

Phylogenetic relationships, larval morphology, and chaetotaxy of the subfamily Coptotominae (Coleoptera: Dytiscidae)

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Abstract—Larval morphology of the monogeneric subfamily Coptotominae (Coleoptera: Dytiscidae) is described and illustrated in detail, with particular emphasis on morphometry and chaetotaxy. Larvae of *Coptotomus* Say are unique within Dytiscidae in the presence of tracheal gills on the abdominal segments I–VI, a short bifid horn or nasale in instar I, long spinulae on the urogomphus in instar I, and rows of natatory setae on both the internal and external margins of the urogomphus in instars II and III. A cladistic analysis based on 125 larval characters sampled among representatives of other dytiscid subfamilies supports a sister-group relationship between Coptotominae and Laccophilinae based on the shared absence of setae LA10 and LA12 on the second labial palpomere and of pore ABc on the abdominal segment VIII. The clade Coptotominae + Laccophilinae resolved as sister to Lancetinae, all three subfamilies sharing the presence of an unusually low number of lamellae clypeales in the first instar (a condition called four-peg-pattern), postulated to have evolved secondarily within Dytiscidae.

Résumé—La morphologie larvaire de la sous-famille monogénérique Coptotominae (Coleoptera: Dytiscidae) est décrite et illustrée en détail, en portant une attention particulière à la morphométrie et la chétotaxie. Les larves de *Coptotomus* Say se distinguent de celles des autres Dytiscidae par la présence de branchies trachéales sur les segments abdominaux I–VI, d’une corne bifide ou nasale ainsi que de spinules allongées sur les urogomphes chez la larve de stade I, et par la présence de soies natatoires sur les marges internes et externes des urogomphes de la larve de stade II et III. Une analyse cladistique effectuée à partir de 125 caractères larvaires répertoriés parmi des représentants des autres sous-familles de Dytiscidae suggère une origine monophylétique des Coptotominae et des Laccophilinae en raison de l’absence chez les larves de ce groupe des soies LA10 et LA12 sur le deuxième article du palpe labial ainsi que du pore ABc sur le segment abdominal VIII. La sous-famille Lancetinae est suggérée à titre de groupe consoeur du groupe monophylétique Coptotominae + Laccophilinae en raison de la présence chez les larves de stade I de ce groupe d’un nombre anormalement faible de ‘lamellae clypeales’ (une condition appelée ‘four-peg-pattern’) ce qui paraît représenter une évolution secondaire chez les Dytiscidae.

Introduction

The monogeneric dytiscid subfamily Coptotominae (Coleoptera: Dytiscidae) is a Nearctic endemic group of diving beetles that includes five medium-sized (6.0–8.0 mm long) species generally inhabiting weedy areas of permanent ponds, lakes, and slowly flowing streams (Larson *et al.* 2000; Nilsson 2001). The genus *Coptotomus* Say has a broad range of distribution

in North America being found from southern Canada to northern Mexico (Larson *et al.* 2000).

The taxonomic placement of *Coptotomus* within the Dytiscidae has been the matter of much debate. Traditionally, *Coptotomus* has been treated as a monotypic tribe in the subfamily Colymbetinae (*e.g.*, Brinck 1948; Burmeister 1976; Ruhnau and Brancucci 1984). In a comparative study of the Hydradephaga ovipositor, Burmeister (1976) found a close relationship

Received 25 June 2012. Accepted 14 August 2012. First published online 1 May 2013.

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Subject editor: Patrice Bouchard

doi:10.4039/tce.2012.100

between *Coptotomus* and the colymbetine genera *Colymbetes* Clairville, *Rhantus* Dejean, and *Meladema* Laporte. More recently, Miller (2001) provided convincing arguments in support of the elevation of *Coptotomus* to subfamily level based mainly on female genitalia and postulated a sister-group relationship between *Coptotomus* and a clade comprised the subfamilies Copelatinae, Hydrodytinae, and Hydroporinae. Barman (2004), based on larval characters, postulated a sister-group relationship between Coptotominae and Laccophilinae, and Ribera *et al.* (2008), based on molecular data, found *Coptotomus* nested within Copelatinae, as sister to the genus *Agaporomorphus* Zimmermann.

The study of larval morphology provides another line of evidence to test phylogenetic hypotheses. As different expressions of the same genotype, larval characters help to complement adult characters, which have been traditionally the primary basis for classification. Recent studies have demonstrated the taxonomic and phylogenetic value of chaetotaxy in studying larval Dytiscidae (e.g., Alarie *et al.* 2000, 2009, 2011; Alarie and Michat 2007; Michat *et al.* 2007; Michat and Alarie 2009; Michat and Torres 2009). There is an overall pattern of setae and pores, which is widespread among taxa, though it is modified in a variety of groups. This generalised pattern is consistent enough to be used for phylogenetic analysis and yet sufficiently variable to allow for taxonomic distinction.

Knowledge of the larval morphology of *Coptotomus* is scanty. Larvae of two species have been described: *Coptotomus interrogatus* (Fabricius) (Wilson 1923; De Marzo 1976; Barman 2004) and *Coptotomus longulus lenticus* Hilsenhoff (Bacon *et al.* 2000, only the mature larva). Descriptions at the genus level were also provided by Ruhnau and Brancucci (1984), Nilsson (1988) (only leg primary chaetotaxy), and Larson *et al.* (2000). Most of these treatments, however, lack comparative precision and detail and can hardly be used in the context of the recent system developed for the Dytiscidae (e.g., Alarie 1995, 1998; Alarie *et al.* 2002, 2009, 2011; Michat and Torres 2009). The aims of this paper therefore are: (i) to describe and illustrate all larval instars of *C. longulus lenticus* including detailed morphometric and chaetotaxic analyses of the cephalic capsule, head appendages,

legs, last abdominal segment, and urogomphus; (ii) to propose the ground plan pattern of primary setae and pores for the subfamily Coptotominae; and (iii) to revisit the relative phylogenetic placement of the Coptotominae within Dytiscidae based on an extensive data set of larval characters.

Materials and methods

Material examined

Eight specimens of instar I, five of instar II, and three of instar III of *C. longulus lenticus* were used for the descriptions. Larvae were collected in association with adults at the following locality: Canada, Trois-Rivières area (46°22'N, 72°31'W), which is the southern border of the region (41 794 km²) drained by the St-Maurice River, midway between the cities of Québec and Montréal. Sampling sites included open pools surrounded by willow shrubs (*Salix bebbiana* Sargent and *Salix discolor* Mühlenberg (Salicaceae)) and cattails (*Typha angustifolia* Linnaeus and *Typha latifolia* Linnaeus (Typhaceae)). The identification is firm as *C. longulus lenticus* is the only species of *Coptotomus* collected at that locality. *Coptotomus loticus* Hilsenhoff, the only other species of the genus present in Canada, was never collected in the region where the larvae and adults were collected. Also, that species has been most frequently found in flowing waters.

Methods

Specimens were cleared in lactic acid, dissected and mounted on glass slides with polyvinyl-lactoglycerol. Observation (at magnifications up to 1000×) and drawings were made using an Olympus CX31 (Olympus Corporation, Tokyo, Japan) compound microscope equipped with a camera lucida. Drawings were scanned and digitally inked using a Genius PenSketch tablet (KYE Corporation, Taipei, Taiwan). The material is held in the larval collection of Y. Alarie (Laurentian University, Sudbury, Ontario, Canada).

Morphometric analysis

We employed the terms used in previous papers dealing with the larval morphology of Agabinae and Colymbetinae (Michat 2005; Alarie *et al.* 2009; Michat and Archangelsky 2009). Paired structures of each individual were considered independently. The following measurements were

taken (with abbreviations shown in parentheses). Head length (HL) (total HL including the frontoclypeus, measured medially along the epicranial stem); maximum head width (HW); length of frontoclypeus (FRL) (from apex of nasale to the joint of frontal and coronal sutures); occipital foramen width (OCW) (maximum width measured along dorsal margin); coronal line length (COL); length of mandible (MNL) (measured from laterobasal angle to apex); width of mandible (MNL) (maximum width measured at base); length of maxillary palpi (PPF); length of galea (GA). Length of antenna (A), 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, including the longest claw (CL), 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). The length of trochanter includes only the proximal portion, considered from the base to the beginning of the femur. The legs were considered as being composed of six articles following Lawrence (1991). Length of last abdominal segment (LAS) (measured dorsally along midline from anterior to posterior margin). Length of urogomphus (U) (total length from base to apex). These measurements were used to calculate several ratios that characterise body shape.

Chaetotaxic analysis

Primary (present in first instar) and secondary (added in later instars) setae and pores were distinguished in the cephalic capsule, head appendages, legs, LAS, and urogomphus. Sensilla were coded by two capital letters, in most cases corresponding to the first two letters of the name of the structure on which are located, and a number (setae) or a lower case letter (pores). The following abbreviations were used: AB, abdominal segment VIII; AN, antenna; CO, coxa; FE, femur; FR, frontoclypeus; LA, labium; MN, mandible; MX, maxilla; PA, parietal; PT, pretarsus; TA, tarsus; TI, tibia; TR, trochanter; UR, urogomphus. Setae and pores present in first instars were labelled by comparison with the groundplan of chaetotaxy of the subfamilies

Agabinae and Colymbetinae (Alarie 1995, 1998; Michat 2005; Alarie *et al.* 2009; Michat and Archangelsky 2009). Homologies were recognised using the criterion of similarity of position (Wiley 1981). Setae located at the apices of the antenna and maxillary and labial palpi were extremely difficult to distinguish due to their position and small size. Accordingly, they are not well represented in the drawings.

Phylogenetic analysis

The phylogenetic relationships of *Coptotomus* (Coptotominae) were analysed cladistically using the program TNT (Goloboff *et al.* 2008) and considering the character set provided by the larval chaetotaxy and morphology. A broad sampling of taxa was included comprising members of eight of the remaining nine dytiscid subfamilies. The subfamily Hydrodytinae was not included because their larvae are unknown. Data were scored directly from the observation of the specimens except for *C. interrogatus*; data for this species were scored from Barman (2004). In order to avoid the problem of comparing different semaphoronts (see Meier and Lim 2009), in the codification of each character the same instars were compared. The tree was rooted in Aspidytidae (another family of aquatic Adephaga) to allow the dytiscid taxa to vary freely, thus testing the relationships of Coptotominae with the other dytiscid subfamilies. All characters were treated as unordered and equally weighted. Multistate characters were treated as nonadditive. A heuristic search was implemented using “tree bisection reconnection” as algorithm, with 200 replicates and saving 100 trees per replication (previously setting “hold 20000”). 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 sub-optimal cladograms by one step, until all Bremer values were obtained (Kitching *et al.* 1998).

Results

Description of the larvae of *C. longulus lenticus* Hilsenhoff

Diagnosis

The larvae of *Coptotomus* are characterised by the following combination of characters: occipital

suture absent (Figs. 2, 16); egg bursters elongate (Fig. 2); anterior margin of FR projected forward in a horn (nasale) (Figs. 2, 16); instar I bearing two lamellae clypeales (Fig. 2); A3 with a ventroapical spinula (Fig. 5); cleaning device present on proTI and proTA (Figs. 17–18); abdominal segments I–VI bearing tracheal gills (Fig. 1); U long, one-segmented, bearing rows of elongate spinulae (instar I, Fig. 15) or setae (instars II–III, Fig. 19) on lateral margins; seta AN3 located basally (Fig. 5); A3 without additional pores (Figs. 4–5); stipes with one additional seta contiguous to MX5 and MX6 (Fig. 7); prementum with 5–7 additional spine-like setae (Figs. 9–10); setae LA10 and LA12 absent (Figs. 9–10); seta CO7 inserted proximally on all legs (Fig. 11); seta FE1 inserted submedially (Fig. 11); setae FE5 and TI6 short, spine-like (Fig. 12); seta TI7 elongate, hair-like (Fig. 12); seta AB13 and pore ABc absent (Figs. 13–14); TI and TA with natatory setae (instars II–III) (Fig. 18); LAS with a row of natatory setae on each lateral margin (instars II–III) (Fig. 19).

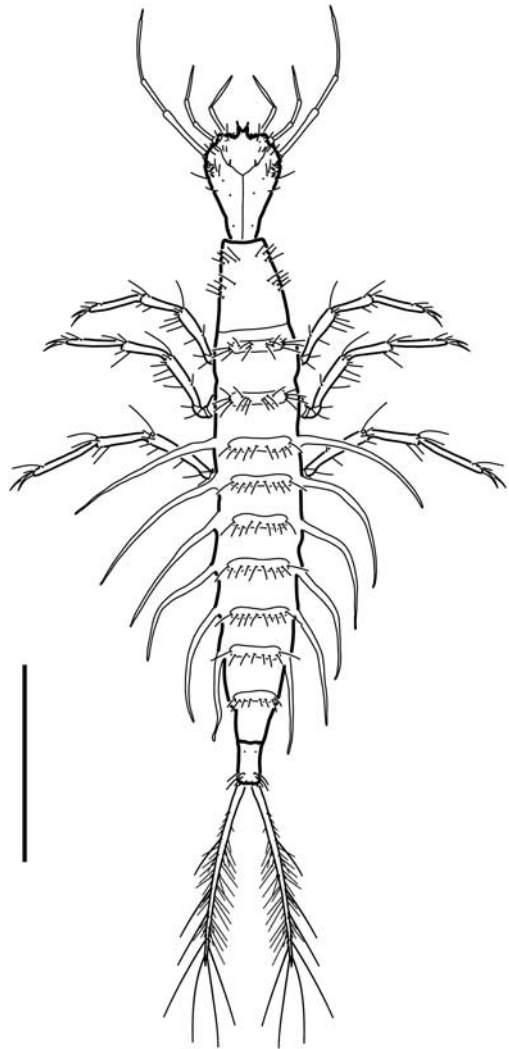
First instar (Figs. 1–15)

Colour (alcohol-preserved specimens). Larva uniformly light brown to brown; cephalic capsule, thorax and abdomen sometimes darker than head appendages, legs, and urogomphi.

Body. Subcylindrical, widest at metathorax, narrowing anteriorly and posteriorly (Fig. 1). Measurements and ratios that characterise the body shape are shown in Table 1.

Head. *Cephalic capsule* (Figs. 2–3). Subtriangular, longer than broad; posterodorsal and lateral surfaces covered with minute spinulae; maximum width at level of stemmata, narrowing towards occipital foramen; neck constriction present, moderately marked; occipital suture absent; ecdysial line visible, coronal line long; occipital foramen deeply emarginate ventrally; posterior tentorial pits visible ventrally; FR subtriangular, with one elongate, slightly prominent egg burster on each posterolateral margin, anterior margin projected forward in a V-shaped horn (nasale) bearing two well-developed lamellae clypeales; anterolateral lobes well developed, not projected beyond nasale; six stemmata at each side, arranged in two vertical rows. *Antenna* (Figs. 4–5). Very long, slender, much longer than HW, composed of four

Fig. 1. *Coptotomus longulus lenticus*, first instar, habitus, dorsal aspect. Scale bar = 1.50 mm.



antennomeres; A4 the shortest, A1 and A3 the longest, subequal, A3 with a ventroapical spinula; A3' short. *Mandible* (Fig. 6). Prominent, broad basally, distal half projected inwards, apex sharp, basoexternal margin with minute spinulae, ventrointernal margin with denticles; mandibular channel present. *Maxilla* (Figs. 7–8). Cardo small, subovate; stipes short, broad, with minute spinulae on dorsointernal and ventrointernal surfaces, anterointernal angle prominent; galea well developed, subconical, lacinia absent; PPF short, palpomere-like; MP elongate, composed of three palpomeres, MP1 and MP2 the shortest, subequal,

Table 1. Measurements and ratios for the three larval instars of *Coptotomus longulus lenticus*.

Measure	Instar I (<i>n</i> = 3)	Instar II (<i>n</i> = 3)	Instar III (<i>n</i> = 3)
HL (mm)	0.84–0.92	1.38–1.44	2.18–2.25
HW (mm)	0.58–0.59	0.88–0.93	1.50–1.52
FRL (mm)	0.35–0.38	0.54–0.55	0.78–0.82
OCW (mm)	0.19–0.22	0.36–0.41	0.64–0.69
HL/HW	1.44–1.55	1.48–1.62	1.43–1.49
HW/OCW	2.68–3.03	2.29–2.47	2.17–2.37
COL/HL	0.57–0.59	0.60–0.61	0.62–0.65
FRL/HL	0.41–0.43	0.39–0.40	0.35–0.38
A/HW	2.08–2.18	1.79–1.86	1.21–1.29
A3/A1	1.02–1.11	0.90–0.92	0.74–0.77
A3/A2	1.53–1.64	1.36–1.43	1.31–1.37
A4/A3	0.15–0.17	0.11–0.12	0.10
A3'/A4	0.31–0.36	0.36–0.46	0.50
MNL/MNW	2.18–2.29	2.30–2.57	2.46–2.61
MNL/HL	0.40–0.42	0.38–0.40	0.36–0.37
A/MP	2.02–2.08	2.02–2.12	1.93–1.98
PPF/MP1	0.14–0.16	0.13	0.14–0.15
GA/MP1	0.21–0.25	0.18–0.22	0.18–0.22
MP2/MP1	0.97–1.06	0.85–0.92	0.75–0.79
MP2/MP3	0.63–0.67	0.76–0.80	0.84–0.90
MP/LP	1.06–1.09	1.05–1.07	1.03–1.06
LP2/LP1	1.00–1.06	0.84–0.88	0.68–0.76
L3 (mm)	2.17–2.34	3.36–3.42	4.95–5.14
L3/L1	1.19–1.22	1.22–1.26	1.24–1.28
L3/L2	1.13–1.14	1.13–1.15	1.16–1.18
L3/HW	3.71–4.01	3.61–3.89	3.24–3.42
L3 (CO/FE)	0.86–0.89	0.90–0.92	0.95–0.98
L3 (TI/FE)	0.75–0.80	0.74–0.77	0.69–0.73
L3 (TA/FE)	1.01–1.04	0.88–0.92	0.71–0.79
L3 (CL/TA)	0.42–0.44	0.34–0.37	0.24–0.32
LAS (mm)	0.36–0.47	1.05–1.25	2.25–2.37
LAS/HW	0.60–0.80	1.13–1.35	1.47–1.57
U (mm)	1.42–1.55	1.98–2.07	2.72–2.81
U/LAS	3.11–4.36	1.61–1.93	1.18–1.21
U/HW	2.42–2.64	2.13–2.26	1.79–1.87

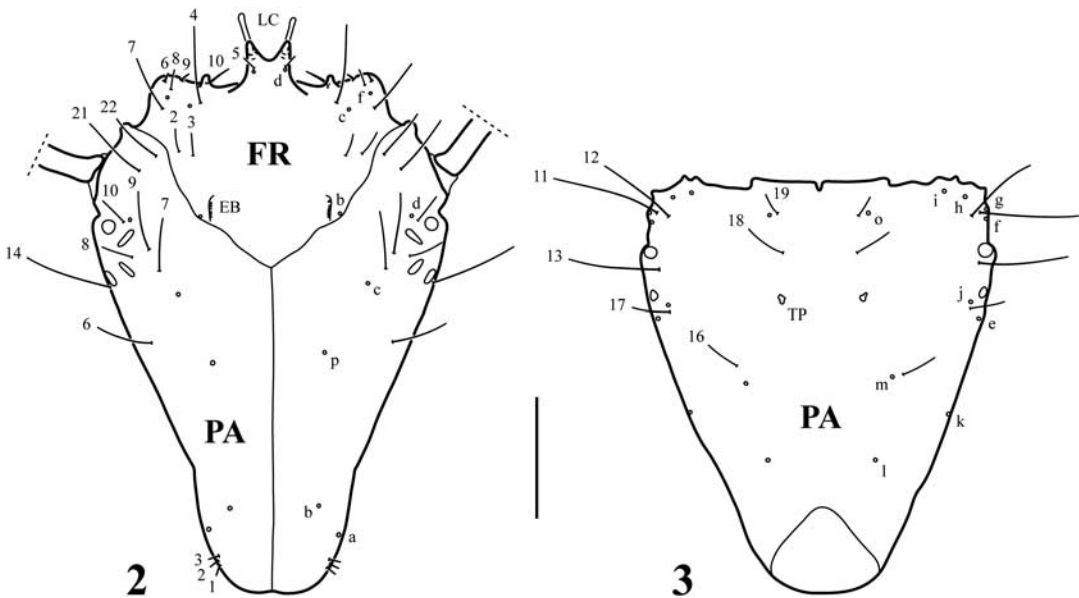
HL, Head length; HW, head width; FRL, frontoclypeus length; OCW, occipital foramen width; COL, coronal line length; MNL, length of mandible; MNW, width of mandible; MP, maxillary palpi; PPF, length of maxillary palpi; GA, length of galea; LP, labial palpi; LAS, last abdominal segment.

MP3 the longest. *Labium* (Figs. 9–10). Prementum well developed, subrectangular, broader than long, with minute spinulae dorsally; LP long, composed of two palpomeres subequal in length.

Thorax. Terga convex, pronotum about as long as mesonotum and metanotum combined, mesonotum and metanotum subequal, wider than pronotum; protergite short, subrectangular, not covering anterior and posterior parts of pronotum, more developed than mesotergite and metatergite; mesotergite and metatergite small,

transverse, not covering the whole dorsal surface; all sclerites without anterior transverse carina, with sagittal line; thoracic sterna membranous; spiracles absent. *Legs* (Figs. 11–12). Long, composed of six segments, L1 the shortest, L3 the longest; CO robust, elongate, TR transversely divided into two parts by an annulus, FE, TI, and TA slender, subcylindrical, PT with two long, slender, slightly curved claws, posterior claw shorter than anterior one; surface of CO (dorsoproximal), FE, TI, and TA covered with minute slender spinulae; ventral

Figs. 2–3. *Coptotomus longulus lenticus*, first instar. (2) Cephalic capsule, dorsal aspect; (3) Cephalic capsule, ventral aspect. FR, frontoclypeus; PA, parietal; EB, egg bursters; LC, lamellae clypeales; TP, tentorial pits. Scale bar = 0.20 mm.



surface of TI and TA with a row of long spinulae; spinulae on anteroventrodistal portion of proTI and anteroventroproximal portion of proTA dense, forming weakly delimited patches (cleaning device).

Abdomen. Eight-segmented; segments I–VI sclerotised dorsally, membranous ventrally, bearing a long tracheal gill on each lateral; segment VII narrower, sclerotised dorsally, membranous ventrally; tergites I–VII small, narrow, transverse, rounded laterally, without sagittal line; all sclerites without anterior transverse carina, covered with minute slender spinulae; spiracles absent on segments I–VII; LAS (Figs. 13–14) the narrowest, truncate posteriorly, lacking a siphon; completely sclerotised, ring-like, covered with minute slender spinulae. *Urogomphus* (Fig. 15). Very long, composed of one urogomphomere, much longer than LAS, surface covered with minute slender spinulae, lateral margins with rows of elongate spinulae.

Chaetotaxy (Figs. 1–15). Similar to that of generalised Agabinae and Colymbetinae larvae (Alarie 1995, 1998; Michat 2005; Alarie *et al.* 2009; Michat and Archangelsky 2009) except for the following features: pore FRe absent; seta

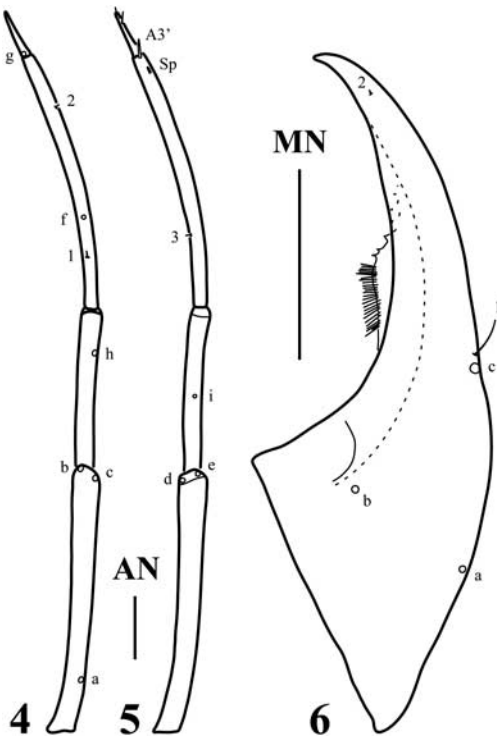
AN3 located basally; pore ANg proximal; A3 without additional pores; seta MN1 close to pore MNC; stipes with one additional seta contiguous to MX5 and MX6; MP3 with a minute structure (possibly a spinula) on ventrodistal margin; prementum with 4–6 additional spine-like setae on anterodorsal and anteroventral surfaces, and one additional pore on dorsal surface; setae LA10 and LA12 absent; LP2 with a minute structure (possibly a pore) on ventral surface, near seta LA11; seta CO7 inserted proximally on all legs; seta FE1 inserted submedially; seta FE5 short, spine-like; additional setae occasionally present on dorsal and ventral surfaces of mesoFE and metaFE; seta TI6 short, spine-like; seta TI7 long, hair-like; seta TA7 inserted apically on proTA and mesoTA; TI and TA without additional setae; seta AB13 and pore ABc absent; seta AB15 inserted medially to seta AB11; seta UR7 long, hair-like.

Second instar

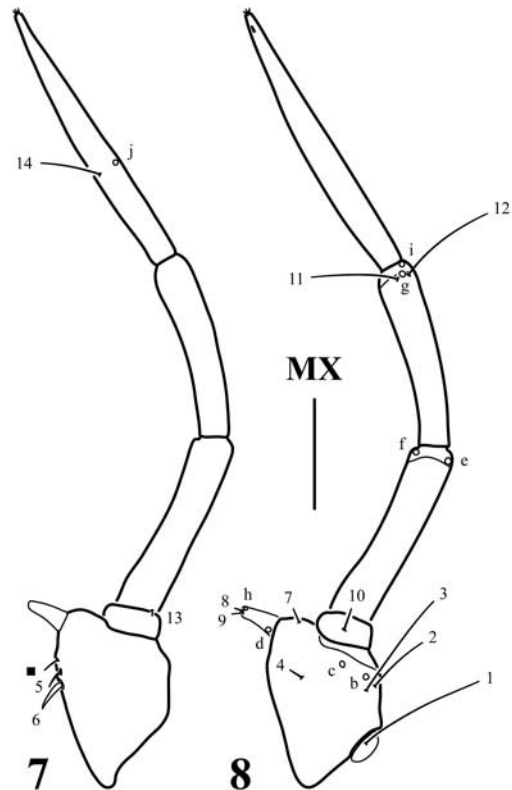
As for first instar except for the following features:

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

Figs. 4–6. *Coptotomus longulus lenticus*, first instar. (4) Right antenna, dorsal aspect; (5) left antenna, ventral aspect; (6) right mandible, dorsal aspect. MN, mandible; AN, antenna; SP, spinula. Scale bars = 0.10 mm.



Figs. 7–8. *Coptotomus longulus lenticus*, first instar. (7) Right maxilla, dorsal aspect; (8) left maxilla, ventral aspect. Solid square refers to additional seta. MX, maxilla. Scale bar = 0.10 mm.



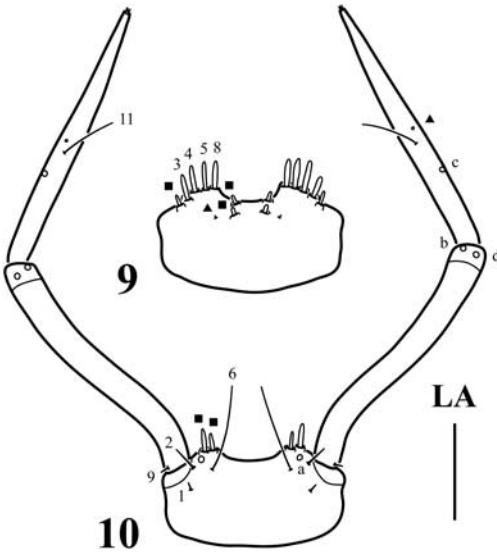
Head. *Cephalic capsule*. Subovate, with well-developed neck (neck constriction well marked); egg bursters absent; anterior margin of nasale rounded, bearing 11–13 lamellae clypeales. *Antenna*. A1 the longest, A3 somewhat shorter than A1. *Maxilla*. MP2 the shortest, MP1 slightly longer than MP2, MP3 slightly longer than MP1. *Labium*. LP1 somewhat longer than LP2.

Thorax. Protergite, mesotergite, and metatergite covering the whole pronota; mesotergite and metatergite with anterior transverse carina. *Legs*. Surface spinulae of FE, TI, and TA less abundant; spinular patches on anteroventrodistal portion of proTI and anteroventroproximal portion of proTA (cleaning device) well developed.

Abdomen. Siphon present, very short, rounded apically. *Urogomphus*. Long spinulae on lateral margins absent.

Chaetotaxy. Cephalic capsule with some minute or hair-like secondary setae scarcely distributed on dorsal and ventral surfaces; PA with 9–10 spine-like secondary setae on each lateral margin and 1–2 spine-like secondary setae on each side of ventral surface; MN with one hair-like, secondary seta on basoexternal margin, proximal to pore MNa; stipes with one minute secondary seta on ventral surface, near setae MX2 and MX3; prementum with one secondary pore on ventral surface, near seta LA1; thoracic tergites with several hair-like secondary setae; secondary leg setation detailed in Table 2; TI and TA with a row of natatory setae on posterodorsal margin; CO with one secondary pore on posterior surface; TR with one secondary pore on proximal portion; abdominal sclerites I–VII with some secondary setae mainly on lateral and posterior margins;

Figs. 9–10. *Coptotomus longulus lenticus*, first instar. (9) Prementum, dorsal aspect; (10) labium, ventral aspect. Solid squares refer to additional setae; solid triangles refer to additional pores. LA, labium. Scale bar = 0.10 mm.



LAS with several spine-like secondary setae on dorsal surface, 0–1 spine-like secondary setae on ventral surface, and a row of natatory setae on each lateral margin; U with a row of natatory setae on each lateral margin.

Third instar (Figs. 16–19)

As for second instar except for the following features:

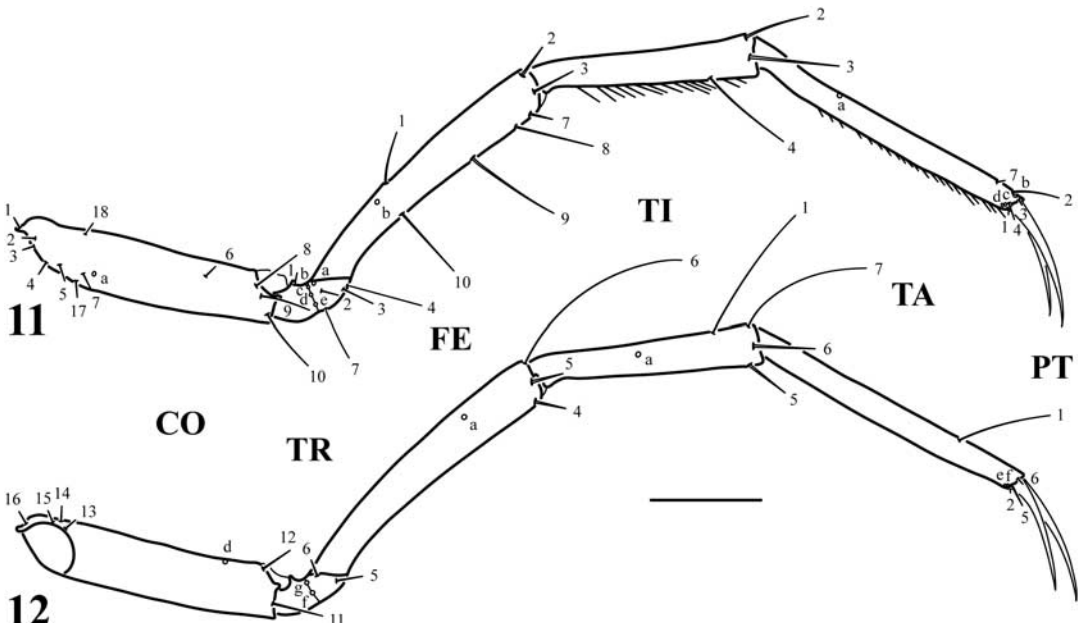
Body. Measurements and ratios that characterise the body shape are shown in Table 1.

Head (Fig. 16). *Cephalic capsule*. Maximum width posterior to stemmata; anterior margin of nasale bearing 21 lamellae clypeales. *Mandible*. Without spinulae. *Maxilla*. MP3 slightly longer than MP2, MP1 slightly longer than MP3.

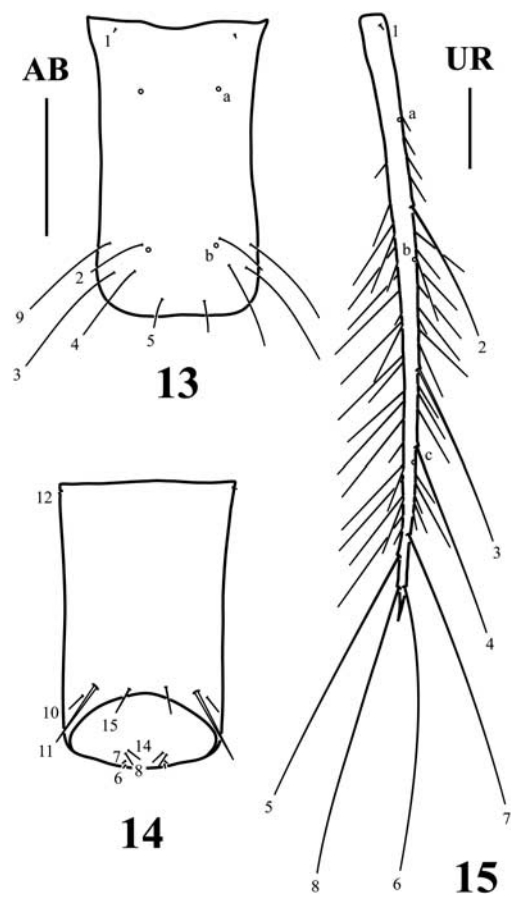
Thorax. Spiracles present on mesothorax. *Legs* (Figs. 17–18). Surface spinulae of FE, TI, and TA less abundant.

Abdomen. Tergites I–VII more developed, sclerites I and II with poorly delimited anterior transverse carina; spiracles present on segments I–VII. *Urogomphus* (Fig. 19). Somewhat longer than LAS.

Figs. 11–12. *Coptotomus longulus lenticus*, first instar. (11) Left metathoracic leg, anterior aspect; (12) right metathoracic leg, posterior aspect. CO, coxa; TR, trochanter; FE, femur; TI, tibia; TA, tarsus; PT, pretarsus. Scale bar = 0.15 mm.



Figs. 13–15. *Coptotomus longulus lenticus*, first instar. (13) Abdominal segment VIII, dorsal aspect; (14) abdominal segment VIII, ventral aspect; (15) right urogomphus, dorsal aspect. AB, abdominal segment VIII; UR, urogomphus. Scale bars = 0.20 mm.



Chaetotaxy. Secondary setae on cephalic capsule somewhat more abundant; PA with 7–9 spine-like secondary setae on each lateral margin and one spine-like secondary seta on each side of ventral surface; MN with some minute secondary setae on external margin; stipes with 1–2 minute secondary setae on ventral surface, near setae MX2 and MX3; prementum with two secondary pores on ventral surface, near seta LA1; secondary leg setation detailed in Table 2 and Figs. 17–18; CO with 1–2 secondary pores on posterior surface; LAS with numerous spine-like secondary setae on dorsal and ventral surfaces (Fig. 19).

Remarks

With the exception of *C. longulus lenticus* whose larval stages are described in this article, *C. interrogatus* represents the only other species of *Coptotomus* for which the first larval stage is known (Barman 2004). Interestingly, Barman (2004) postulated that the urogomphus of the first instar of *C. interrogatus* characterised by the presence of additional setae, which is contrary to the condition observed in *C. longulus lenticus*. Indeed the urogomphus of *C. longulus lenticus* has eight primary setae and three primary pores along with several elongate and seta-like spinulae. Species of *Coptotomus* have long been recognised for their overall morphological similarity (Hilsenhoff 1980; Larson *et al.* 2000). It is therefore postulated that the additional setae reported in *C. interrogatus* are in fact elongate hair-like spinulae similar to the condition

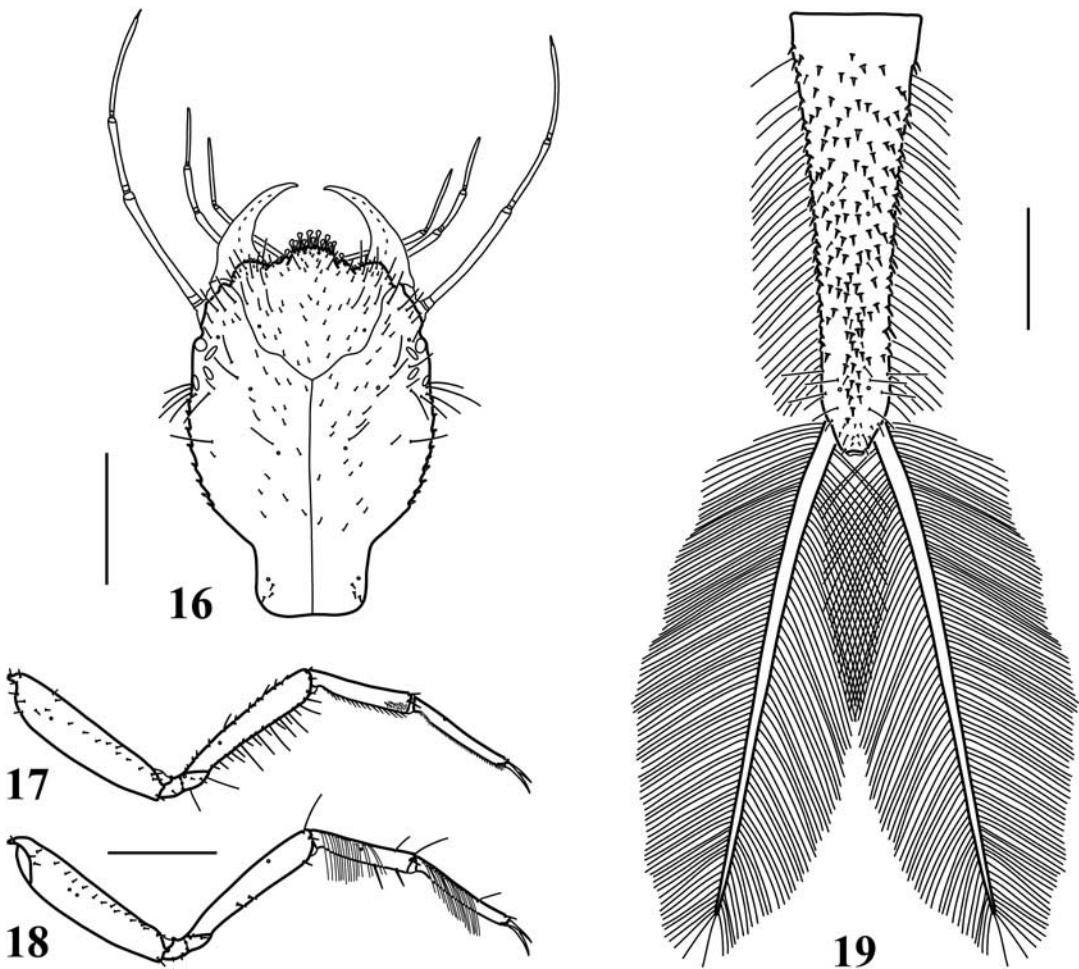
Table 2. Number and position of secondary setae on the legs of larvae of *Coptotomus longulus lenticus*.

Segment	Position	Instar II (n = 3)	Instar III (n = 3)
Coxa	A	0/0/0–1	9–11/7–10/6–11
	PD	2–4/1–3/1–3	10–12/7–10/8–10
	Total	2–4/1–3/1–3	20–21/16–19/15–20
Trochanter	Pr	0/0–1/0–1	2–3/1–3/1–2
	Total	0/0–1/0–1	2–3/1–3/1–2
Femur	AD	4–7/5–8/5–8	9–12/9–12/8–11
	AV	6–8/8–12/9–15	13–19/22–24/19–25
	PV	0/0/0	0–2/0/0
	Total	11–15/14–18/14–22	25–30/32–36/30–35

Numbers between slash marks refer to prothoracic, mesothoracic, and metathoracic leg, respectively. Total, total number of secondary setae on the segment (excluding primary and natatory setae).

A, anterior; D, dorsal; P, posterior; Pr, proximal; V, ventral.

Figs. 16–19. *Coptotomus longulus lenticus*, third instar. (16) Head, dorsal aspect; (17) left prothoracic leg, anterior aspect; (c) right prothoracic leg, posterior aspect; (19) abdominal segment VIII and urogomphi, dorsal aspect. Scale bars = 0.70 mm.



observed in the closely related species *C. longulus lenticus*. These spinulae can easily be confounded with setae when observed at low magnifications. Similarly, Bacon *et al.* (2000) reported the presence of two prothoracic sternites and of a completely sclerotised abdominal segment VII in the third instar of *C. longulus lenticus*. Both these character traits are not corroborated in this study.

Character analysis

The final data matrix included 25 taxa and 125 characters (94 binary and 31 multistate) (Tables 3–4). The parsimony analysis with TNT yielded two most parsimonious cladograms of

374 steps (consistency index = 0.45; retention index = 0.69). Both trees differed in the relative position of the agabine genera *Agabinus* Crotch and *Ilybius* Erichson only. The strict consensus tree was therefore calculated (Fig. 20) in which Coptotominae was recovered as part of the clade Lancetinae + (Coptotominae + Laccophilinae). The derived nodes were generally better supported than the most basal ones. Character states were mapped for each clade (Fig. 20).

Discussion

The larvae of *C. longulus lenticus* described in this paper turn out to be characterised by several

Table 3. Characters and states used for the cladistic analysis.

(000)	<i>Parietal (at level of occipital suture) (instar I):</i> (0) not constricted; (1) constricted
(001)	<i>Parietal (at level of occipital suture) (instar III):</i> (0) not constricted; (1) constricted
(002)	<i>Occipital suture (instar I):</i> (0) absent; (1) present
(003)	<i>Occipital suture (instar III):</i> (0) absent; (1) present
(004)	<i>Frontal line (instar III):</i> (0) straight to moderately sinuate; (1) strongly sinuate
(005)	<i>Nasale (instars I–III):</i> (0) absent; (1) present
(006)	<i>Anterolateral lobes of frontoclypeus (instar III):</i> (0) absent; (1) inconspicuous; (2) well developed, not projected beyond anterior margin; (3) well developed, projected beyond anterior margin
(007)	<i>Egg bursters (instar I):</i> (0) located submedially; (1) located basally
(008)	<i>Seta FR7 (instar I):</i> (0) spiniform; (1) setiform
(009)	<i>Pore FRe (instar I):</i> (0) absent; (1) present
(010)	<i>Lamellae clypeales on anterior margin of frontoclypeus (instar I):</i> (0) absent; (1) two; (2) at least four
(011)	<i>Seta PA3 (instar I):</i> (0) inserted contiguous to setae PA1 and PA2; (1) inserted far from setae PA1 and PA2
(012)	<i>Seta PA7 (instar I):</i> (0) present; (1) absent
(013)	<i>Seta PA18 (larva I):</i> (0) present; (1) absent
(014)	<i>Pore PAb (instar I):</i> (0) inserted contiguous to seta PA3; (1) inserted far from seta PA3, close to coronal or frontal lines
(015)	<i>Pore PA1 (instar I):</i> (0) present; (1) absent
(016)	<i>Pore PAp (instar I):</i> (0) present; (1) absent
(017)	<i>Secondary spine-like setae on ventral surface of parietal (instar III):</i> (0) present; (1) absent
(018)	<i>Ventroapical spinula on antennomere 3 (instars I–III):</i> (0) absent; (1) small; (2) strongly developed
(019)	<i>Apical lateroventral process of antennomere 3 (instars I–III):</i> (0) protruding; (1) not protruding
(020)	<i>Antennomere 4 (instar I):</i> (0) shorter than antennomere 3; (1) subequal to slightly longer than antennomere 3
(021)	<i>Antennomeres 2 and 3 (instar III):</i> (0) not subdivided; (1) subdivided
(022)	<i>Seta AN1 (instar I):</i> (0) inserted medially or distally; (1) inserted proximally
(023)	<i>Seta AN3 (larva I):</i> (0) inserted distally; (1) inserted submedially
(024)	<i>Pore ANf (instar I):</i> (0) present; (1) absent
(025)	<i>Pore ANg (instar I):</i> (0) inserted basally; (1) inserted medially
(026)	<i>Pore ANi (instar I):</i> (0) present; (1) absent
(027)	<i>Additional ventroapical pores on antennomere 3 (instar I):</i> (0) present; (1) absent
(028)	<i>Secondary setae on antennomere 1 (instar III):</i> (0) absent; (1) present
(029)	<i>Mandible (instars I–III):</i> (0) not oriented obliquely; (1) oriented obliquely
(030)	<i>Mandibular channel (instars I–III):</i> (0) more or less closed; (1) widely open; (2) absent
(031)	<i>Seta MN1 (instar I):</i> (0) distal to pore MNc; (1) proximal to pore MNc
(032)	<i>Sensillum MN2 (instar I):</i> (0) hair-like; (1) pore-like
(033)	<i>Pore MNa (instar I):</i> (0) inserted at about the same level as pore MNb; (1) inserted distally to pore MNb
(034)	<i>Internal margin of stipes (instars I–III):</i> (0) without robust spinulae; (1) with three robust spinulae
(035)	<i>Galea (instars I–III):</i> (0) well developed, subconical; (1) well developed, spine-like; (2) very short, subconical; (3) absent
(036)	<i>Palpifer (instars I–III):</i> (0) inconspicuous, not clearly differentiated from the stipes; (1) palpomere-like, clearly differentiated from the stipes
(037)	<i>Maxillary palpomere 2 (instar III):</i> (0) not subdivided; (1) subdivided
(038)	<i>Maxillary palpomere 3 (instar III):</i> (0) not subdivided; (1) subdivided
(039)	<i>Seta MX4 (instar I):</i> (0) present; (1) absent
(040)	<i>Seta MX6 (instar I):</i> (0) present; (1) absent
(041)	<i>Seta MX8 (instar I):</i> (0) inserted apically on the galea; (1) inserted subapically on the galea; (2) absent
(042)	<i>Seta MX9 (instar I):</i> (0) inserted apically on the galea; (1) inserted subapically on the galea; (2) absent
(043)	<i>Seta MX10 (instar I):</i> (0) present; (1) absent
(044)	<i>Pore MXa (instar I):</i> (0) present; (1) absent
(045)	<i>Pore MXd (instar I):</i> (0) present; (1) absent

Table 3. *Continued*

(046)	<i>Pore MXh (instar I)</i> : (0) inserted on the galea; (1) inserted on the stipes
(047)	<i>Pore MXk (instar I)</i> : (0) present; (1) absent
(048)	<i>Additional setae on the stipes (instar I)</i> : (0) absent; (1) a single seta contiguous to MX6; (2) two setae contiguous to MX6 and to MX2 and MX3 respectively; (3) row(s) of elongate spine-like setae
(049)	<i>Anterior margin of prementum (instar I)</i> : (0) straight to slightly emarginate; (1) deeply emarginate; (2) projected forward
(050)	<i>Labial palpomeres 1 and 2 (instar III)</i> : (0) not subdivided; (1) subdivided
(051)	<i>Seta LA3 (instar I)</i> : (0) inserted distally or subdistally; (1) inserted proximally; (2) absent
(052)	<i>Seta LA5 (instar I)</i> : (0) elongate, hair-like; (1) short, spine-like; (2) elongate, spine-like
(053)	<i>Setae LA4 and LA8 (instar I)</i> : (0) short, spine-like; (1) elongate, hair-like; (2) elongate, spine-like
(054)	<i>Seta LA10 (instar I)</i> : (0) inserted submedially; (1) inserted distally; (2) absent
(055)	<i>Seta LA12 (instar I)</i> : (0) inserted submedially; (1) inserted distally; (2) absent
(056)	<i>Setae LA10 and LA12 (instar I)</i> : (0) elongate; (1) short to very short; (2) inapplicable
(057)	<i>Pore LAc (instar I)</i> : (0) present; (1) absent
(058)	<i>Additional setae on dorsal surface of prementum (instar I)</i> : (0) absent; (1) present
(059)	<i>Additional pore on dorsal surface of prementum (instar I)</i> : (0) absent; (1) present
(060)	<i>Secondary setae on prementum (instar III)</i> : (0) absent; (1) present
(061)	<i>Secondary pores on ventral surface of prementum (instar III)</i> : (0) absent; (1) present
(062)	<i>Ventral sclerites on prothorax (instars I–III)</i> : (0) absent; (1) one; (2) two
(063)	<i>Seta CO7 on mesocoxa and metacoxa (instar I)</i> : (0) inserted distally; (1) inserted proximally
(064)	<i>Seta TR2 (instar I)</i> : (0) present; (1) absent
(065)	<i>Seta TR3 (instar I)</i> : (0) absent; (1) present
(066)	<i>Seta FE1 (instar I)</i> : (0) inserted proximally; (1) inserted subproximally or submedially
(067)	<i>Seta FE4 (instar I)</i> : (0) present; (1) absent
(068)	<i>Seta FE5 on mesofemur (instar I)</i> : (0) short, spine-like; (1) elongate, hair-like; (2) absent
(069)	<i>Seta FE5 on metafemur (instar I)</i> : (0) short, spine-like; (1) elongate, hair-like; (2) absent
(070)	<i>Seta FE6 (instar I)</i> : (0) absent; (1) inserted distally; (2) inserted subdistally
(071)	<i>Additional posteroventral setae on femur (instar I)</i> : (0) absent; (1) present
(072)	<i>Natatory setae on femur (instar I)</i> : (0) absent; (1) present
(073)	<i>Natatory dorsal setae on femur (instar III)</i> : (0) absent; (1) present
(074)	<i>Natatory ventral setae on femur (instar III)</i> : (0) absent; (1) present
(075)	<i>Secondary anterodorsal setae on femur (instar III)</i> : (0) absent; (1) present
(076)	<i>Secondary posteroventral setae on mesofemur and metafemur (instar III)</i> : (0) absent; (1) present
(077)	<i>Seta TI1 (instar I)</i> : (0) inserted distally; (1) inserted subdistally or submedially
(078)	<i>Seta TI4 (instar I)</i> : (0) more proximal on leg 1; (1) not more proximal on leg 1
(079)	<i>Seta TI5 (instar I)</i> : (0) spine-like; (1) hair-like
(080)	<i>Seta TI6 on pro- and mesotibia (instar I)</i> : (0) elongate, hair-like; (1) short, spine-like
(081)	<i>Seta TI6 on metatibia (instar I)</i> : (0) elongate, hair-like; (1) short, spine-like
(082)	<i>Seta TI7 (instar I)</i> : (0) short, spine-like; (1) elongate, hair-like
(083)	<i>Additional anteroventral setae on tibia (instar I)</i> : (0) absent; (1) present
(084)	<i>Secondary setae on tibia (instar III)</i> : (0) absent; (1) present
(085)	<i>Natatory dorsal setae on tibia (instar I)</i> : (0) absent; (1) present
(086)	<i>Natatory ventral setae on tibia (instars I–III)</i> : (0) absent; (1) present
(087)	<i>Seta TA1 (instar I)</i> : (0) short to very short; (1) elongate to very elongate; (2) absent
(088)	<i>Seta TA1 (instar I)</i> : (0) inserted distally or subdistally; (1) inserted submedially; (2) inapplicable
(089)	<i>Additional anteroventral setae on tarsus (instar I)</i> : (0) absent; (1) present
(090)	<i>Secondary setae on anterodorsal margin of protarsus (instar III)</i> : (0) absent; (1) present
(091)	<i>Secondary setae on posteroventral margin of protarsus (instar III)</i> : (0) absent; (1) present
(092)	<i>Secondary setae on posteroventral margin of metatarsus (instar III)</i> : (0) absent; (1) present
(093)	<i>Natatory dorsal setae on tibia and tarsus (instars II–III)</i> : (0) absent; (1) present
(094)	<i>Basoventral patch of dense slender spinulae on protarsus (cleaning device) (instars I–III)</i> : (0) absent; (1) present
(095)	<i>Basoventral spinulae on claws (instar I)</i> : (0) absent; (1) present

Table 3. *Continued*

(096)	<i>Abdominal segments I–VI (instars I–III):</i> (0) not bearing tracheal gills; (1) bearing tracheal gills
(097)	<i>Abdominal tergites I–VI (instar I):</i> (0) with anterotransverse carina; (1) without anterotransverse carina
(098)	<i>Ventral surface of abdominal segments II–IV (instar III):</i> (0) membranous; (1) sclerotised
(099)	<i>Ventral surface of abdominal segment VI (instar I):</i> (0) membranous; (1) sclerotised
(100)	<i>Abdominal segment VII (instar I):</i> (0) sclerotised dorsally, membranous ventrally; (1) completely sclerotised except for a narrow longitudinal membranous band ventrally; (2) sclerotised dorsally and ventrally, with ventral sclerite independent from dorsal sclerite; (3) completely sclerotised
(101)	<i>Abdominal sclerite VII (instar I):</i> (0) with anterotransverse carina; (1) without anterotransverse carina
(102)	<i>Abdominal segment VII (instar III):</i> (0) without a lateral row of natatory setae; (1) with a lateral row of natatory setae
(103)	<i>Siphon (instars I–III):</i> (0) very short, slightly projected backwards; (1) moderately long, projected backwards; (2) very long, urogomphomere-like; (3) absent
(104)	<i>Seta AB4 (instar I):</i> (0) not distinctly developed; (1) very long, strongly developed
(105)	<i>Seta AB7 (instar I):</i> (0) small; (1) well developed to strongly developed; (2) absent
(106)	<i>Seta AB9 (instar I):</i> (0) inserted dorsolaterally; (1) inserted ventrolaterally
(107)	<i>Seta AB11 (instar I):</i> (0) spine-like; (1) hair-like
(108)	<i>Seta AB11 (instar I):</i> (0) inserted contiguous to ventroapical margin; (1) inserted far from ventroapical margin
(109)	<i>Seta AB15 (instar I):</i> (0) absent; (1) inserted contiguous to ventroapical margin; (2) inserted far from ventroapical margin
(110)	<i>Pore ABC (instar I):</i> (0) present; (1) absent
(111)	<i>Additional setae on abdominal segment VIII (excluding natatory setae) (instar I):</i> (0) absent; (1) a single spine-like seta inserted on lateral margin; (2) numerous
(112)	<i>Natatory setae on lateral margin of abdominal segment VIII (instar I):</i> (0) absent; (1) present
(113)	<i>Natatory setae on lateral margin of abdominal segment VIII (instar III):</i> (0) absent; (1) present
(114)	<i>Urogomphus (instars I–III):</i> (0) composed of one urogomphomere; (1) composed of two urogomphomeres
(115)	<i>Elongate spinulae on urogomphus (instar I):</i> (0) absent; (1) present
(116)	<i>Number of primary setae on urogomphus (excluding natatory setae) (instar I):</i> (0) nine; (1) eight; (2) seven; (3) numerous
(117)	<i>Setae UR2, UR3, and UR4 (instar I):</i> (0) inserted contiguously; (1) not inserted contiguously; (2) only UR2 and UR3 contiguous; (3) only UR3 and UR4 contiguous
(118)	<i>Seta UR4 (instar I):</i> (0) shorter than seta UR2; (1) subequal in length to seta UR2; (2) longer than seta UR2
(119)	<i>Seta UR7 (instar I):</i> (0) elongate, hair-like; (1) short, spine-like
(120)	<i>Seta UR8 (instar I):</i> (0) inserted terminally on urogomphomere 2; (1) inserted subapically on urogomphomere 2; (2) inserted submedially on urogomphomere 2; (3) inserted proximally on urogomphomere 2; (4) absent; (5) inserted on urogomphomere 1
(121)	<i>Pore URb (instar I):</i> (0) proximal to seta UR2; (1) contiguous to seta UR2; (2) distal to seta UR2; (3) absent
(122)	<i>Pore URc (instar I):</i> (0) not inserted terminally on urogomphomere 1; (1) inserted terminally on urogomphomere 1
(123)	<i>Secondary setae on urogomphus (instar III):</i> (0) absent; (1) present
(124)	<i>Row of natatory setae on urogomphus (instar III):</i> (0) absent; (1) present

unique character states: (1) the presence of well-developed tracheal gills on the lateral margins of the abdominal segments I–VI (character 96.1); (2) the presence of elongate seta-like spinulae on the urogomphus of the first instar (character 115.1); (3) the presence of a short nasale (character 5.1);

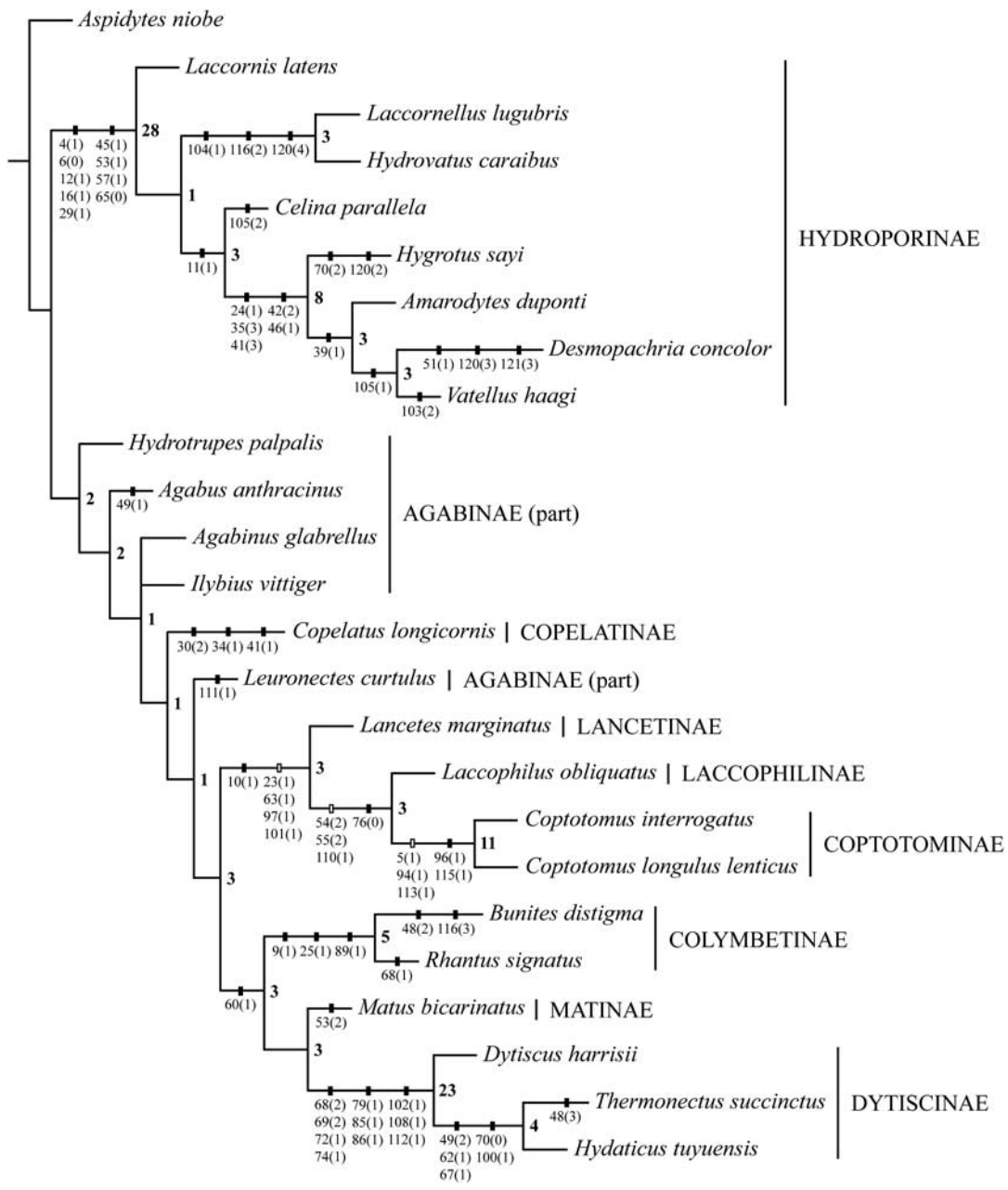
(4) the presence of a row of natatory setae on the lateral margin of abdominal segment VIII (character 113.1); and (5) the presence of a basoventral patch of dense slender spinulae on the protarsus (character 94.1), postulated to represent either a cleaning or a device for

Table 4. Data matrix used for the cladistic analysis.

Species	Character												
	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	1111111111	1111111111	11111
	0000000000	1111111111	2222222222	3333333333	4444444444	5555555555	6666666666	7777777777	8888888888	9999999999	0000000000	1111111111	22222
	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	01234
<i>Aspidytes niobe</i> Ribera, Beutel, Balke, and Vogler	0000003010	0000010011	0001000000	1010001000	1100000100	0010222011	0000011000	1000000010	1100000220	0000000011	3003???101	0200100310	02010
<i>Agabinus glabrellus</i> (Motschulsky)	1100002010	2000000010	0010000100	0000001000	0000000100	0010111010	0100011000	1100011000	1100000000	0000000000	2000001001	0000001010	52000
<i>Agabus anthracinus</i> Mannerheim	0100003010	2000000010	0000000100	0000001000	0000000111	0010111000	0100011000	1100011000	1100100000	0000000000	2000001001	0000101010	52000
<i>Hydrotrupes palpalis</i> Sharp	0000003010	2000000010	0000000100	1000001000	0000000110	0010111010	0100011000	1000001000	1100000000	0000000000	2000000001	0000101300	52100
<i>Ilybius vittiger</i> (Gyllenhal)	1100002010	2000000010	0000000100	0000001000	0000000110	0010011010	0100011000	1100011000	1100100000	1110000000	0000001001	0000101000	51000
<i>Leuronectes curtulus</i> Régimbart	1100002010	2000000011	0000000000	0000001000	0000100110	0010001000	0100011000	1100011000	1100000000	0000000000	0000000001	0100101110	50000
<i>Bunites distigma</i> (Brullé)	1?1?00?011	2000000?11	1?10010000	0000001??0	0000100120	?010111010	???0010000	110????010	0011?00001	????0100?0	00?0000001	020?003???	5?0??
<i>Rhantus signatus</i> (Fabricius)	1111002011	2000000011	1010010000	0000001000	0000100100	0010111010	1100010011	1101011010	0011100001	1011010000	0000000001	0000001111	50011
<i>Copelatus longicornis</i> Sharp	1101001010	2000000010	0000000100	2100111000	1110100100	0010111011	0100010000	1100001000	1100000100	0000000100	0100000101	0000101000	10000
<i>Coptotomus interrogatus</i> (Fabricius)	110001201?	1000?0?0?0	00?0???000	0??001000	0000100110	001022201?	?221011000	1000010110	1110000100	0001101?00	0?0000????	1001011???	5??11
<i>Coptotomus longulus lenticus</i> Hilsenhoff	1100012010	1000100010	0011000100	0000001000	0000100110	0010222011	01?1011000	1000010110	1110000100	0001101100	0100000001	1001011110	52011
<i>Thermonectus succinctus</i> (Aubé)	1111003010	2000010021	0110000110	0001010010	?010100132	0010111011	1011110122	00111110?1	0010111100	1001000000	1110000112	1011001301	52000
<i>Dytiscus harrisii</i> Kirby	111100301?	2000000011	0110000010	0001001110	0000100110	1010?00000	?1?1010022	11111110?1	0011111100	1001000000	0110000112	001100????	5??11
<i>Hydaticus tuyuensis</i> Trémouilles	1111003010	2000000010	0101000010	0101001110	0000100112	0010111000	1111010122	00111110?1	0010111100	1001110000	1010000112	0011001210	52000
<i>Amarodytes duponti</i> (Aubé)	0011110000	2111111110	0001010101	0110030001	0221111100	0001000100	0000100000	1000011110	1100100100	0000000100	21010?1000	1000101110	10110
<i>Laccornellus lugubris</i> (Aubé)	0?0??1?000	2011111?00	0?010011?1	0111020??0	0001110000	?001001100	???0000000	100????210	1100?00100	????0000?0	20?1100002	000?102010	411??
<i>Hydrovatus caraibus</i> Sharp	0000110110	2011111100	0001001101	0101020000	0001110000	0201000100	0000100000	1000001110	1100000100	0000010011	2001100000	0000102000	41100
<i>Hygrotus sayi</i> Balfour-Browne	0001110000	2111111000	0001010101	0111030000	0221111000	0001000100	0000100000	2000011110	1100100100	0110000100	2101001000	0000101210	21100
<i>Desmopachria concolor</i> Sharp	0001110010	2111111010	0001010101	0111030001	1221111000	0101110110	0000100000	1001011110	1110100100	1111000111	2101011000	0000101010	33110
<i>Laccornis latens</i> (Fall)	0001110000	2011111000	0000001101	0111020000	0001110100	0001000100	0000000000	1000001110	1100000100	0000000000	2001001000	0000101010	01100
<i>Celina parallela</i> (Babington)	0111110000	2110111100	0011001101	0101020000	0000110000	0201110100	0000000000	1000011110	1100100100	1110000000	2001021100	0000101020	01100
<i>Vatellus haagi</i> Wehncke	0001110010	2110111110	0001010111	0111030001	0221111?00	0021222100	0000101000	1000011110	1110100100	1111000100	2102011000	0000101110	01111
<i>Laccophilus obliquatus</i> Régimbart	0100002110	1000000000	0001001100	0100001000	0000100110	0220222000	0101011000	1000000000	0000100110	1001000100	0101001001	1000101111	50110
<i>Lancetes marginatus</i> (Steinheil)	1100003010	1000000010	1111000000	0000001110	0000100100	1010011011	0101011000	1000001000	0001100000	0001000100	0100000001	0000001110	50011
<i>Matus bicarinatus</i> (Say)	0011001010	2000000020	0010000010	0000001000	0000000100	0022111000	1000010001	1001011110	1000100110	1111000000	2001000001	0200001200	52010

Missing data coded “?”.

Fig. 20. Strict consensus cladogram with unique or interesting character changes mapped. Solid rectangles indicate unique character state transformations; open rectangles indicate homoplastic character state transformations. Bremer support values are indicated on each branch.



spreading a microbial growth inhibitor (De Marzo and Nilsson 1986), which is less evident in the first instar (Fig. 20).
The presence of abdominal tracheal gills is noteworthy as this trait resembles those observed

in the larvae of Gyrinidae (Michat *et al.* 2010). Indeed all gyrinid larvae share the presence of tracheal gills on the abdominal segments I–IX. Larvae of *Coptotomus*, however, differ from those of Gyrinidae in that the gills are devoid of

spinulae along the lateral edges (Fig. 1). This condition in combination with the fact that larvae of Coptotominae are clearly Dytiscidae allows us to conclude that the presence of abdominal gills in both Gyrinidae and Coptotominae is the result of convergent evolution.

The presence of elongate seta-like spinulae on the urogomphus of the first instar (Fig. 15) represents another putative synapomorphy for the Coptotominae. Whereas such a character state could not be corroborated for *C. interrogatus* (Barman 2004), we do believe that the trait is generalised within the subfamily and that the additional setae described by Barman (2004) refer to these elongate spinulae. Interestingly, the second and third instars of *C. longulus lenticus* do not have spinulae on the urogomphus but bear a row of natatory setae on both the internal and external margins (Fig. 19). Such setal fringes resemble those of *Dytiscus* Linnaeus and *Hyderodes* Hope (Alarie *et al.* 2011), although in those dytiscine genera the natatory setae are restricted to the external margin only.

The presence of a frontoclypeal projection (=nasale) in both the Coptotominae and Hydroporinae is also noteworthy. Compared with the Hydroporinae, which are characterised by an extensive nasale reaching approximately the tip of the mandibles, the nasale of *Coptotomus* barely gets beyond the level of the lateral lobes (Figs. 2, 16). Moreover, as it arises post marginally from within the frontoclypeal interior in the Coptotominae (Barman 2004), compared with being strictly an elongation of the distal margin within the Hydroporinae, it is reasonable to postulate that both conditions evolved independently. It is worth mentioning that the structure sometimes described as a “clypeal horn” in the Coptotominae reflects the typical bifid shape of the first instar nasale, which varies through the ontogenetic development of the larva (compare Figs. 2 and 16).

The presence of a basoventral patch of spinulae on the ventroproximal portion of the protarsus (which is accompanied by another patch on the anteroventrodistal portion of protibia (Fig. 17) to form the device mentioned above) and the presence of a row of natatory setae on the lateral margin of abdominal segment VIII are other interesting features of Coptotominae larvae. No other dytiscid has these features except members of the subfamily Dytiscinae (Alarie *et al.* 2011). Whereas these character states support a

closer relationship between both subfamilies, our results suggest that the conditions found in Coptotominae and Dytiscinae are the result of convergent evolution.

Several different hypotheses have been proposed as to where to place the Coptotominae within Dytiscidae (Brinck 1948; Burmeister 1976; Ruhnau and Brancucci 1984; Miller 2001; Barman 2004; Ribera *et al.* 2008). Although not strongly supported (Bremer value = 3), our study suggests a monophyletic origin of Lancetinae + Laccophilinae + Coptotominae. This relationship is interesting from an evolutionary point of view, as the larvae of these subfamilies share a unique feature within Dytiscidae, the presence of an unusually low number of lamellae clypeales in the first instar (character 10.1) (Fig. 2). Such a character trait is somewhat related to the “four-peg-pattern” suggested by Ruhnau and Brancucci (1984), which combines the two anteromedial lamellae of the frontoclypeus with the two sublateral spines labelled as FR10 within the Dytiscidae. The “four-peg-pattern” is also observed in the Carabidae and Gyrinidae, where the two anteromedial lamellae are labelled FR11 (Bousquet and Goulet 1984; Michat *et al.* 2010). Ruhnau and Brancucci (1984) postulated that the “four-peg-pattern” has arisen independently in Laccophilinae, Coptotominae, and Lancetinae, and that it represents a reversal to the ancestral condition found in Carabidae and Gyrinidae. This reinforces the idea that the presence of only two lamellae clypeales within the Dytiscidae evolved secondarily from patterns composed of a larger number of lamellae. Our results, however, do not support an independent acquisition of this pattern in each of the three subfamilies as long as they form a distinct clade in our analysis. See De Marzo and Nilsson (1986) and Friis *et al.* (2003) for hypotheses on the possible function of the lamellae clypeales.

The monophyletic origin of Lancetinae + Laccophilinae + Coptotominae proposed in our study is also supported by: (1) the submedial insertion of seta AN3 on the third antennomere (character 23.1); (2) the proximal insertion of seta CO7 on the meso- and metacoxa (character 63.1); and (3) the absence of an anterior transverse carina on abdominal tergites I–VII in the first instar (characters 97.1 and 101.1, shared with Copelatinae). These characters must be treated with caution

knowing that they are also present in some taxa of Hydroporinae and Dytiscinae. However, as both subfamilies are strongly supported, monophyletic groups (Fig. 20) they most probably represent independent evolutionary processes.

Our study gives moderate support (Bremer value = 3) to a monophyletic origin of Laccophilinae + Coptotominae, which reinforces the hypothesis formulated by Barman (2004) based on a smaller data set. This grouping is supported by the absence of setae LA10 and LA12 on the second labial palpomere (characters 54.2 and 55.2, homoplastic in *Vatellus* Aubé), and the absence of pore ABC on the LAS (character 110.1, homoplastic in *Amarodytes* Régimbart and *Thermonectus* Dejean) (Fig. 20). Both character states, however, must be seen as less meaningful evolutionarily because they are present in other dytiscid taxa. The primary setae LA10 and LA12 are well developed in the Hydroporinae except Vatellini (see Michat and Torres 2005, 2011) compared with very small to minute in the Colymbetinae, Copelatinae, Dytiscinae, and Lancetinae (Michat *et al.* 2005; Alarie *et al.* 2009, 2011; Michat and Torres 2009). On the other hand, the pore ABC is also absent in the hydroporine tribe Bidessini (Michat and Alarie 2008) and in the dytiscine tribes Aciliini and Eretini (Alarie *et al.* 2011).

Acknowledgements

We thank the anonymous referees for their useful comments on the manuscript. Laboratory work by M. C. Michat was supported by an external scholarship from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and projects PIP 112-200801-02759 from CONICET and PICT-2007-01438 from the Agencia Nacional de Promoción Científica y Tecnológica. Financial support was also provided by the Natural Sciences and Engineering Research Council of Canada in the form of an operating research grant to Y. Alarie.

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