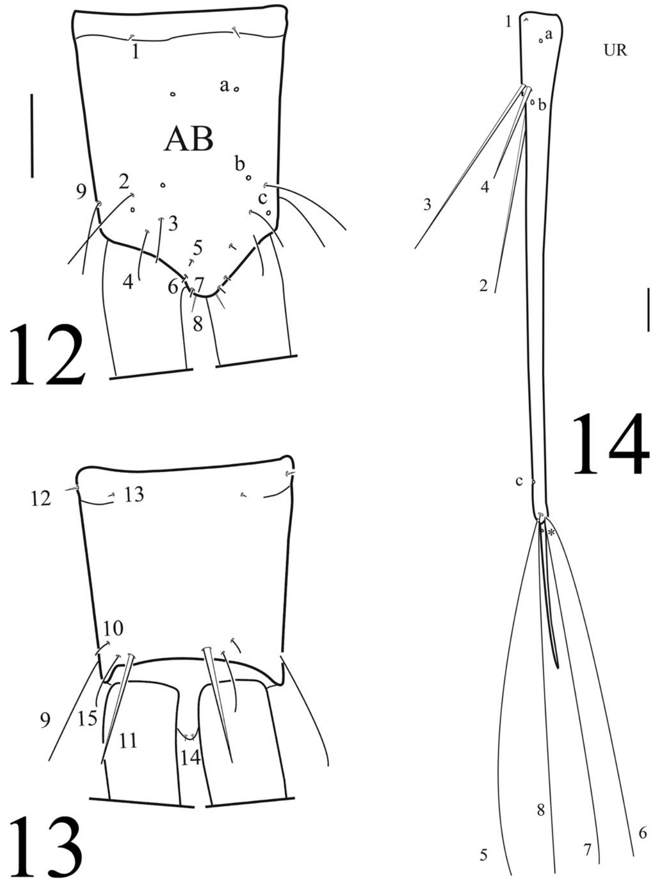
Figures 3–7. *Agabus* (s. str.) *disintegratus* (Crotch, 1873), head appendages, instar I: (3–4) antenna; (3) dorsal aspect; (4) ventral aspect; (5) mandible, dorsal aspect; (6–7) labium; (6) dorsal aspect; (7) ventral aspect. AN: antenna; LA: labium; MN: mandible; sp: spinula. Numbers and lowercase letters refer to primary setae and pores, respectively; * = additional sensilla. Scale bar = 0.1 mm.

Head capsule. HL = 0.60–0.82 mm; HW = 0.69–0.72 mm; FRL = 0.35–0.41 mm. Flattened, rounded or sub-ovate, broader than long or longer than broad (HL/HW = 0.84–1.19), maximum width at level of stemmata, sides distinctly converging posteriorly; neck constriction absent; occipital suture absent; ecdysial line well marked, coronal line long, 0.42–0.50 times HL; occipital foramen broadly emarginate ventrally, HW/OCW = 1.62–1.71; frontoclypeus subtriangular, rounded mesally, not reaching level of anterolateral lobes of frontoclypeus (= adnasalia of Beutel 1994); dorsal



Figures 8–11. *Agabus* (s. str.) *disintegratus* (Crotch, 1873), instar I: (8–9) maxilla; (8) dorsal aspect; (9) ventral aspect; (10–11) metathoracic leg; (10) anterior surface; (11) posterior surface. CO, coxa; FE: femur; MX: maxilla; PT: pretarsus; TA: tarsus; TI: tibia; TR: trochanter. Numbers and lowercase letters refer to primary setae and pores, respectively. * = additional sensilla. Scale bar = 0.1 mm.

surface with two spine-like egg bursters (= ruptor ovi of Bertrand 1972) posteriorly, less than half as broad basally than maximum width of antennomere 1; anterior margin with 7–15 either spine-like or spatulate setae (= *lamellae clypeales* of Bertrand (1972)) variable in size; gular suture visible; ocularium present, with six stemmata, four visible dorsally and two ventrally, arranged into two curved vertical rows; tentorial pits visible ventrally on each side at about mid-length.



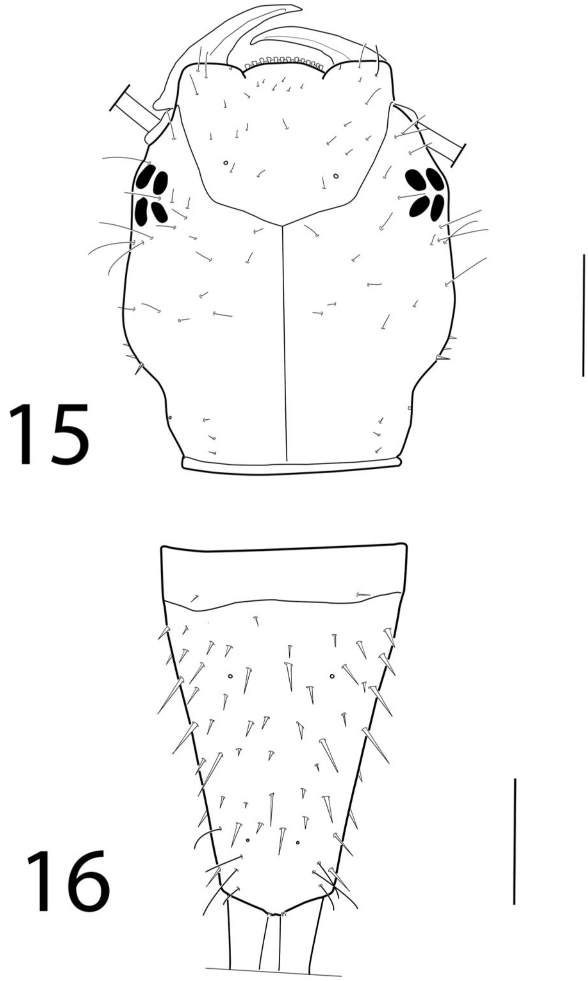
Figures 12–14. *Agabus* (s. str.) *disintegratus* (Crotch, 1873), instar I: (12–13) abdominal segment VIII; (12) dorsal aspect; (13) ventral aspect; (14) urogomphus, dorsal aspect. AB: abdominal segment VIII; UR: urogomphus. Numbers and lowercase letters refer to primary setae and pores, respectively. * = additional sensilla. Scale bars = 0.5 mm.

Antenna **(Figures 3–4, 19–20).** Slender, shorter than HW (A/HW = 0.68–0.75), composed of four antennomeres; A1 the shortest; A3 the longest (A3/A4 = 1.19–1.35); A3' protruding, bulge-like.

Mandible **(Figures 5, 21).** Prominent, broad basally, distal half projected inwards, sharp or rounded apically, 0.47–0.78 times as long as HL; inner margin toothed or not; mandibular channel present.

Maxilla **(Figures 8–9, 22–23).** Cardo small, subovate; stipes short, broad, with minute spinulae proximad to galea; galea well developed, subconical, lacinia absent; palpifer short, palpomere-like, with dorsodistal spinulae; palpus 3-segmented, shorter than antenna (A/MP = 1.22–1.40), MP1 the shortest, MP3 the longest (MP3/MP2 = 1.43–1.57).

Labium **(Figures 6–7).** Prementum subrectangular, broader than long, with minute spinulae on dorsal and ventral surfaces; palpus elongate, 2-segmented, subequal to



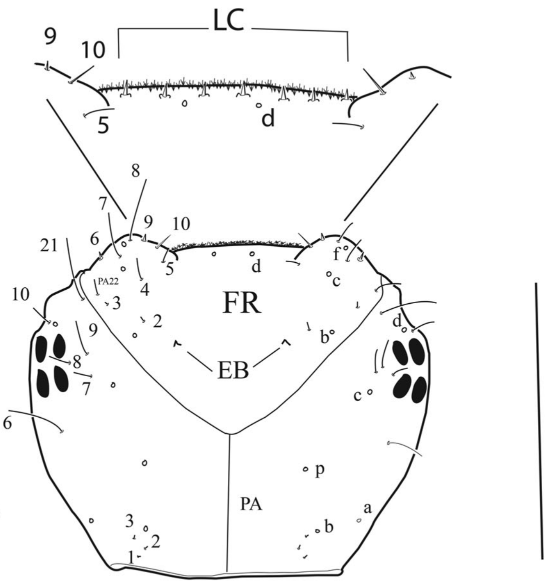
Figures 15–16. *Agabus* (s. str.) *disintegratus* (Crotch, 1873), instar III: (15) head capsule, dorsal aspect (colour pattern not represented); (16) abdominal segment VIII, dorsal aspect. Scale bars =0.5 mm.

slightly shorter than maxillary palpus (MP/LP = 1.07–1.14); LP2 longer than LP1 (LP2/LP1 = 1.35–1.45).

Thorax

Terga convex, pronotum slightly shorter than meso- and metanotum combined, meso- and metanotum subequal, wider than pronotum; protergite subrectangular, margins rounded, more developed than meso- and metatergites; meso- and metatergites transverse, with anterotransverse carina; sagittal line visible on the three tergites; sterna membranous, spiracles absent.

Legs **(Figures 10–11, 24–25).** Long, well developed, composed of six segments (including pretarsus), L1 the shortest, L3 the longest; L3 = 1.59–2.68 mm, L3/HW = 2.22–3.87; CO robust, elongate, TR divided into two parts, FE, TI and TA slender,



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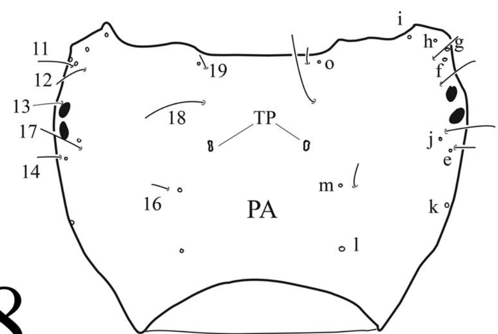

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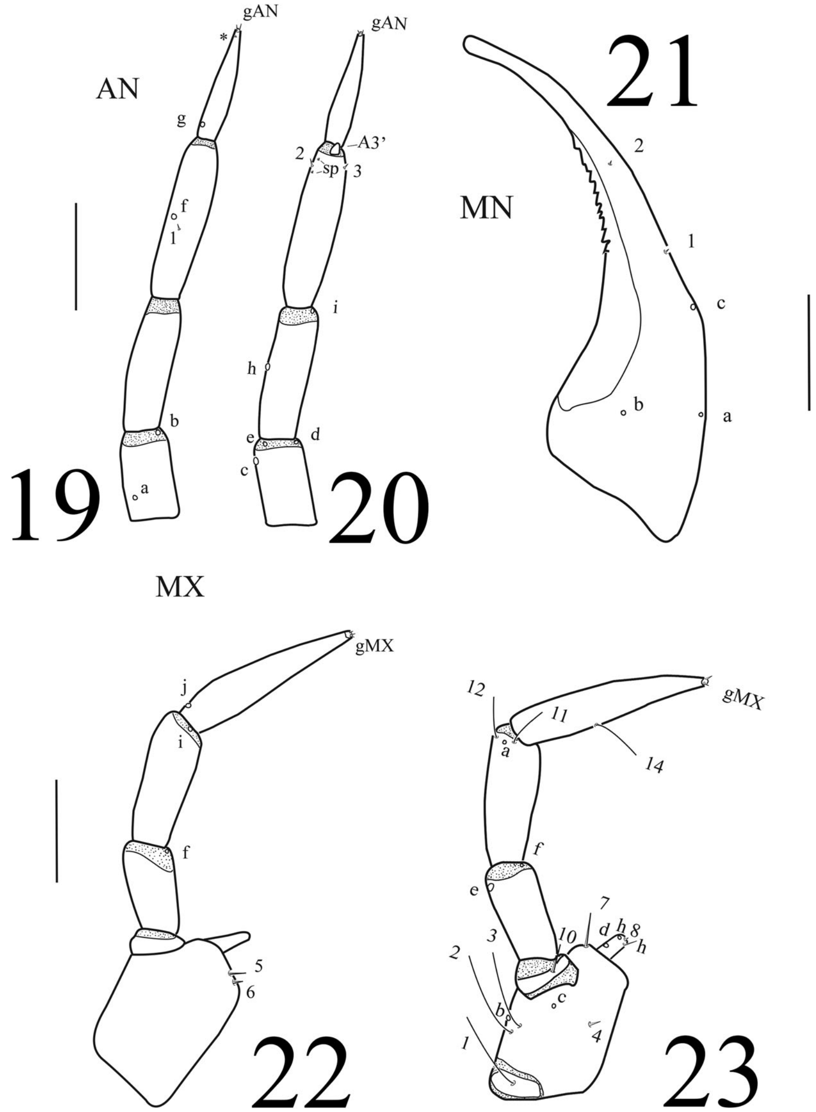
Figures 17–18. *Agabus* (s. str.) *falli* (Zimmermann, 1934), head capsule, instar I: (17) dorsal aspect; (8) ventral aspect. EB: egg bursters; FR: frontoclypeus; LC: lamellae clypeales; PA: parietale; TP: tentorial pits. Numbers and lowercase letters refer to primary setae and pores, respectively. Scale bar = 0.1 mm.

subcylindrical; tibiae and tarsi with strong spinulae along ventral margin; pretarsus with two long, slender, slightly curved claws, posterior claw shorter than anterior one on L1 and L2, claws subequal in length on L3; claws lacking basoventral spinulae.

Abdomen

(Figures 12–13, 26–27)

Eight-segmented; segments I–VII sclerotised dorsally, membranous ventrally; tergites I–VII narrow, transverse, rounded laterally, with sagittal line, with anterotransverse carina, covered with minute spinulae; spiracles absent on segments I–VII; segment VIII (=LAS) short, subquadrate or subcylindrical, completely sclerotised, ring-like, with anterotransverse carina dorsally and laterally (LAS = 0.33–0.45 mm,



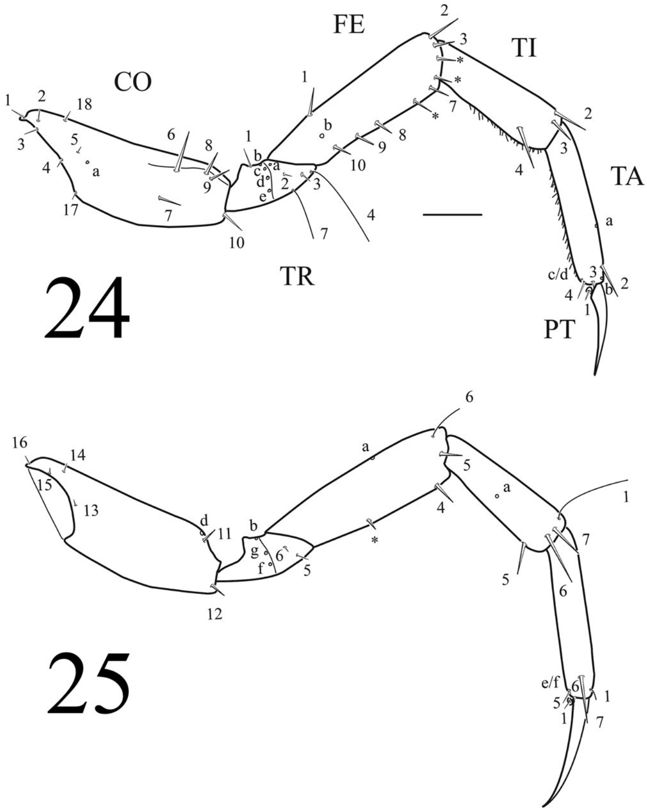
Figures 19–23. *Agabus* (s. str.) *falli* (Zimmermann, 1934), head appendages, instar I: (19–20) antenna; (19) dorsal aspect; (20) ventral aspect; (21) mandible, dorsal aspect; (22–23) maxilla; (22) dorsal aspect; (23) ventral aspect. AN: antenna; MN: mandible; MX: maxilla; sp: spinula. Numbers and lowercase letters refer to primary setae and pores, respectively; * = additional sensilla. Scale bar = 0.1 mm.

LAS/HW = 0.46–0.63), covered with minute spinulae; extending dorsally into a well-developed siphon.

***Urogomphus* (Figures 14, 28).** U = 0.56–1.58 mm, U/HW = 0.78–2.18, two-segmented, subequal to distinctly longer than LAS (U/LAS = 1.23–4.75), with microspinulae.

Chaetotaxy

(Figures 1–14, 17–28)



Figures 24–25. *Agabus* (s. str.) *falli* (Zimmermann, 1934), metathoracic leg, instar I: (24) anterior surface; (25) posterior surface. CO: coxa; FE: femur; TA: tarsus; PT: pretarsus; TA: tarsus; TI: tibia; TR: trochanter. Numbers and lowercase letters refer to primary setae and pores, respectively. * = additional sensilla. Scale bar = 0.1 mm.

Similar to that of generalised Agabinae larva (Alarie et al. 1998; Alarie 1995, 1998; Alarie and Larson 1998; Michat and Archangelsky 2009) except for the following characteristics: pore ANi located more distally; A3 with one minute spinula anterodistally; a minute structure (possibly a pore) present on dorsal surface of A4; prementum with one additional seta on dorsal surface; seta FE1 inserted either proximally or submedially; femora with a variable number of additional setae (Table 2); setae UR2, UR3 and UR4 contiguously articulated; seta UR4 shorter than setae UR2 and UR3.

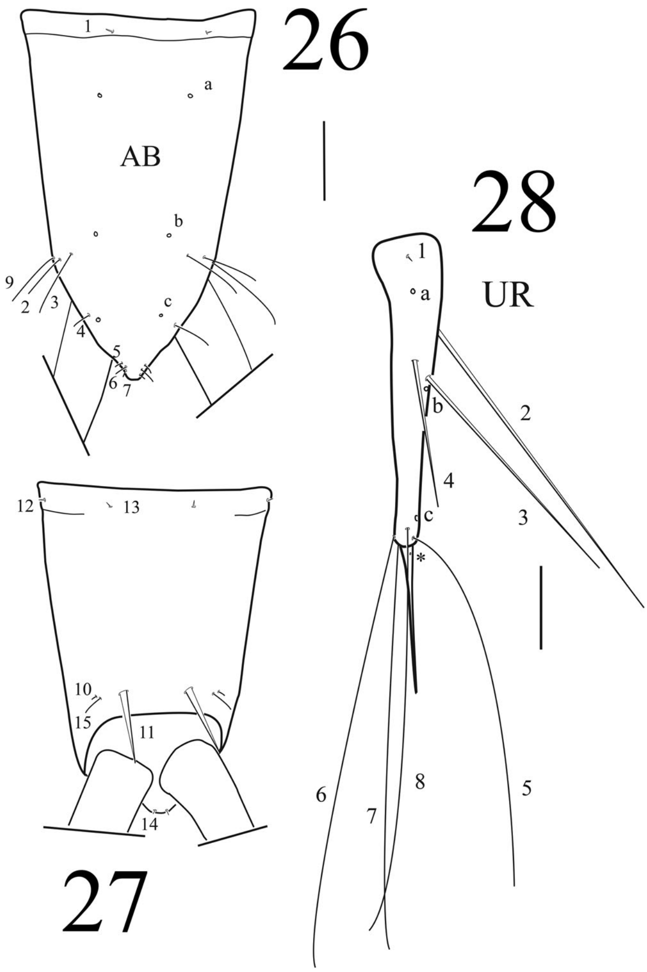
Description of instar II

As instar I except as follows:

Head

HL = 0.88–1.21 mm; HW = 1.03–1.09 mm; FRL = 0.40–0.56 mm.

Head capsule. Sub-ovate to subtrapezoidal (HL/HW = 0.86–1.19, HW/OCW = 1.40–1.89, constricted at level of occiput; frontoclypeus with 17–32 spatulate or spine-like lamellae clypeales; egg bursters lacking.



Figures 26–28. *Agabus* (s. str.) *falli* (Zimmermann, 1934), instar I: (26–27) abdominal segment VIII; (26) dorsal aspect; (27) ventral aspect; (28) urogomphus, dorsal aspect. AB: abdominal segment VIII; UR: urogomphus. Numbers and lowercase letters refer to primary setae and pores, respectively. * = additional sensilla. Scale bars = 0.1 mm.

Antenna. A/HW = 0.60–0.66; A3 and A2 subequal in length (A3/A2 = 1.05–1.11).

Maxilla. MP3 subequal to slightly longer than MP2 (MP3/MP2 = 1.05–1.22);

Labium. MP/LP = 1.06–1.19; LP2 subequal or slightly longer than LP1 (LP2/LP1 = 1.06–1.18).

Thorax

Legs. L3 = 2.57–4.13 mm, L3/HW = 2.50–3.97; ventral marginal spinulae of tibiae and tarsi spine-like, more strongly developed.

Table 1. Measurements and ratios for the larvae of selected species of *Agabus* s. str. Leach, 1817: ABIF = *A. bifarius* (Kirby, 1837), ADIS = *A. disintegratus* (Crotch, 1873), and AFAL = *A. falli* (Zimmermann, 1934).

Measure	ABIF	ADIS			AFAL		
	Instar III (*n* = 1)	Instar I (*n* = 2)	Instar II (*n* = 3)	Instar III (*n* = 3)	Instar I (*n* = 1)	Instar II (*n* = 1)	Instar III (*n* = 1)
HL (mm)	1.54	0.82	1.19–1.21	1.67–1.71	0.60	0.88	1.27
HW (mm)	1.40	0.69–0.72	1.04–1.09	1.38–1.48	0.71	1.03	1.38
FRL (mm)	0.61	0.41–0.43	0.54–0.56	0.67–0.71	0.35	0.40	0.61
OCW (mm)	0.93	0.41–0.45	0.67–0.75	0.89–0.91	0.43	0.55	0.87
HL/HW	1.10	1.14–1.19	1.09–1.15	1.15–1.22	0.84	0.86	0.92
HW/OCW	1.50	1.62–1.71	1.40–1.55	1.54–1.62	1.67	1.89	1.58
COL/HL	0.60	0.48–0.50	0.53–0.55	0.58–0.60	0.42	0.55	0.52
FRL/HL	0.40	0.50–0.52	0.45–0.47	0.40–0.42	0.58	0.45	0.48
A/HW	0.65	0.74–0.75	0.60–0.66	0.55–0.60	0.68	0.65	0.68
A3/A1	1.00	1.35–1.47	1.19–1.29	0.96–1.07	1.74	1.35	1.00
A3/A2	1.00	1.13–1.15	1.05–1.11	0.88–0.94	1.20	1.10	1.00
A3/A4	1.85	1.19–1.22	1.40–1.61	1.81–1.83	1.35	1.97	2.08
MNL/MNW	2.46	2.34–2.35	2.36–2.44	2.42–2.80	3.13	3.11	3.02
MNL/HL	0.48	0.47–0.49	0.46–0.48	0.44–0.46	0.78	0.79	0.65
A/MP	1.34	1.32–1.40	1.40–1.41	1.38–1.47	1.22	1.25	1.28
GA/MP1	0.31	0.44–0.53	0.39–0.47	0.37–0.41	0.30	0.25	0.23
PPF/MP1	0.29	0.31–0.33	0.33–0.41	0.41–0.43	0.34	0.29	0.24
MP2/MP1	1.09	1.60	1.42–1.50	1.44–1.48	1.51	1.22	1.02
MP3/MP2	0.94	1.43–1.57	1.15–1.22	0.81–0.85	1.50	1.05	0.85
MP/LP	1.12	1.11–1.14	1.10–1.19	1.10–1.14	1.07	1.06	1.05
LP2/LP1	0.74	1.42–1.45	1.14–1.18	0.96–1.06	1.35	1.06	0.81
L3 (mm)	4.11	2.68	4.03–4.13	5.65–5.72	1.59	2.57	3.50
L3/L1	1.33	1.40	1.43–1.47	1.46–1.47	1.17	1.23	1.28
L3/L2	1.20	1.19	1.19–1.24	1.21–1.22	1.12	1.14	1.18
L3/HW	2.94	3.87	3.70–3.97	3.83–4.15	2.22	2.50	2.55
L3(CO/FE)	1.15	0.98	1.08–1.11	1.09–1.11	1.11	1.07	1.09
L3(TI/FE)	0.70	0.77	0.76–0.78	0.73–0.76	0.73	0.71	0.68
L3(TA/FE)	0.69	0.87	0.77–0.80	0.68–0.74	0.83	0.71	0.65
L3(CL/TA)	0.47	0.76	0.63–0.72	0.54–0.58	0.69	0.62	0.48
LAS (mm)	1.51	0.33–0.34	0.71–0.75	1.38–1.45	0.45	0.91	1.38
LAS/HW	1.08	0.46–0.49	0.66–0.72	0.93–1.05	0.63	0.88	1.00
U (mm)	1.04	1.44–1.58	2.25–2.42	3.04–3.09	0.56	0.68	0.52
U/LAS	0.70	4.26–4.75	3.00–3.34	2.10–2.21	1.23	0.75	0.38
U/HW	0.75	2.07–2.18	2.11–2.30	2.06–2.22	0.78	0.66	0.38

Table 2. Number and position of additional setae of selected species of *Agabus* s. str. Leach, 1817: ADIS = *A. disintegratus* (Crotch, 1873); AFAL = *A. falli* (Zimmerman, 1934); AV = anteroventral, PV = posteroventral, Total = total number of additional setae on segment; *n* = number of specimens examined.

Segment	Position	ADIS (*n* = 3)	AFAL (*n* = 1)
Femur	AV	3–4/3/3–4	3/3/3
	PV	1/1/1	1/1/1
	Total	4–5/4/4–5	4/4/4

Numbers between slash marks refer to pro-, meso- and metathoracic leg, respectively.

Abdomen

Segment VII completely sclerotised, ring-like; LAS = 0.71–0.91 mm, LAS/HW = 0.66–0.88.

Table 3. Number and position of secondary setae on the legs of selected species of *Agabus* s. str. Leach, 1817: ABIF = *A. bifarius* (Kirby, 1837); ADIS = *A. disintegratus* (Crotch, 1873); AFAL = *A. falli* (Zimmerman, 1934); AV = anteroventral, PV = posteroventral, Total = total number of additional and secondary setae on segment; *n* = number of specimens examined.

		Instar II		Instar III		
Segment	Position	ADIS (*n* = 2)	AFAL (*n* = 1)	ABIF (*n* = 1)	ADIS (*n* = 3)	AFAL (*n* = 1)
Coxa	PD	4–5/4–5/3–4	2/2/2	5/6/4	5–7/4–5/5–7	2/2/3
	A	2–5/4–5/4–5	2/2/2	4/7/7	3–4/3–6/4–7	2/3/3
	Total	7–10/8–10/8	4/4/4	9/13/11	8–10/8–10/11–12	4/5/6
Trochanter	Di	0–1/1/1	0/0/0	0/0/0	0–1/1/1	0/0/0
Femur	AD	3/3–4/3	2/1/2	3/5/4	3–4/3–5/4–5	1/2/1
	AV	4–5/8/7–8	3/4/7	7/5/7	3–5/6–8/5–10	3/5/7
	PV	5/6/8	6/5/5	5/9/9	5–6/7–9/8–11	5/6/5
	Total	10–13/17–18/18–19	11/10/14	15/19/20	12–14/17–20/17–25	9/13/13
Tibia	AD	0/1/3	0/0/0	0/0/0	0/1–2/3	0/0/0
	AV	0/1/2–3	0/0/2	0/2/2	0/0–3/4	0/0/2
	PD	1/3/5	0/0/0	0/0/0	1/2–3/4–5	0/0/0
	Total	1/5/10–11	0/0/2	0/2/2	1/4–7/11–12	0/0/2
Tarsus	AD	0/3/1–2	0/0/0	0/0/0	0/0–1/2–4	0/0/0
	AV	0/0/3–4	0/0/2	0/0/3	0/0/4–5	0/0/3
	PD	1–2/0/3–5	0/0/0	0/0/0	1–2/2–3/3–6	0/0/0
	Total	1–2/3/8–10	0/0/2	0/0/3	1–2/2–3/10–14	0/0/3

Numbers between slash marks refer to pro-, meso- and metathoracic leg, respectively. A = anterior; AD = anterodorsal; AV = anteroventral; Di = distal; PD = posterodorsal; PV = posteroventral; Total, total number of secondary setae on the segment (excluding primary setae but additional setae).

Urogomphus. U = 0.68–2.42 mm, U/HW = 1.27–1.62, shorter or longer than LAS (U/LAS = 0.75–3.34).

Chaetotaxy

Cephalic capsule with several minute secondary setae; parietale with 3–5 spine-like secondary setae on lateral margin and ventral surface; mandible with several minute secondary setae along external margin; prementum with one secondary pore on ventral surface, near seta LA1; secondary leg setation detailed in Table 3. LAS with several secondary setae.

Description of instar III

(Figures 15–16, 29–38)

As instar II except as follows:

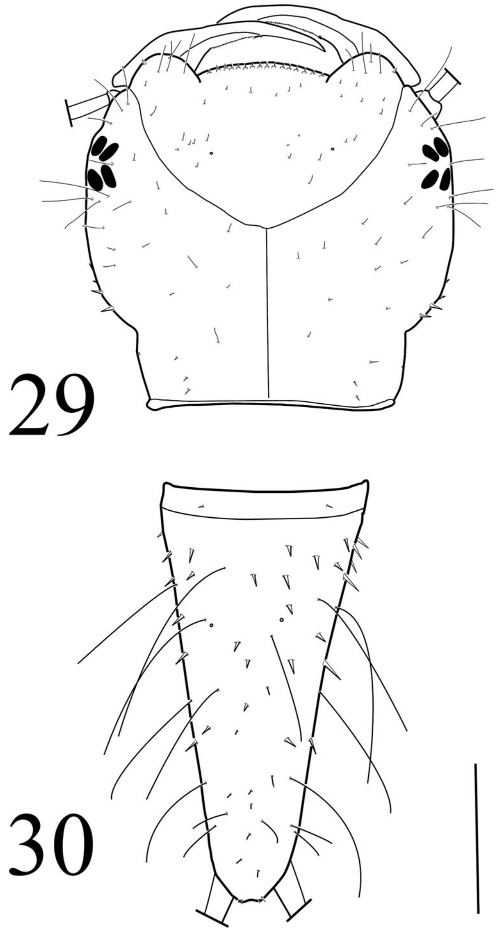
Head

(Figures 15, 29, 31)

HL = 1.27–1.71 mm; HW = 1.03–1.48 mm; FRL = 0.61–0.71 mm. Frontoclypeus with 28–32 spatulate or spine-like *lamellae clypeales*.

Antenna. A1, A2 and A3 the longest, subequal in length.

Maxilla. MP2 the longest, MP1 and MP3 subequal in length.



Figures 29–30. *Agabus* (s. str.) *falli* (Zimmermann, 1934), instar III: (29) head capsule, dorsal aspect (colour pattern not represented); (30) abdominal segment VIII, dorsal aspect. Scale bars =0.5 mm.

Labium. LP2 shorter to slightly longer than LP1 (LP2/LP1 = 0.74–1.06).

Thorax

Spiracular openings present on mesothorax.

***Legs* (Figures 33–38).** L3 = 3.50–5.72 mm; L3/HW = 2.55–4.15.

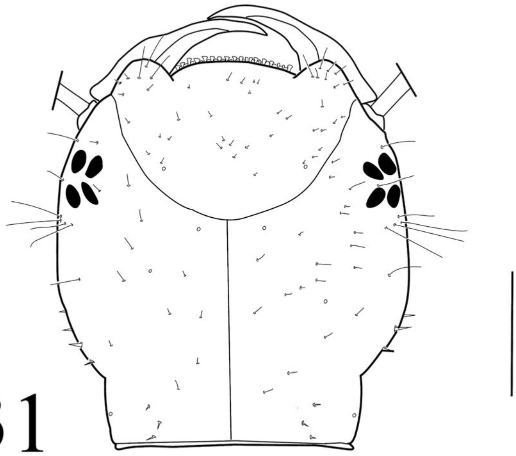
Abdomen

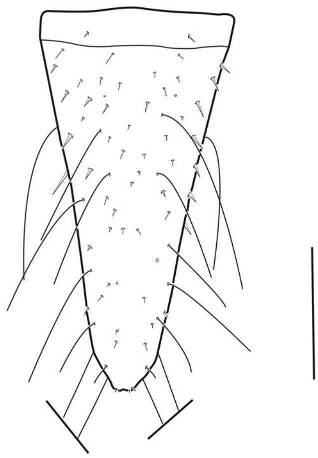
Spiracular openings present on segments I–VII; LAS = 1.38–1.51 mm, LAS/HW = 0.93–1.08.

Urogomphus. U = 0.52–3.09 mm, U/HW = 0.38–2.22; U/LAS = 0.38–2.21.

Chaetotaxy

Secondary leg setation detailed in Table 3 and Figures 33–38.





Figures 31–32. *Agabus* (s. str.) *bifarius* (Kirby, 1837), instar III: (31) head capsule, dorsal aspect (colour pattern not represented); (32) abdominal segment VIII, dorsal aspect. Scale bars =0.5 mm.

Agabus disintegratus (Crotch, 1873)

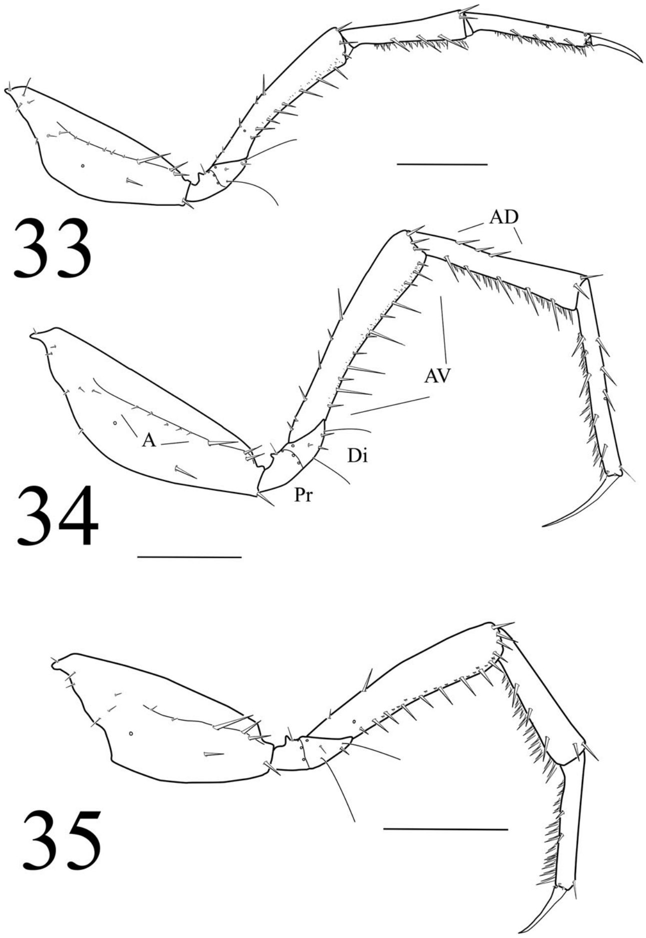
(Figures 1–16, 34, 37)

Material studied

Five instar I, five instar II, five instar III collected in association with adults at the following locality: Canada, Ontario, North of Dealtown at Hwy 6, ca. 1 km of junction to Hwy 8, pond, 04.V.1996, Y. Alarie leg.

Diagnostic combination

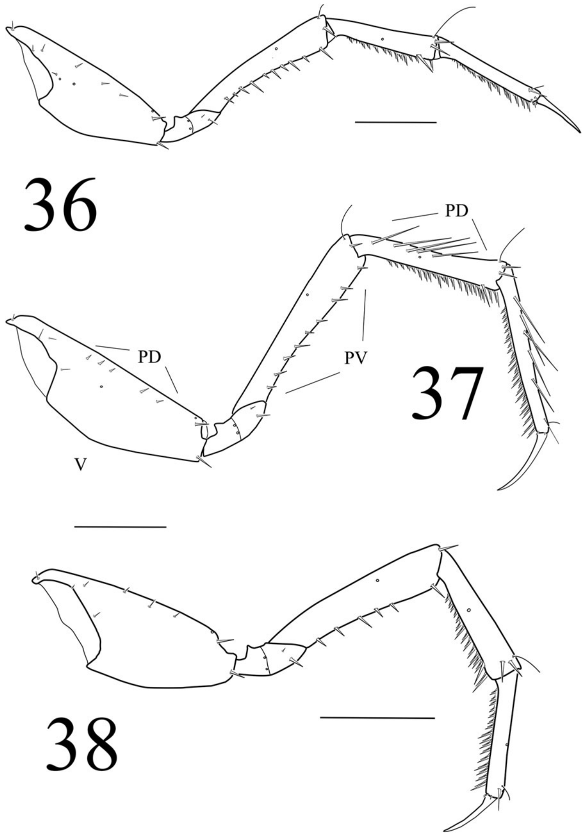
Larvae of *A. disintegratus* are readily distinguished from those of the other two species studied in this paper by the following combination of characters: larger size (Table 1); head capsule longer than broad, sub-ovate (instar I) (Figure 1) or subtrapezoidal (instar II, III) (Figure 15); instar I with more than 10 *lamellae clypeales*; *lamellae clypeales* spatulate (Figure 1); mandible less than 0.50 times HL



Figures 33–35. Metathoracic leg of selected species of *Agabus* s. str. Leach, 1817, anterior surface, instar III: (33) *A. bifarius* (Kirby, 1837); (34) *A. disintegratus* (Crotch, 1873); (35) *A. falli* (Zimmermann, 1934). CO: coxa; FE: femur; MX: maxilla; PT: pretarsus; TA: tarsus; TI: tibia; TR: trochanter. Scale bars = 0.5 mm.

(instar III), acute apically, not toothed along inner margin (Figure 5); maxillary palpomere 2 more than 1.50 times as long of maxillary palpomere 1 (instar III); metathoracic legs elongate, more than 3.70 times HW; pro- and mesothoracic legs with secondary setae on tibia and tarsus (Table 3; Figures 34, 37); primary seta FE1 inserted submedially (instar I) (Figure 10); metatibia and metatarsus with more than eight secondary setae (Table 3; Figures 34, 37); LAS with short spine-like secondary setae only (Figure 16); urogomphus elongate more than two times LAS and HW.

Description of instar I
(Figures 1–14)



Figures 36–38. Metathoracic leg of selected species of *Agabus* s. str. Leach, 1817, posterior surface, instar III: (36) *A. bifarius* (Kirby, 1837); (37) *A. disintegratus* (Crotch, 1873); (38) *A. falli* (Zimmermann, 1934). PD: posterodorsal; PV: posteroventral. Scale bars = 0.5 mm.

Body. Measurements and ratios that characterise the body shape are shown in Table 1.

Head. Head capsule (Figures 1–2) sub-ovate, longer than broad; frontoclypeus with 14–15 spatulate *lamellae clypeales*.

Mandible. 0.50 times as long as HL, acute apically, not toothed along inner margin (Figure 5).

Thorax. Legs. L3/L1 = 1.40; L3/HW = 3.90.

Abdomen. LAS/HW = 0.46–0.49. Urogomphus. U/LAS = 4.26–4.75; U/HW = 2.07–2.18.

Chaetotaxy. Position and number of additional setae on legs detailed in Table 2.

Description of instar II

As instar I except as follows:

Body. Measurements and ratios that characterise the body shape are shown in Table 1.

Head. Head capsule subtrapezoidal, lateral margin slightly diverging posteriorly; frontoclypeus with 28–32 *lamellae clypeales*.

Thorax. Legs. L3/L1 = 1.43–1.47; L3/HW = 3.70–3.97.

Abdomen. LAS/HW = 0.66–0.72. Urogomphus. U/LAS = 3.00–3.34; U/HW = 2.11–2.30.

Chaetotaxy. Parietale with 3–4 spine-like secondary setae on lateral margin; position and number of secondary setae on legs detailed in Table 3. LAS with only short and spine-like secondary setae (as in Figure 16).

Description of instar III

(Figures 15–16, 34, 37)

As instar II except as follows:

Body. Measurements and ratios that characterise the body shape are shown in Table 1.

Head. Head capsule (Figure 15). Frontoclypeus with 30–34 *lamellae clypeales*.

Thorax. Legs. L3/L1 = 1.46–1.47; L3/HW = 3.83–4.15.

Abdomen. LAS/HW = 0.83–1.05.

Urogomphus. U/LAS = 2.10–2.21; U/HW = 2.06–2.22.

Chaetotaxy. Position and number of secondary setae on legs detailed in Table 3.

Ecology

Agabus disintegratus is a species of temporary ponds. Apparently overwintering adults lay eggs in the springs, which hatch and complete development into adults by early summer (Larson, Alarie, and Roughley 2000). Garcia and Hagen (1985, 1987) and Garcia, Hagen, and Voigt (1990) on the one hand and Barman et al. (2000) on the other hand provided detailed information on the ecology of this species in California and Central Georgia, respectively.

Agabus falli (Zimmermann, 1934)

(Figures 17–30, 35, 38)

Material studied

One instar I, one instar II, one instar III reared *ex ovo* from adults collected at the following locality: Canada, Québec, Trois-Rivières at Boulevard des Récollets, pond with *Salix* sp., 14.V.1986, 28.V1986, Y. Alarie leg.

Diagnostic combination

Larvae of *A. falli* are readily distinguished from the other two *Agabus* species studied in this paper by the following combination of characters: smaller size (Table 1); head capsule broader than long, sub-rounded (Figures 17, 29); instar I with less than 10 *lamellae clypeales*; *lamellae clypeales* spine-like (Figures 17, 29); mandible more than 0.60 times HL (instar III), bluntly rounded apically (more obvious in instar I), toothed along inner margin (Figure 21); maxillary palpomere 2 about as long as maxillary palpomere 1 (instar III); metathoracic legs shorter, less than 2.60 times HW; pro- and mesothoracic legs lacking secondary setae on tibia and tarsus (Table 3; Figures 35, 38); primary seta FE1 inserted proximally (instar I) (Figure 24); metatibia and metatarsus with less than three secondary setae (Table 3; Figures 35, 38); LAS with a mix of short spine-like and elongate hair-like secondary setae (Figure 30); urogomphus very short, less than 0.40 times LAS and HW.

Description of instar I

(Figures 17–28)

Body. Measurements and ratios that characterise the body shape are shown in Table 1.

Head. Head capsule (Figures 17–18) sub-rounded, broader than long; frontoclypeus with 7 spine-like *lamellae clypeales*.

Mandible. 0.80 times as long as HL, bluntly rounded apically, toothed along inner margin (Figure 21).

Thorax. Legs. L3/L1 = 1.20; L3/HW = 2.20.

Abdomen. LAS/HW = 0.63. Urogomphus. U/LAS = 1.23; U/HW = 0.78.

Chaetotaxy. Position and number of additional setae on legs are shown in Table 2.

Description of instar II

As instar I except as follows:

Body. Measurements and ratios that characterise the body shape are detailed in Table 1.

Head. Head capsule sub-rounded, lateral margin distinctly converging posteriorly; frontoclypeus with 17 *lamellae clypeales*.

Thorax. Legs. L3/L1 = 1.23; L3/HW = 2.50.

Abdomen. LAS/HW = 0.88.

Urogomphus. U/LAS = 0.75; U/HW = 0.66.

Chaetotaxy. Parietale with 5 spine-like secondary setae on lateral margin; position and number of secondary setae on legs are detailed in Table 3. LAS with a mix of spine-like and hair-like secondary setae (as in Figure 30).

Description of instar III

(Figures 29–30, 35, 38)

As instar II larva except as follows:

Body. Measurements and ratios that characterise the body shape are detailed in Table 1.

Head. Head capsule (Figure 29). Frontoclypeus with 28 *lamellae clypeales*.

Thorax. Legs. L3/L1 = 1.28; L3/HW = 2.55.

Abdomen. LAS/HW = 1.00.

Urogomphus. U/LAS = 0.38; U/HW = 0.38.

Chaetotaxy. Position and number of secondary setae on legs are detailed in Table 3.

Ecology

Agabus falli is generally collected in ponds. Adults, which are the overwintering stage, occur very early in the spring, just when melting snow has filled the pond basin and flooded surrounding grasses and sedges (Larson et al. 2000).

Agabus bifarius (Kirby, 1837)

(Figures 31–33, 36)

Material studied

One instar III reared *ex ovo* from adults collected at the following locality: Canada, Québec, Saint-Louis-de-France, Tourbière des Grandes Prairies, bog with *Carex oligosperma* Michx., 18.VI.1986, Y. Alarie leg.

Diagnostic combination (instar III)

The instar III larva of *A. bifarius* can be distinguished from the other two species studied by the following combination of characters: smaller size (Table 1); head capsule longer than broad, sub-rounded (Figure 31); *lamellae clypeales* spatulate (Figure 31); mandible less than 0.50 times HL, acute apically, not toothed along inner margin; maxillary palpomere 2 about as long as palpomere 1; metathoracic legs shorter, less than 3.00 times HW; pro- and mesothoracic legs lacking secondary setae on tibia and tarsus (Table 3; Figures 33, 36); metatibia and metatarsus with less than three secondary setae (Table 3; Figures 33, 36); LAS with a mix of short spine-like and elongate hair-like secondary setae (Figure 32); urogomphus shorter, less than 0.80 times LAS and HW.

Description of instar III

(Figures 31–33, 36)

Body. Measurements and ratios that characterise the body shape are shown in Table 1.

Head. Head capsule (Figure 31). Frontoclypeus with 32 *lamellae clypeales*.

Thorax. Legs. L3/L1 = 1.33; L3/HW = 2.94.

Abdomen. LAS/HW = 1.08.

Urogomphus. U/LAS = 0.70; U/HW = 0.75.

Chaetotaxy. Position and number of secondary setae on legs are detailed in Table 3.

Ecology

Agabus bifarius is generally collected amongst emergent grasses and sedges of a variety of lentic habitats. Adults become active very early in the spring and the larval stages are completed by early summer (Larson et al. 2000).

Phylogenetic analysis

Fifty-five characters (47 binary and eight multistate) (Table 4) were coded for larvae of 17 species of Agabinae and two outgroups. The analysis of the data matrix (Table 5) with TNT resulted in five most parsimonious trees of 115 steps (CI = 0.55; RI = 0.69). The strict consensus was calculated (Figure 39). Character state changes are mapped on one of the most parsimonious trees (Figure 40).

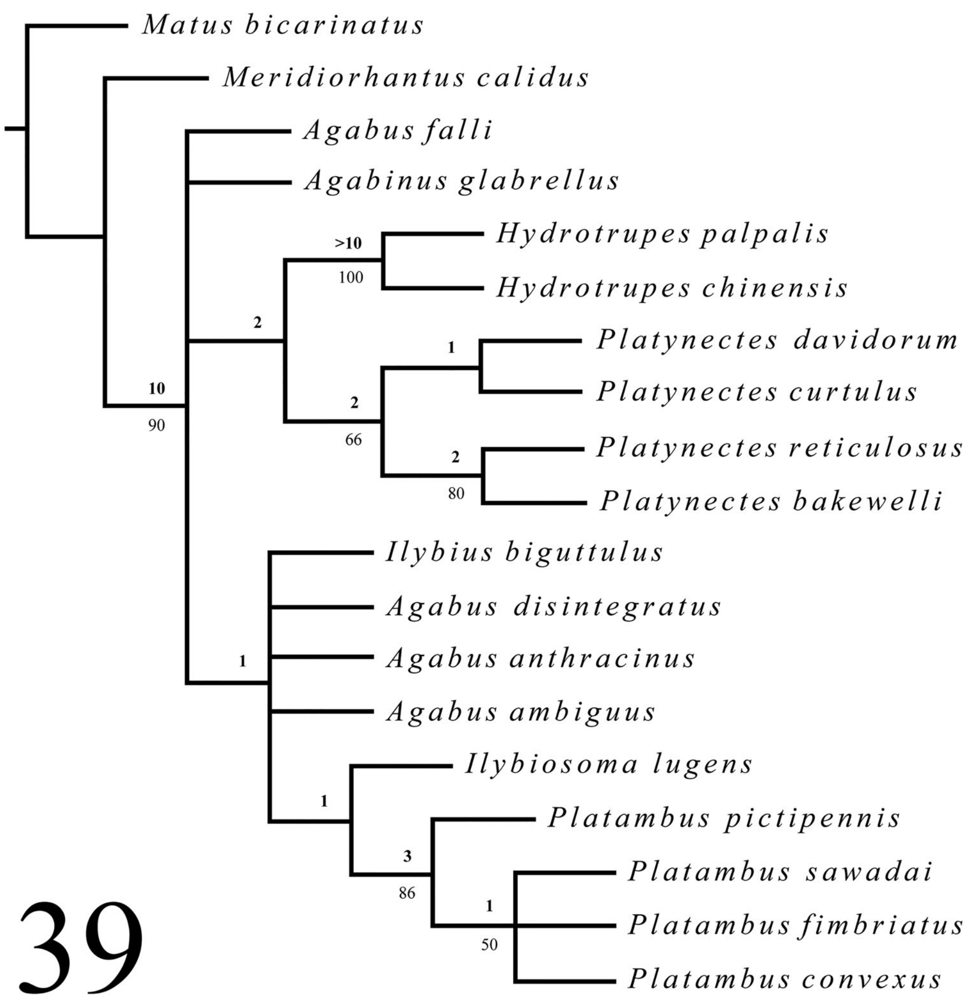


Figure 39. Strict consensus cladogram obtained from the cladistic analysis of 17 terminal taxa of Agabinae and two outgroups, with Bremer support values indicated above branches and Jackknife support values indicated below branches.

Discussion

Given the great diversity of the genus *Agabus* our study must be seen as provisional owing to the limited number of taxa included. It is obviously open to reconsideration as larvae of additional Agabinae taxa will be described.

Following this study, a placement of the three species of *Agabus* s. str. within Agabinae seems rather clear based on larval morphology. High Jackknife (0.90) and Bremer (10) values (Figure 39) indicate strong support for the monophyly of Agabinae with respect to the chosen out-groups. Larvae of *Agabus* s. str. share with other Agabinae studied the following unique character states (Figure 40): (1) absence of an occipital suture (Figures 1, 15, 17, 29, 31; character 08); (2) absence of natatory setae on legs (Figures 33–38; characters 31, 37, 41), and (3) spine-like appearance of the primary seta TI6 (Figures 11, 25; character 34).

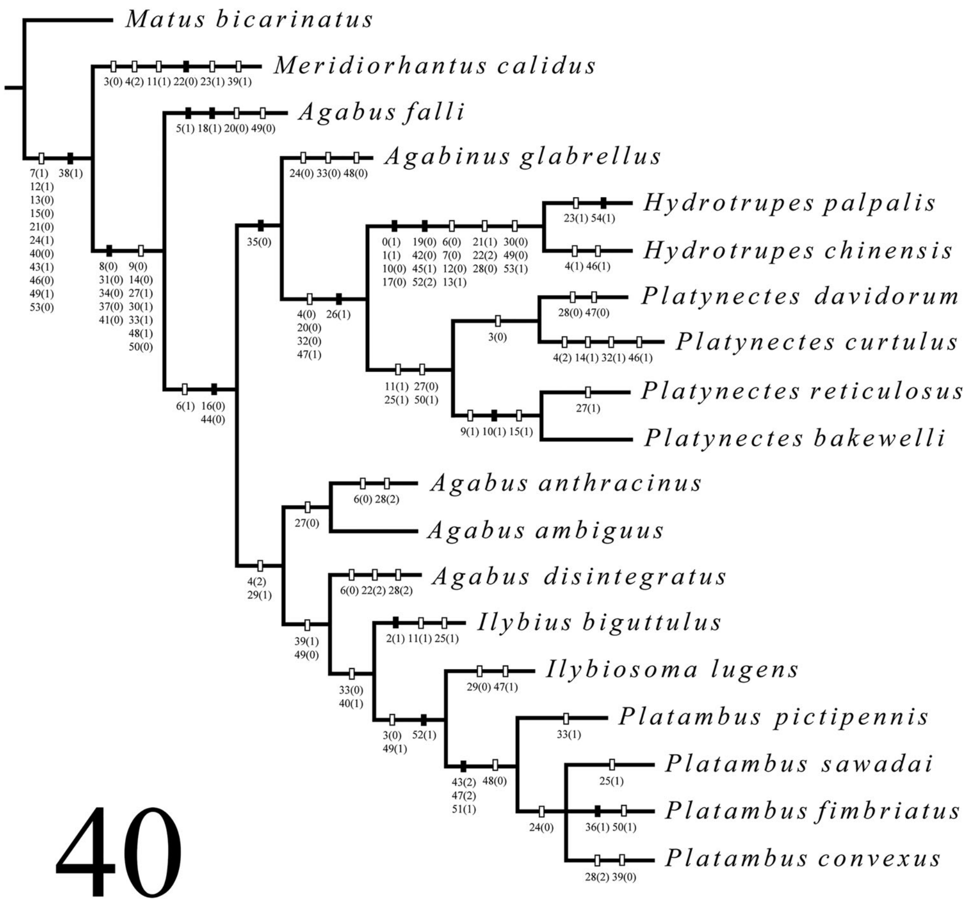


Figure 40. One of the most parsimonious trees obtained from the cladistic analysis of 17 terminal taxa of Agabinae and two outgroups, with character changes mapped for each clade. Solid rectangles indicate unique character state transformations; open rectangles indicate homoplastic character state transformations.

Quite interestingly, *Agabus falli* stands out as part of a polytomy together with *Agabinus glabrellus* (Motschulsky, 1859) and two large clades, one including *Hydrotrupes* and *Platynectes*, and the other comprising *Ilybius*, *Ilybiosoma*, *Platambus* and the other species of *Agabus* (Figure 39). Such positioning, however, is likely explained by the unique appearance of the larva of this species in comparison with all other Agabinae studied. *Agabus falli* indeed is characterised by the unique presence of spine-like *lamellae clypeales* (character 5; Figures 17, 29) added to a mandible bluntly rounded apically (character 18; Figure 21). Compared to the other Agabini studied (*Agabus* (*Acatodes*), *A.* (*Gaurodytes*), *Agabinus*, *Ilybiosoma*, *Ilybius*, *Platambus*), the larva of *Agabus falli* is also distinguished by a reduced number of *lamellae clypeales* (Figure 1), a toothed mandible (Figure 21) as well as by the presence of elongate secondary hair-like setae on the last abdominal segments (Figure 30) (also observed in *A. bifarius* (Figure 32)). With so many unique features added to the limited number of species included in our study the relative position of *Agabus falli* must be interpreted cautiously.

Table 4. Characters used for the phylogenetic analysis and the coding of states using the genera *Matus* Aubé, 1836–1838 and *Meridiorhantus* Balke et al., 2017 as outgroups.

00. Cephalic capsule (instar I): (0) maximum width at level of stemmata; (1) maximum width posterior to stemmata.
01. Coronal suture (instar III): (0) COL/HL > 0.50; (1) COL/HL < 0.40.
02. Egg bursters (instar I): (0) located close to frontal suture; (1) located far from frontal suture.
03. Seta FR5 (instar I): (0) inserted anteriorly; (1) inserted anterolaterally.
04. Number of lamellae clypeales (excluding FR10) (instar I): (0) four; (1) six to eight; (2) 10 or more.
05. Lamellae clypeales (instars I–III): (0) Bluntly rounded to spatulate; (1) spine-like.
06. Parietals (at level of occiput) (instar I): (0) not constricted; (1) constricted.
07. Parietals (at level of occiput) (instars II–III): (0) not constricted; (1) constricted.
08. Occipital suture (instar I): (0) absent; (1) present.
09. Occipital suture (instars II–III): (0) absent; (1) present.
10. Antenna (instar III). (0) A/HW < 0.50; (1) $0.50 <$ A/HW < 0.60 (2) A/HW > 0.60.
11. Apical lateroventral process of antennomere 3 (instars I–III): (0) protruding; (1) not protruding.
12. Antennomere 3 (instar III): (0) Longer than antennomere I, A3/A1 > 1.50; (1) about as long as antennomere I, A3/A1 $= 0.80 - 1.10$.
13. Pore ANc (instar I): (0) inserted distally; (1) inserted submedially.
14. Additional ventroapical pores on antennomere 3 (instar I): (0) absent; (1) present.
15. Secondary setae on antennomere 1 (instars II–III): (0) absent; (1) present.
16. Mandible (instar III): (0) shorter, MNL/HL < 0.50; (1) longer, MNL/HL > 0.60.
17. Mandible (instars II–III): (0) with one secondary seta; (1) with several secondary setae.
18. Apex of mandible (instar I): (0) Acute;(1) bluntly rounded.
19. Mandibular channel (instars I–III): (0) absent; (1) present.
20. Inner ventral margin of mandible (instars I–III): (0) serrate; (1) not serrate.
21. Galea (instar III): (0) short, <0.40 length of maxillary palpomere 1; (1) long, > 0.50 length of maxillary palpomere 1.
22. Maxillary palpomere 2 (instar III): (0) shorter than maxillary palpomere 1, MP2/MP1 < 0.90; (1) as long as maxillary palpomere 1, MP2/MP1 $= 0.90 - 1.10$; (2) much longer than maxillary palpomere 1, MP2/MP1 > 1.40.
23. Maxillary palpomere 3 (instar III): (0) about as long as maxillary palpomere 2, MP2/MP3 $= 0.80 - 1.20$; (1) much shorter than maxillary palpomere 2, MP2/MP3 > 1.40.
24. Additional setae on stipes (instar I): (0) absent; (1) a single seta inserted contiguously to seta MX6.
25. Seta LA10 (instar I): (0) inserted distally; (1) inserted submedially.
26. Seta LA12 (instar I): (0) inserted distally; (1) inserted submedially.
27. Additional setae on dorsal surface of prementum (instar I): (0) absent; (1) present.
28. Metathoracic legs (instar III): (0) L3/HW < 2.40; (1) L3/HW $= 2.45 - 3.50$; (2) L3/HW > 3.60.
29. Seta FE1 (instar I): (0) inserted proximally; (1) inserted submedially.
30. Additional posteroventral setae on femur (instar I): (0) absent; (1) present.
31. Natatory posterodorsal setae on femur (instars II–III): (0) absent; (1) present.
32. Secondary anterodorsal setae on femur (instars II–III): (0) absent; (1) present.
33. Seta TI4 (instar I): (0) not inserted more proximally on protibia than on metatibia; (1) inserted more proximally on protibia than on metatibia.
34. Seta TI6 on metatibia (instar I): (0) spine-like; (1) hair-like.
35. Secondary setae on tibiae (instars II–III): (0) absent; (1) present.
36. Primary setae TI2, TI3, TI6, TI7, TA6, TA7 (instars II–III): (0) acute; (1) bluntly pointed.
37. Natatory posterodorsal setae on tibia (instars II–III): (0) absent; (1) present.
38. Seta TA1 (instar I): (0) elongate to very elongate; (1) very short.
39. Secondary dorsal setae on protarsus (instar III): (0) absent; (1) present.
40. Secondary posteroventral setae on pro- and metatarsus (instar III): (0) absent; (1) present.
41. Natatory posterodorsal setae on tarsus (instars II–III): (0) absent; (1) present.
42. Metatibia and metatarsus (instar III): (0) lacking spine-like spinulae along ventral margin; (1) with well-developed spine-like spinulae along ventral margin.
43. Abdominal segment 8 (instar III): (0) extensively extending posteriorly beyond bases of urogomphi; (1) moderately extending posteriorly beyond bases of urogomphi; (2) not extending posteriorly beyond bases of urogomphi.
44. Abdominal segment 8 (instars II–III): (0) with almost only more or less elongate spine-like secondary setae; (1) with a mix of short spine-like and elongate hair-like secondary setae.
45. Seta AB14 (instar I): (0) not lanceolate; (1) lanceolate.
46. Additional setae on abdominal segment VIII (instar I): (0) absent; (1) 1–2 spine-like setae inserted on dorsolateral surface; (2) numerous.
47. Siphon (instars I): (0) rounded; (1) deeply sinuate medially; (2) lacking.
48. Urogomphus (instars I): (0) composed of one urogomphomere; (1) composed of two urogomphomeres.
49. Seta UR4 (instar I): (0) shorter than seta UR3; (1) as long or longer than seta UR3.
50. Seta UR4 (instar I): (0) articulated close to setae UR2 and UR3; (1) articulated distad to setae UR2 and UR3.
51. Seta UR5 (instar I): (0) elongate, as long as seta UR7; (1) short, much shorter than seta UR7.
52. Seta UR6 (instar I): (0) elongate, as long as seta UR7; (1) short, much shorter than seta UR7.
53. Seta UR8 (instar I): (0) elongate, hair-like; (1) elongate, spine-like; (2) short, spine-like.
54. Additional setae on urogomphomere 1 (instar I): (0) absent; (1) 1–2 spine-like setae inserted dorsolaterally.

Table 5. Data matrix used for the cladistic analysis. Missing data coded '?'

Species	00	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Matus bicarinatus	0	0	0	1	1	0	0	0	1	1	2	0	0	1	1	1	1	1	0	1	1	1	1	0	0	0	0	0
Meridiorhantus calidus	0	0	0	0	2	0	0	1	1	1	2	1	1	0	1	0	1	1	0	1	1	0	0	1	1	0	0	0
Agabinus glabrellus	0	0	0	1	1	0	1	1	0	0	2	0	1	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1
Agabus (G.) ambiguus	0	0	0	1	2	0	1	1	0	0	2	0	1	0	0	0	0	1	0	1	1	0	1	0	1	0	0	0
Agabus (A.) anthracinus	0	0	0	1	2	0	0	1	0	0	2	0	1	0	0	0	0	1	0	1	1	0	1	0	1	0	0	0
Agabus (s. str.) falli	0	0	0	1	1	1	0	1	0	0	2	0	1	0	0	0	1	1	1	1	0	0	1	0	?	0	0	1
Agabus (s. str.) disintegratus	0	0	0	1	2	0	0	1	0	0	2	0	1	0	0	0	0	1	0	1	1	0	2	0	?	0	0	1
Hydrotrupes chinensis	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	2	0	1	0	1	1
Hydrotrupes palpalis	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	2	1	1	0	1	1
Ilybiosoma lugens	0	0	0	0	2	0	1	1	0	0	2	0	1	0	0	0	0	1	0	1	1	0	1	0	1	0	0	1
Ilybius biguttulus	0	0	1	1	2	0	1	1	0	0	2	1	1	0	0	0	0	1	0	1	1	0	1	0	1	1	0	1
Platynectes bakewelli	0	0	0	1	0	0	1	1	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	0	1	1	1	0
Platynectes curtulus	0	0	0	0	2	0	1	1	0	0	2	1	1	0	1	0	0	1	0	1	0	0	1	0	1	1	1	0
Platynectes reticulosus	0	0	0	1	0	0	1	1	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	0	1	1	1	1
Platynectes davidorum	0	0	0	0	0	0	1	1	0	0	2	1	1	0	0	0	0	1	0	1	0	0	1	0	?	1	1	0
Platambus convexus	0	0	0	0	2	0	1	1	0	0	2	0	1	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1
Platambus fimbriatus	0	0	0	0	2	0	1	1	0	0	2	0	1	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1
Platambus pictipennis	0	0	0	0	2	0	1	1	0	0	2	0	1	0	0	0	0	1	0	1	1	0	1	0	1	0	0	1
Platambus sawadai	0	0	0	0	2	0	1	1	0	0	2	0	1	0	0	0	0	1	0	1	1	0	1	0	0	1	0	1

Species	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Matus bicarinatus	1	0	0	1	1	0	1	1	0	1	0	0	1	1	1	0	1	0	2	0	0	0	1	0	0	1	0
Meridiorhantus callidus	1	0	0	1	1	0	1	1	0	1	1	1	0	1	1	1	1	0	0	0	0	1	1	0	0	0	0
Agabinus glabrellus	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0
Agabus (G.) ambiguus	1	1	1	0	1	1	0	1	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0
Agabus (A.) anthracinus	2	1	1	0	1	1	0	1	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0
Agabus (s. str.) falli	1	0	1	0	1	1	0	1	0	0	1	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0
Agabus (s. str.) disintegratus	2	1	1	0	1	1	0	1	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0
Hydrotrupes chinensis	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	1	1	1	0	0	0	2	1	0
Hydrotrupes palpalis	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	0	2	1	1
Ilybiosoma lugens	1	0	1	0	1	0	0	1	0	0	1	1	1	0	1	1	0	0	0	1	1	1	0	0	1	0	0
Ilybius biguttulus	1	1	1	0	1	0	0	1	0	0	1	1	1	0	1	1	0	0	0	0	1	0	0	0	0	0	0
Platynectes bakewelli	1	0	1	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	1	1	1	0	0	0	0
Platynectes curtulus	1	0	1	0	1	1	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0
Platynectes reticulosus	1	0	1	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	1	1	1	0	0	0	0
Platynectes davidorum	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	1	1	0	0	0	0
Platambus convexus	2	1	1	0	1	?	0	1	0	0	1	0	1	0	1	2	0	0	0	2	0	1	0	1	1	0	0
Platambus fimbriatus	1	1	1	0	1	?	0	1	1	0	1	1	1	0	1	2	0	0	0	2	0	1	1	1	1	0	0
Platambus pictipennis	1	1	1	0	1	1	0	1	0	0	1	1	1	0	1	2	0	0	0	2	0	1	0	1	1	0	0
Platambus sawadai	1	1	1	0	1	?	0	1	0	0	1	1	1	0	1	2	0	0	0	2	0	1	0	1	1	0	0

It is worth noting that excluding *Agabinus* and *Agabus falli* makes Agabini monophyletic. Although weakly supported (Bremer support = 1), members of this clade are characterised by (1) a larger number (>10) of *lamellae clypeales* in instar I (character 04; Figure 1) and by the more distal articulation of the primary seta FE1 (character 29; Figure 10 as compared to Figure 24). These two traits may be useful in the future study of other Agabinae larvae.

Although topologically close, *Agabus* (s. str.) *disintegratus*, *A.* (*Acatodes*) *anthracinus* Mannerheim, 1852 and *A.* (*Gaurodytes*) *ambiguus* (Say, 1823) are part of an unresolved polytomy along with *Ilybius biguttulus* (Germar, 1824), all four sister to a weakly supported clade comprised of *Ilybiosoma* and *Platambus* (Figure 40). This result is, to some extent, not surprising knowing that Agabini is a difficult group, which always has shown bad resolution in recent analysis of the Dytiscidae based on larval morphology (cf., Michat, Alarie, and Miller 2017). It is worth noting, however, that members of Hydrotrupini and Platynectini stand out as monophyletic in our analysis sister to Agabini minus *Agabinus* and *Agabus falli*, which supports the observations made in the context of previous studies (Ribera, Nilsson, and Vogler 2004; Ribera, Vogler, and Balke 2008; Miller and Bergsten 2014; Alarie et al. 2019). In this context, it will be interesting to continue the study of Agabinae larval morphology by integrating a larger number of species and characters.

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