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PRIMARY RESEARCH PAPER

A preliminary study on the phylogenetic relationships of *Copelatus* Erichson (Coleoptera: Dytiscidae: Copelatinae) based on larval chaetotaxy and morphology

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Abstract The phylogenetic relationships of the diving-beetle (Dytiscidae) subfamily Copelatinae are not well known. Some authors postulated a sister-group relationship between *Copelatus* Erichson and the remaining Dytiscidae, relying mainly on the absence of a mandibular channel in *Copelatus*. Other authors suggested a more derived position of the genus. Larval characters have been underutilized in phylogenetic studies, mainly because the larvae of many taxa within the family and, in particular, within Copelatinae are unknown. A comprehensive approach aimed to study the phylogenetic placement of a member of this subfamily based on larval characters has not been produced so far. In this study, the phylogenetic relationships of *Copelatus* are explored based on a cladistic analysis of 24 taxa and 120 chaetotaxic and morphological characters from larvae. For this purpose, larvae of *Copelatus longicornis* Sharp are described and illustrated in detail for the first time, with particular emphasis on morphometry and chaetotaxy, with the latter being unexplored until now. The results support a derived position of *Copelatus* within Dytiscidae, with a sister-group relationship between this genus and a clade formed by the subfamilies Lancetinae, Coptotominae, Laccophilinae, Colymbetinae, Matinae, and Dytiscinae, and part of Agabinae. No evidence was found for a sister-group relationship between *Copelatus* and the remaining Dytiscidae so that the absence of a mandibular channel in this genus is likely a reduction. *Copelatus* is supported by three apomorphies within Dytiscidae: mandibular channel absent, internal margin of the stipes with three robust spinulae, and seta MX8 inserted subapically on the galea.

Keywords Diving beetles · Larvae · Phylogenetic relationships · Chaetotaxy

Introduction

The genus *Copelatus* Erichson includes more than 430 species (Nilsson, 2001) and is thus the most speciose genus within Dytiscidae. It includes small- to medium-sized species distributed worldwide, though much more species rich in the tropics (Spangler, 1962; Miller & Balke, 2003; Balke et al., 2004). On the basis of the number of elytral striae, the genus was divided into species groups (Sharp, 1882; Guéorguiev, 1968). Although the resulting classification presents some utility, the groups defined on the basis of this character

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are probably not natural (Balke et al., 2004). Copelatines were traditionally considered a tribe, although they are often treated today as a subfamily (Miller, 2001; Nilsson, 2001). Seven genera are included: *Agaporomorphus* Zimmermann, *Aglymbus* Sharp, *Copelatus*, *Exocelina* Broun (=*Papuadytes* Balke, see Nilsson, 2007), *Lacconectus* Motschulsky, *Liopterus* Dejean, and the recently described *Madaglymbus* Shaverdo & Balke (Miller, 2001; Nilsson, 2001; Balke et al., 2004; Shaverdo et al., 2008). Copelatinae is a speciose and morphologically homogeneous group within Dytiscidae, with some 540 species listed in Nilsson's (2001) catalog, though since then a large number of species have been (or are to be) described, mainly in the genera *Copelatus* and *Exocelina* (Balke et al., 2004, 2007). As suggested by previous authors (e.g., Miller & Balke, 2003), *Copelatus* is in need of a complete revision of its species at a worldwide scale.

Whereas the monophyly of Copelatinae seems well supported (Miller, 2001; Balke et al., 2004), the phylogenetic position of the group within Dytiscidae has been a topic of controversy over the last decades. Some authors (Ruhnau & Brancucci, 1984; De Marzo & Nilsson, 1986; Beutel, 1994, 1995) considered *Copelatus* the sister group of the remaining Dytiscidae. Others, however, found no evidence for this relationship, and argued in favor of a more derived position of the genus (Miller, 2001; Ribera et al., 2002, 2008; Balke et al., 2004). These contrasting results point to the need of further studies, involving character sets that are not explored so far. Immatures have been underutilized in phylogenetic studies, though there is considerable evidence for the utility of characters from larvae (Meier & Lim, 2009). As demonstrated over the past recent years, larval chaetotaxy is a particularly significant source of characters for the study of the phylogenetic relationships within Dytiscidae (Alarie, 1998; Michat, 2005, 2006; Michat & Torres, 2005, 2006; Alarie & Michat, 2007; Michat et al., 2007), and the development of a system of nomenclature to name primary sensilla (setae and pores) in first-instar larvae of the Dytiscidae has brought great progress because it allows for exploration of an extensive set of characters that is phylogenetically very useful. Unfortunately, prior to our study, the larval primary chaetotaxy was still very poorly known for copelatines, and there were few articles analyzing this character set for members of this subfamily.

Descriptions of larvae of *Copelatus* have been presented by several authors, with different degrees in detail. Williams (1936) briefly described the larva of *Copelatus parvulus* (Boisduval) and added interesting biological observations. The three larval instars of *Liopterus haemorrhoidalis* (Fabricius) (as *Copelatus haemorrhoidalis* (Fabricius)) were described in detail by De Marzo (1976b). The third-instar larva of *C. glyphicus* (Say) was described by Spangler (1962), and the larva of *C. australiae* Clark (as *C. extensus* Sharp) was described by Watts (1963), who also included brief comments on habitat and biology. A description of the three larval instars at the genus level was provided by Larson et al. (2000), and several brief descriptions of different species of *Copelatus* were presented by Bertrand (summarized in Bertrand, 1972). Finally, detailed studies of the mandible, the sucking apparatus, and the leg chaetotaxy of *L. haemorrhoidalis* (as *C. haemorrhoidalis*) were presented by De Marzo (1976a, c, 1979) and Nilsson (1988).

With the exception of the legs (Nilsson, 1988), primary chaetotaxy of a member of Copelatinae remained unexplored until now. Therefore, the hypotheses on the relationships of the members of this subfamily remained untested using this source of information. The recent finding of the larvae of *Copelatus longicornis* Sharp thus gives the opportunity to study in detail, for the first time, the larval chaetotaxy of a member of Copelatinae, including also a detailed morphometric analysis of selected structures. A cladistic analysis based on larval characters is also included, with the objective of testing previous hypotheses on the phylogenetic position of *Copelatus*. The absence of detailed descriptions of the remaining genera of Copelatinae, however, makes the analysis presented here preliminary, aimed mainly to identify the larval characters potentially useful in studying relationships within the group.

Materials and methods

Material examined

Five specimens of instar I and three of instar III were used for the descriptions. Larvae were collected in association with adults at the following locality: Argentina, Buenos Aires City, (1) 11-v-2000 and 18-

ix-2000, permanent pond with littoral vegetation; (2) 7-i-2002 and 17/19-iv-2002, temporary rain pools of a variety of sizes with clear water, soil bottom, and abundant vegetation (mainly grasses). The association is firm, as larvae and adults of *C. longicornis* were taken together from the collecting sites, and no other *Copelatus* species was present.

Methods

Specimens were cleared in lactic acid, dissected, and mounted on glass slides with polyvinyl–lactoglycerol. Observation (at magnifications up to 1,000×) and drawings were made using an Olympus CX31 compound microscope equipped with a camera lucida. Drawings were scanned and digitally edited. The material is stored in the larval collection of M.C. Michat (Laboratory of Entomology, Buenos Aires University, Argentina).

Morphometric analysis

We employed, with minimal modifications and additions, the terms used in previous articles dealing with the larval morphology of Agabinae and Colymbetinae (Alarie & Larson, 1998; Alarie et al., 1998; Michat, 2005). Paired structures of each individual were considered independently. The following measurements were taken (with abbreviations shown in parentheses). Total length (excluding urogomphi) (TL), maximum width (MW), head length (HL) (total head length including the frontoclypeus, measured medially along the epicranial stem), maximum head width (HW), length of frontoclypeus (FRL) (from apex of nasale to posterior margin of ecdysial suture), occipital foramen width (OCW) (maximum width measured along dorsal margin of occipital foramen), coronal line length (COL), length of mandible (MNL) (measured from laterobasal angle to apex), width of mandible (MNW) (maximum width measured at base). 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 (L), 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, and the length of distal portion is included in the femoral length. The legs were considered as being composed of six segments following Lawrence (1991). Dorsal length of the last abdominal segment (LAS) was measured along the midline from anterior to posterior margin. Length of urogomphus (U) was derived by adding the lengths of the individual segments; each segment is denoted by the letter U followed by a number (e.g., U1, first urogomphomere). These measurements were used to calculate several ratios that characterize the body shape.

Chaetotaxic analysis

Primary (present in first-instar larva) and secondary (added in later instars) setae and the so-called pores were distinguished in the cephalic capsule, head appendages, legs, last abdominal segment, 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 they are located, and a number (setae) or a lowercase 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; and UR, urogomphus. Setae and pores present in first-instar larva were labeled by comparison with the ground plan of chaetotaxy of the subfamilies Agabinae and Colymbetinae (Alarie, 1995, 1998). Homologies were recognized using the criterion of similarity of position (Wiley, 1981). Setae located at the apices of the 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 *Copelatus* were analyzed cladistically using the program TNT (Goloboff et al., 2008) and considering the character set provided by the larval chaetotaxy and morphology. Data from other character sources (e.g., adults, molecules) could not be used in combination with the larval data due to the lack of taxon overlap. With the objective of testing the previous hypotheses on the position of *Copelatus*, a broad sampling of taxa was included comprising members of eight of the remaining nine dytiscid subfamilies (Table 3). The subfamily Hydrodytinae, as well as the remaining six copelatine genera were not included because their larvae are unknown (*Agaporomorphus*, *Aglymbus*, *Exocelina*, *Lacconectus*, *Madaglymbus*) or well known, but without sufficient chaetotaxic detail (*Liopterus*). Data for most species (*Leuronectes curtulus* Régimbart, *Bunites distigma* (Brullé), *Rhantus signatus* (Fabricius), *Copelatus longicornis*, *Thermonectus succinctus* (Aubé), *Dytiscus harrisii* Kirby, *Hydaticus tuyuensis* Trémouilles, *Amarodytes duponti* (Aubé), *Laccornellus lugubris* (Aubé), *Hydrovatus caraibus* Sharp, *Desmopachria concolor* Sharp, *Celina parallela* (Babington), *Vatellus haagi* Wehncke, *Laccophilus obliquatus* Régimbart, and *Lancetes marginatus* (Steinheil)) were scored directly from the observation of the specimens, and data for the remaining species were scored from the literature (Table 3). In order to avoid the problem of comparing different semaphoronts (see Meier & Lim, 2009), in the codification of each character, the same instars were compared, based on the fact that Dytiscidae larvae pass through three instars distinguishable by the presence of egg bursters (instar I), the presence of spiracular openings on mesothorax and abdominal segments I–VII (instar III), or the absence of both characters (instar II). The tree was rooted in Aspidytidae (another family of aquatic Adephaga) to allow the dytiscid taxa to vary freely, thus testing the relationships of *Copelatus* with the other dytiscid subfamilies. All the 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 by increasing the length of the suboptimal cladograms by one step, until all the Bremer values were obtained (Kitching et al., 1998). Jackknife values were calculated with 2,000 replicates and P (removal probability) = 36.

Results

Description of the larvae of *Copelatus longicornis* Sharp

Diagnosis

Larvae of *Copelatus* are characterized by the following combination of characters: cephalic capsule with slightly marked neck constriction (Figs. 2–3, 16); occipital suture absent in instar I (Fig. 2), present in instar III (Fig. 16); anterolateral lobes of FR indistinct, serrate marginally (Figs. 2, 16); A3 with a ventroapical spinula (Fig. 5); A3′ short (Figs. 4–5); internal margin of MN with denticles (Fig. 6); mandibular channel absent (Fig. 6); stipes with three robust spinulae on internal margin, at the base of the GA (Figs. 7–8); GA strongly developed, spiniform (Figs. 7–8); prementum broader than long (Figs. 9–10); legs without natatory setae (Figs. 11–12, 17–18); siphon short (Figs. 13, 19); U composed of two urogomphomeres (Figs. 15, 19); U2 very short, much shorter than U1 (Figs. 15, 19); pore FRe absent (Fig. 2); pore MXa absent (Figs. 7–8); seta MX6 absent (Fig. 8); prementum with one additional seta and one additional pore on dorsal surface (Fig. 9); setae FE5, TI6 and TI7 short, spine-like (Fig. 12); setae UR2, UR3, and UR4 inserted contiguous (Fig. 15); seta UR4 shorter than setae UR2 and UR3 (Fig. 15); seta UR8 inserted on U2 (Fig. 15); thoracic and abdominal tergites with hair-like secondary setae inserted on the apices of stout spinulae (Fig. 19); and U without secondary setae (Fig. 19).

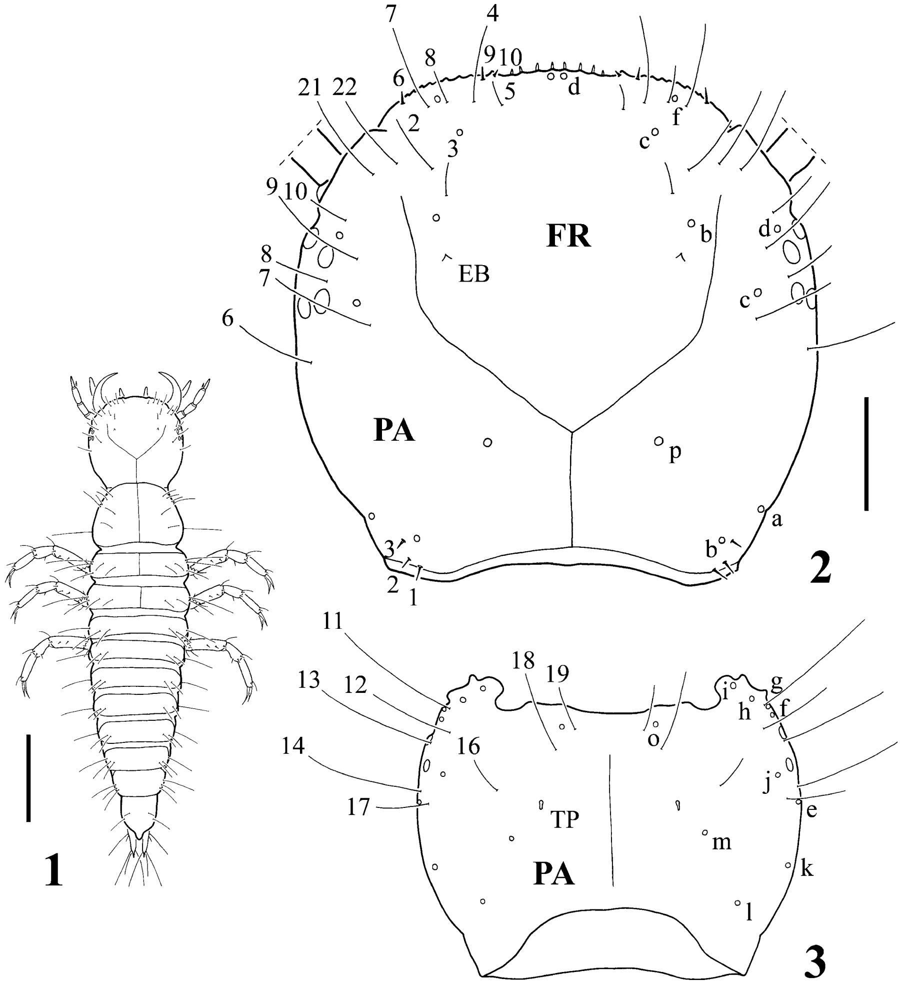
First-instar larva (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15)

Color Larva entirely testaceous, except for distal half of MN light brown.

Body Subcylindrical, narrowing toward abdominal apex (Fig. 1). Measurements and ratios that characterize the body shape are shown in Table 1.

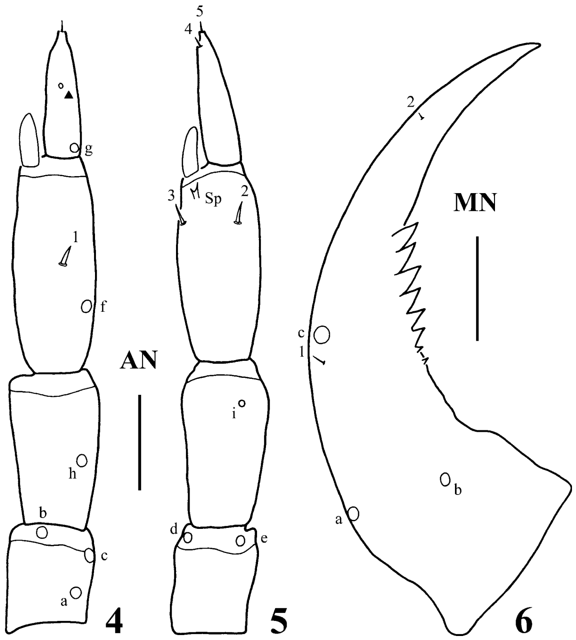
Head Cephalic capsule (Figs. 2–3) subcircular, slightly longer than broad; surface covered with short, stout spinulae except ventromedially; maximum width at

Figs. 1–3 *Copelatus longicornis*, first-instar larva. (1) Habitus, dorsal aspect; (2) Cephalic capsule, dorsal aspect; (3) Cephalic capsule, ventral aspect. *EB* egg burster, *TP* tentorial pit. Scale bars = 0.50 mm for Fig. 1 and 0.10 mm for Figs. 2–3

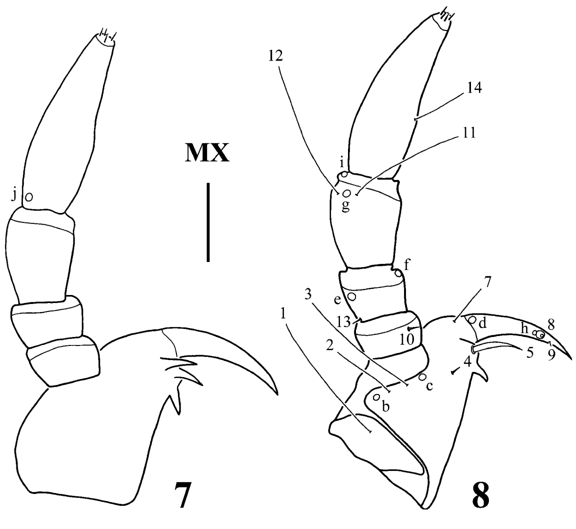


stemmata; with slightly marked neck constriction; occipital suture absent; ecdysial line visible, weakly marked; coronal line short; occipital foramen broadly emarginate ventrally; posterior tentorial pits visible ventrally; FR subtriangular, with one spine-like egg burster on each lateral; anterior margin rounded medially, with eight small spine-like setae, anterolateral lobes indistinct, serrate marginally, not projecting beyond anterior margin; six lateral stemmata arranged in two curved vertical rows at each side. *Antenna* (Figs. 4–5). Short, robust, shorter than HW, composed of four antennomeres; A1 the shortest, A3 the longest, with a ventroapical spinula; A3′ short. *Mandible* (Fig. 6). Prominent, broad basally, distal half projected inwards, apex sharp, internal margin with denticles, dorsointernal ones more developed; mandibular channel absent. *Maxilla* (Figs. 7–8). Cardo elongate, well developed; stipes short, robust, with minute surface spinulae and three robust spinulae on internal margin, at the base of the GA; GA strongly developed, spiniform; PPF short, palpomere-like; MP short, composed of three palpomeres, MP1 the shortest, MP3 the longest. *Labium* (Figs. 9–10). Prementum well developed, subrectangular, broader than long; LP short, composed of two palpomeres, LP2 longer than LP1.

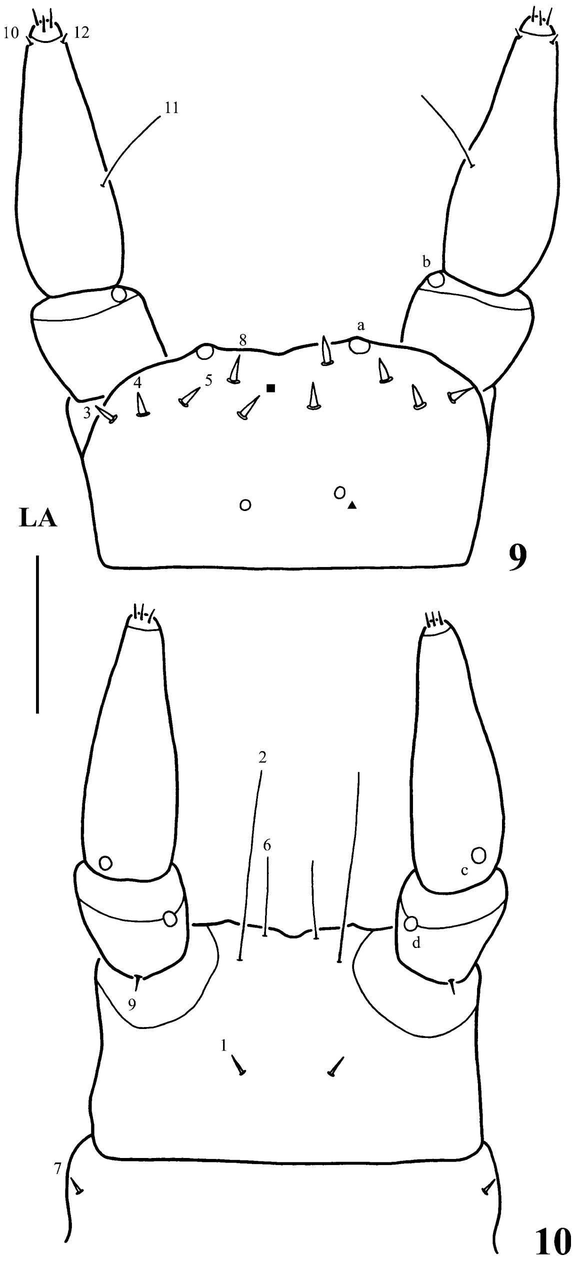
Thorax Terga convex, pronotum about as long as meso- and metanotum combined, meso- and metanotum subequal in length, wider than pronotum; protergite subrectangular, margins rounded, more developed than meso- and metatergite; meso- and metatergite transverse, covered with short spinulae; tergites without anterotransverse carina, with sagittal line slightly visible; thoracic sterna membranous; spiracles absent. *Legs* (Figs. 11-12). Short, composed of six articles, L1 and L2 the shortest, subequal, L3 the longest; CO robust,



Figs. 4–6 *Copelatus longicornis*, first-instar larva. (4) Antenna, dorsal aspect; (5) Antenna, ventral aspect; (6) Mandible, dorsal aspect. *SP* spinula. *Solid triangle* refers to additional pore. Scale bars = 0.04 mm



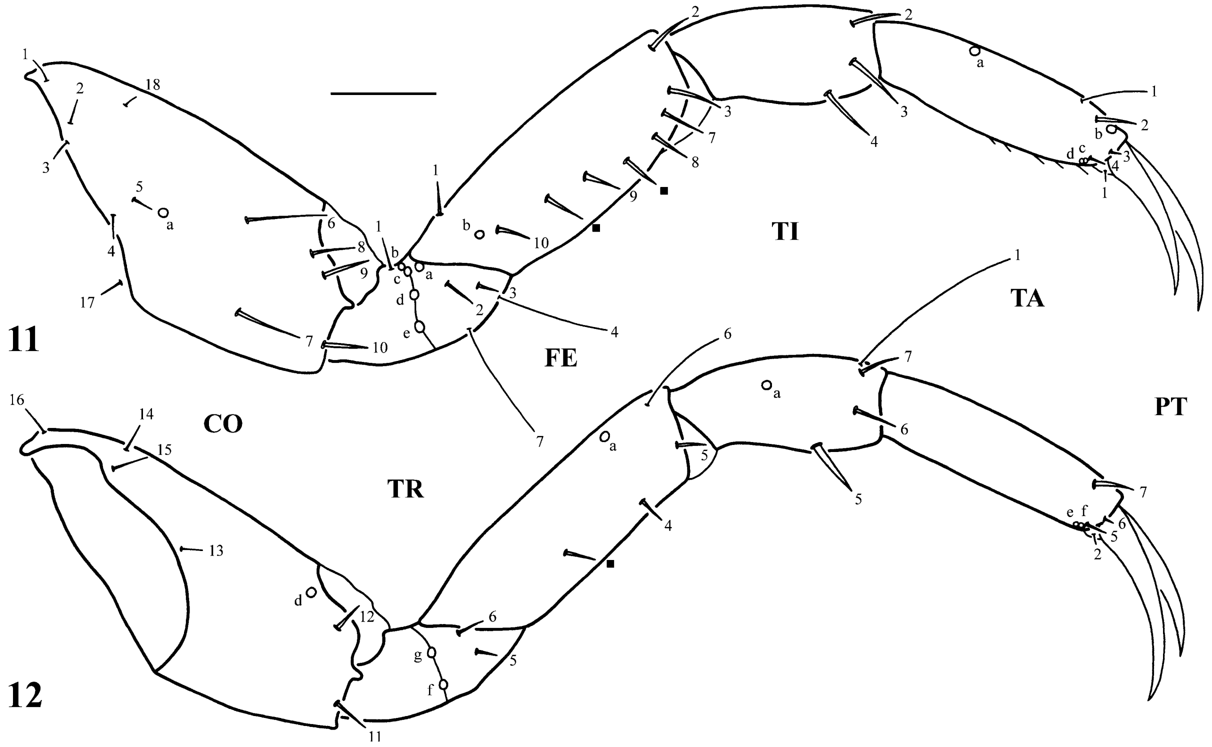
Figs. 7–8 *Copelatus longicornis*, first-instar larva. (7) Maxilla, dorsal aspect; (8) Maxilla, ventral aspect. Scale bar = 0.04 mm



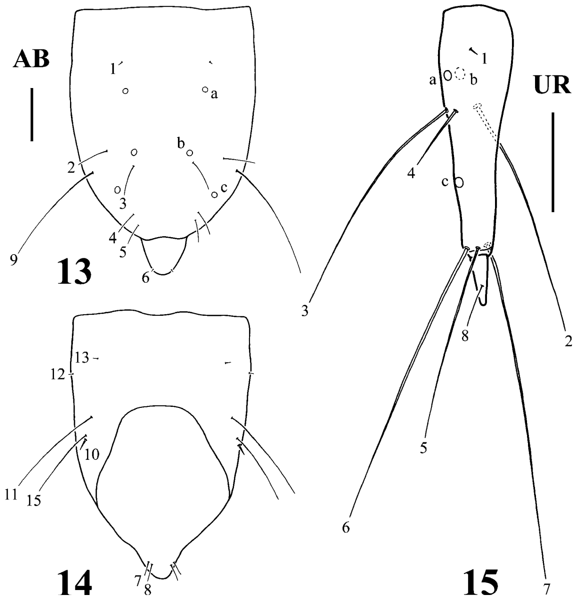
Figs. 9–10 *Copelatus longicornis*, first-instar larva. (9) Labium, dorsal aspect; (10) Labium, ventral aspect. *Solid square* refers to additional seta; *solid triangle* refers to additional pore. Scale bar = 0.04 mm

elongate, TR transversely divided into two parts, FE, TI and TA slender, subcylindrical, PT with two long, slender, slightly curved claws, posterior claw shorter than anterior one on L1 and L2, posterior claw longer than anterior one on L3; surface of FE, TI and TA covered in part with minute scattered spinulae, more developed on ventral surface of TI and TA.

Abdomen Eight-segmented; segments I–VII sclerotized dorsally, membranous ventrally; tergites I–VII



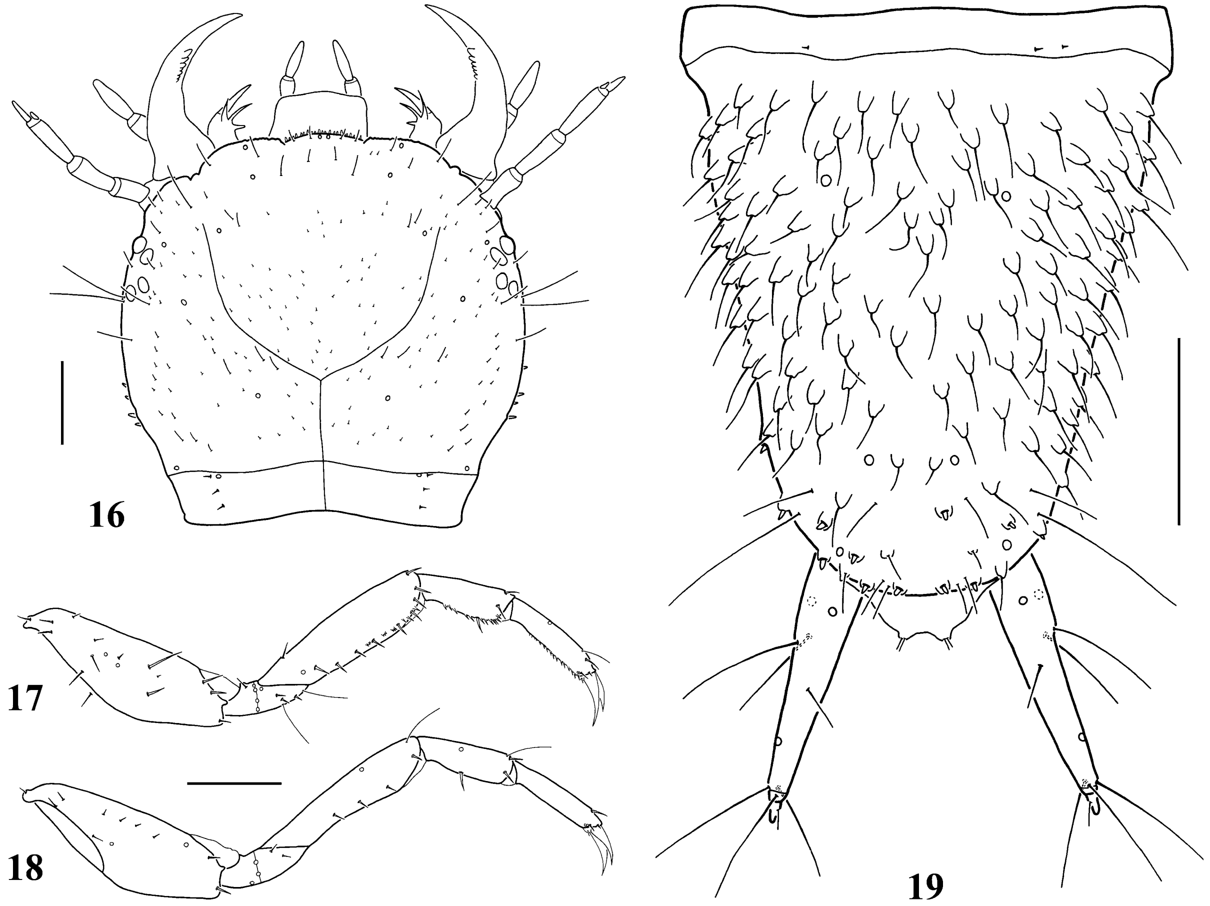
Figs. 11–12 *Copelatus longicornis*, first-instar larva. (11) Metathoracic leg, anterior aspect; (12) Metathoracic leg, posterior aspect. *Solid squares* refer to additional setae. Scale bar = 0.06 mm



Figs. 13–15 *Copelatus longicornis*, first-instar larva. (13) Abdominal segment VIII, dorsal aspect; (14) Abdominal segment VIII, ventral aspect; (15) Urogomphus, dorsal aspect. Scale bars = 0.06 mm

narrow, transverse, rounded laterally, without sagittal line, without anterotransverse carina, covered with short, stout spinulae; spiracles absent on segments I–VII; LAS (Figs. 13–14) the longest, completely sclerotized, ring-like, without anterotransverse carina, covered with short, stout spinulae dorsally and laterally; siphon short. *Urogomphus* (Fig. 15). Short, composed of two urogomphomeres; U1 shorter than LAS, covered with minute spinulae; U2 very short, much shorter than U1.

Chaetotaxy (Figs. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15) Similar to that of generalized Agabinae and Colymbetinae larvae (Alarie, 1995, 1998) except for the following features: pore FRe absent; pore ANg proximal; A3 without additional pores; a minute structure (possibly a pore) present on dorsal surface of A4; seta MN1 close to pore MNc; pore MXa absent; seta MX6 absent; seta MX8 minute, with a pore-like aspect; stipes without additional setae; prementum with one additional seta and one additional pore on dorsal surface; seta LA2 elongate, setiform; setae LA10 and



Figs. 16–19 *Copelatus longicornis*, third-instar larva. (16) Head, dorsal aspect; (17) Prothoracic leg, anterior aspect; (18) Prothoracic leg, posterior aspect; (19) Abdominal segment VIII and urogomphi, dorsal aspect. Scale bars = 0.20 mm

LA12 minute, inserted subapically (we have coded them as present but with some hesitation); setae CO6 and CO7 inserted more proximally on L1; seta FE1 inserted proximally; seta FE4 inserted somewhat more proximally; seta FE5 short, spine-like; seta FE6 hair-like; additional spine-like setae present on anteroventral and posteroventral margins of FE; seta TI4 inserted somewhat more proximally on L1; setae TI6 and TI7 short, spine-like; TI and TA without additional setae; seta AB14 absent; setae UR2, UR3 and UR4 inserted contiguous at about mid-length of U1; seta UR4 shorter than setae UR2 and UR3; setae UR5, UR6 and UR7 elongate, inserted apically on U1; seta UR8 inserted on distal third of U2.

Third-instar larva (Figs. 16, 17, 18, 19)

As first-instar larva except for the following features:

Color Larva entirely testaceous to light brown except the head capsule, mandible, and thoracic and abdominal sclerites brown (abdominal sclerites III–VII with one white macula on each anterolateral angle).

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

Head (Fig. 16) *Cephalic capsule* Slightly broader than long; maximum width posterior to stemmata; spinulae absent in several small rounded areas on the base of FR and on the PA (posterior to stemmata); occipital suture present; ecdysial line well marked; anterior margin of FR with 22 small spine-like setae. *Antenna* A4 the shortest.

Thorax All tergites covered with stout spinulae, with sagittal line well visible; meso- and metatergite

Table 1 Measurements and ratios for the larval instars of *Copelatus longicornis*

Measure	Instar I	Instar III
TL (mm)	2.10–3.00	6.40–7.00
MW (mm)	0.40–0.50	0.90–1.00
HL (mm)	0.42–0.46	0.90–0.93
HW (mm)	0.47–0.49	0.95–1.00
FRL (mm)	0.30–0.33	0.58–0.61
OCW (mm)	0.32–0.34	0.68–0.70
HL/HW	0.89–0.94	0.93–0.95
HW/OCW	1.42–1.53	1.35–1.47
COL/HL	0.27–0.29	0.34–0.36
FRL/HL	0.71–0.73	0.64–0.66
A/HW	0.52–0.55	0.47
A3/A1	1.80–2.25	1.08–1.45
A3/A2	1.13–1.38	1.00–1.11
A4/A3	0.58–0.71	0.34–0.42
A3′/A4	0.33–0.45	0.33–0.42
MNL/MNW	2.75–3.13	2.50–2.76
MNL/HL	0.52–0.54	0.47–0.50
A/MP	1.37–1.49	1.43–1.64
GA/MP1	2.17–2.80	1.43–2.09
PPF/MP1	0.67–0.80	0.57–0.75
MP2/MP1	1.67–2.20	1.57–2.00
MP2/MP3	0.45–0.50	0.88–0.96
MP/LP	1.67–1.90	1.71–1.88
LP2/LP1	2.50–3.00	1.33–1.46
L3 (mm)	0.82–0.84	1.67–1.72
L3/L1	1.02–1.06	1.08–1.12
L3/L2	1.03–1.07	1.08–1.10
L3/HW	1.71–1.77	1.69–1.77
L3 (CO/FE)	1.10–1.21	1.06–1.09
L3 (TI/FE)	0.59–0.66	0.59–0.62
L3 (TA/FE)	0.79–0.84	0.67–0.71
L3 (CL/TA)	0.59–0.69	0.41–0.43
LAS (mm)	0.27–0.34	0.62–0.64
LAS/HW	0.59–0.70	0.64–0.66
U (mm)	0.16–0.19	0.27–0.28
U/LAS	0.51–0.66	0.43–0.44
U/HW	0.34–0.39	0.28–0.29
U1/U2	5.17–7.00	8.33–10.40

with anterotransverse carina; spiracles present on mesothorax.

Abdomen Segment VII completely sclerotized, ring-like; sclerites I–VII with sagittal line, with anterotransverse carina; spiracles present on segments I–VII; LAS (Fig. 19) with anterotransverse carina. *Urogomphus* (Fig. 19). Without spinulae.

Chaetotaxy Cephalic capsule with numerous secondary setae, mainly minute or hair-like, 4–5 spine-like setae on each lateral margin of PA, and 7–10 spine-like setae on each side of ventral surface of PA; MN with 3–4 minute secondary setae along 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; thoracic tergites with numerous hair-like secondary setae, meso- and metatergite with hair-like secondary setae inserted on the apices of stout spinulae; secondary leg setation detailed in Table 2 and Figs. 17–18; CO with 1–3 secondary pores on posterior surface; TR with one secondary pore on proximal portion; abdominal tergites I–VI with numerous hair-like secondary setae inserted on the apices of stout spinulae; sclerites VII and VIII with numerous hair-like secondary setae, those on dorsal and lateral surfaces inserted on the apices of stout spinulae (Fig. 19).

Comparative notes

The descriptions of larvae of *Copelatus* presented by Williams (1936), Watts (1963), and Bertrand (1972) are rather superficial and do not permit in-depth comparisons with the larvae described here. No diagnostic differences in morphology to separate these larvae from that described here were found.

Table 2 Number and position of secondary setae on the legs of third-instar larva of *Copelatus longicornis*

Article	Position	Instar III
Coxa	A	0–3/1–5/1–6
	PD	3–5/3–4/1–4
	Range	3–7/4–8/2–9
Femur	AV	0–1/1/1
	PV	0–1/0–1/0–1
	Range	1–2/1–2/1–2

Numbers between slash marks refer to pro-, meso- and metathoracic leg, respectively. Additional setae were not included in the count of secondary setae. *A* anterior, *D* dorsal, *P* posterior, *V* ventral, *range* total number of secondary setae on the article (excluding primary setae)

Table 3 Taxa coded for parsimony analysis

Taxon	Species	Source
Aspidytidae	*Aspidytes niobe* Ribera, Beutel, Balke & Vogler	Alarie & Bilton (2005)
Dytiscidae		
Agabinae		
Agabini	*Agabus anthracinus* Mannerheim	Alarie (1995, 1998)
	Hydrotrupes palpalis Sharp	Alarie et al. (1998)
	Ilybius vittiger (Gyllenhal)	Alarie (1995, 1998); Nilsson (1983)
	Leuronectes curtulus Régimbart	–
	Platambus glabrellus (Motschulsky)	Alarie & Larson (1998)
Colymbetinae		
Colymbetini	*Bunites distigma* (Brullé)	–
	Rhantus signatus (Fabricius)	–
Copelatinae		
Copelatini	*Copelatus longicornis* Sharp	–
Coptotominae		
Coptotomini	*Coptotomus interrogatus* (Fabricius)	Barman (2004)
Dytiscinae		
Aciliini	*Thermonectus succinctus* (Aubé)	–
Dytiscini	*Dytiscus harrisii* Kirby	–
Hydaticini	*Hydaticus tuyuensis* Trémouilles	–
Hydroporinae		
Bidessini	*Amarodytes duponti* (Aubé)	–
Hydroporini	*Laccornellus lugubris* (Aubé)	–
Hydrovatini	*Hydrovatus caraibus* Sharp	–
Hygrotini	*Hygrotus sayi* J. Balfour-Browne	Alarie & Harper (1990); Alarie et al. (1990a, 1990b); Alarie (1991)
Hyphydrini	*Desmopachria concolor* Sharp	–
Laccornini	*Laccornis latens* (Fall)	Alarie & Harper (1990); Alarie et al. (1990a); Alarie (1989, 1991)
Methlini	*Celina parallela* (Babington)	–
Vatellini	*Vatellus haagi* Wehncke	–
Laccophilinae		
Laccophilini	*Laccophilus obliquatus* Régimbart	–
Lancetinae		
Lancetini	*Lancetes marginatus* (Steinheil)	–
Matinae		
Matini	*Matus bicarinatus* (Say)	Alarie et al. (2001)

The larvae of *C. australiae* described by Watts (1963) seem to have a considerably larger size (HW = 1.88 mm for the third instar) than those described here (HW = 0.95–1.00 mm for the third instar). The description of *C. glyphicus* (Spangler, 1962) is more detailed, but does not include morphometric measures. Spangler (1962), De Marzo (1976b), and Larson et al. (2000) mentioned the presence of one-segmented urogomphi in the larvae. However, in the larvae described here, the urogomphi are two segmented. As the second urogomphomere is very short and may have been overlooked, this difference should be taken with care. The first-instar larva of *L. haemorrhoidalis* (De Marzo, 1976b; Nilsson, 1988)

Table 4 Characters and states used for the parsimony analysis of 24 terminal taxa of Dytiscidae and Aspidytidae

(000) *Parietal (at level of occipital suture) (instar I)*: (0) not constricted; (1) constricted

(001) *Parietal (at level of occipital suture) (instar III)*: (0) not constricted; (1) constricted

(002) *Occipital suture (instar I)*: (0) absent; (1) present

(003) *Occipital suture (instar III)*: (0) absent; (1) present

(004) *Frontal line (instar III)*: (0) straight to moderately sinuate; (1) strongly sinuate

(005) *Anterolateral lobes of frontoclypeus (instar III)*: (0) absent; (1) inconspicuous; (2) well developed, not projected beyond anterior margin; (3) well developed, projected beyond anterior margin

(006) *Egg bursters (instar I)*: (0) located submedially; (1) located basally

(007) *Seta FR7 (instar I)*: (0) spiniform; (1) setiform

(008) *Pore FRe (instar I)*: (0) absent; (1) present

(009) *Lamellae clypeales on anterior margin of frontoclypeus (instar I)*: (0) absent; (1) two; (2) at least four

(010) *Seta PA3 (instar I)*: (0) inserted contiguous to setae PA1 and PA2; (1) inserted far from setae PA1 and PA2

(011) *Seta PA7 (instar I)*: (0) present; (1) absent

(012) *Seta PA18 (larva I)*: (0) present; (1) absent

(013) *Pore PAb (instar I)*: (0) inserted contiguous to seta PA3; (1) inserted far from seta PA3, close to coronal or frontal lines

(014) *Pore PAl (instar I)*: (0) present; (1) absent

(015) *Pore PAp (instar I)*: (0) present; (1) absent

(016) *Secondary spine-like setae on ventral surface of parietal (instar III)*: (0) present; (1) absent

(017) *Ventroapical spinula on antennomere 3 (instars I–III)*: (0) absent; (1) small; (2) strongly developed

(018) *Apical lateroventral process of antennomere 3 (instars I–III)*: (0) protruding; (1) not protruding

(019) *Antennomere 4 (instar I)*: (0) shorter than antennomere 3; (1) subequal to slightly longer than antennomere 3

(020) *Antennomeres 2 and 3 (instar III)*: (0) not subdivided; (1) subdivided

(021) *Seta AN1 (instar I)*: (0) inserted medially or distally; (1) inserted proximally

(022) *Seta AN3 (instar I)*: (0) inserted distally; (1) inserted submedially

(023) *Pore ANf (instar I)*: (0) present; (1) absent

(024) *Pore ANg (instar I)*: (0) inserted basally; (1) inserted medially

(025) *Pore ANi (instar I)*: (0) present; (1) absent

(026) *Additional ventroapical pores on antennomere 3 (instar I)*: (0) present; (1) absent

(027) *Secondary setae on antennomere 1 (instar III)*: (0) absent; (1) present

(028) *Mandible (instars I–III)*: (0) not oriented obliquely; (1) oriented obliquely

(029) *Mandibular channel (instars I–III)*: (0) more or less closed; (1) widely open; (2) absent

(030) *Seta MN1 (instar I)*: (0) distal to pore MNc; (1) proximal to pore MNc

(031) *Sensillum MN2 (instar I)*: (0) hair-like; (1) pore-like

(032) *Pore MNa (instar I)*: (0) inserted at about the same level as pore MNb; (1) inserted distally to pore MNb

(033) *Internal margin of stipes (instars I–III)*: (0) without robust spinulae; (1) with three robust spinulae

(034) *Galea (instars I–III)*: (0) well developed, subconical; (1) well developed, spine-like; (2) very short, subconical; (3) absent

(035) *Palpifer (instars I–III)*: (0) inconspicuous, not clearly differentiated from the stipes; (1) palpomere-like, clearly differentiated from the stipes

(036) *Maxillary palpomere 2 (instar III)*: (0) not subdivided; (1) subdivided

Table 4 continued

(037) *Maxillary palpomere 3 (instar III)*: (0) not subdivided; (1) subdivided

(038) *Seta MX4 (instar I)*: (0) present; (1) absent

(039) *Seta MX6 (instar I)*: (0) present; (1) absent

(040) *Seta MX8 (instar I)*: (0) inserted apically on the galea; (1) inserted subapically on the galea; (2) absent

(041) *Seta MX9 (instar I)*: (0) inserted apically on the galea; (1) inserted subapically on the galea; (2) absent

(042) *Seta MX10 (instar I)*: (0) present; (1) absent

(043) *Pore MXa (instar I)*: (0) present; (1) absent

(044) *Pore MXd (instar I)*: (0) present; (1) absent

(045) *Pore MXh (instar I)*: (0) inserted on the galea; (1) inserted on the stipes

(046) *Pore MXk (instar I)*: (0) present; (1) absent

(047) *Additional setae on the stipes (instar 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

(048) *Anterior margin of prementum (instar I)*: (0) straight to slightly emarginate; (1) deeply emarginate; (2) projected forward

(049) *Labial palpomeres 1 and 2 (instar III)*: (0) not subdivided; (1) subdivided

(050) *Seta LA3 (instar I)*: (0) inserted distally or subdistally; (1) inserted proximally; (2) absent

(051) *Seta LA5 (instar I)*: (0) elongate, hair-like; (1) short, spine-like; (2) elongate, spine-like

(052) *Setae LA4 and LA8 (instar I)*: (0) short, spine-like; (1) elongate, hair-like; (2) elongate, spine-like

(053) *Seta LA10 (instar I)*: (0) inserted submedially; (1) inserted distally; (2) absent

(054) *Seta LA12 (instar I)*: (0) inserted submedially; (1) inserted distally; (2) absent

(055) *Setae LA10 and LA12 (instar I)*: (0) elongate; (1) short to very short; (2) inapplicable

(056) *Pore LAc (instar I)*: (0) present; (1) absent

(057) *Additional setae on dorsal surface of prementum (instar I)*: (0) absent; (1) present

(058) *Additional pore on dorsal surface of prementum (instar I)*: (0) absent; (1) present

(059) *Secondary setae on prementum (instar III)*: (0) absent; (1) present

(060) *Secondary pores on ventral surface of prementum (instar III)*: (0) absent; (1) present

(061) *Ventral sclerites on prothorax (instars I–III)*: (0) absent; (1) one; (2) two

(062) *Seta CO7 on meso- and metacoxa (instar I)*: (0) inserted distally; (1) inserted proximally

(063) *Seta TR2 (instar I)*: (0) present; (1) absent

(064) *Seta TR3 (instar I)*: (0) absent; (1) present

(065) *Seta FE1 (instar I)*: (0) inserted proximally; (1) inserted subproximally or submedially

(066) *Seta FE4 (instar I)*: (0) present; (1) absent

(067) *Seta FE5 on mesofemur (instar I)*: (0) short, spine-like; (1) elongate, hair-like; (2) absent

(068) *Seta FE5 on metafemur (instar I)*: (0) short, spine-like; (1) elongate, hair-like; (2) absent

(069) *Seta FE6 (instar I)*: (0) absent; (1) inserted distally; (2) inserted subdistally

(070) *Additional posteroventral setae on femur (instar I)*: (0) absent; (1) present

(071) *Natatory setae on femur (instar I)*: (0) absent; (1) present

(072) *Natatory dorsal setae on femur (instar III)*: (0) absent; (1) present

(073) *Natatory ventral setae on femur (instar III)*: (0) absent; (1) present

(074) *Secondary anterodorsal setae on femur (instar III)*: (0) absent; (1) present

(075) *Secondary posteroventral setae on femur (instar III)*: (0) absent; (1) present

(076) *Seta TI1 (instar I)*: (0) inserted distally; (1) inserted subdistally or submedially

(077) *Seta TI4 (instar I)*: (0) more proximal on leg 1; (1) not more proximal on leg 1

(078) *Seta TI5 (instar I)*: (0) spiniform; (1) setiform

Table 4 continued

(079) Seta TI6 on pro- and mesotibia (instar I): (0) elongate, hair-like; (1) short, spine-like

(080) Seta TI6 on metatibia (instar I): (0) elongate, hair-like; (1) short, spine-like

(081) Seta TI7 (instar I): (0) short, spine-like; (1) elongate, hair-like

(082) Additional anteroventral setae on tibia (instar I): (0) absent; (1) present

(083) Secondary setae on tibia (instar III): (0) absent; (1) present

(084) Natatory dorsal setae on tibia (instar I): (0) absent; (1) present

(085) Natatory ventral setae on tibia (instars I–III): (0) absent; (1) present

(086) Seta TA1 (instar I): (0) short to very short; (1) elongate to very elongate; (2) absent

(087) Seta TA1 (instar I): (0) inserted distally or subdistally; (1) inserted submedially; (2) inapplicable

(088) Additional anteroventral setae on tarsus (instar I): (0) absent; (1) present

(089) Secondary setae on anterodorsal margin of protarsus (instar III): (0) absent; (1) present

(090) Secondary setae on posteroventral margin of protarsus (instar III): (0) absent; (1) present

(091) Secondary setae on posteroventral margin of metatarsus (instar III): (0) absent; (1) present

(092) Natatory dorsal setae on tibia and tarsus (instar III): (0) absent; (1) present

(093) Basoventral spinulae on claws (instar I): (0) absent; (1) present

(094) Abdominal tergites I–VI (instar I): (0) with anterotransverse carina; (1) without anterotransverse carina

(095) Ventral surface of abdominal segments II–IV (instar III): (0) membranous; (1) sclerotized

(096) Ventral surface of abdominal segment VI (instar I): (0) membranous; (1) sclerotized

(097) Abdominal segment VII (instar I): (0) sclerotized dorsally, membranous ventrally; (1) completely sclerotized except for a narrow longitudinal membranous band ventrally; (2) sclerotized dorsally and ventrally, with ventral sclerite independent from dorsal sclerite; (3) completely sclerotized

(098) Abdominal sclerite VII (instar I): (0) with anterotransverse carina; (1) without anterotransverse carina

(099) Abdominal segment VII (instar III): (0) without a lateral row of natatory setae; (1) with a lateral row of natatory setae

(100) Siphon (instars I–III): (0) very short, slightly projected backwards; (1) moderately long, projected backwards; (2) very long, urogomphomere-like; (3) absent

(101) Seta AB4 (instar I): (0) not distinctly developed; (1) very long, strongly developed

(102) Seta AB7 (instar I): (0) small; (1) well developed to strongly developed; (2) absent

(103) Seta AB9 (instar I): (0) inserted dorsolaterally; (1) inserted ventrolaterally

(104) Seta AB11 (instar I): (0) spine-like; (1) hair-like

(105) Seta AB11 (instar I): (0) inserted contiguous to ventroapical margin; (1) inserted far from ventroapical margin

(106) Seta AB15 (instar I): (0) absent; (1) inserted contiguous to ventroapical margin; (2) inserted far from ventroapical margin

(107) Additional setae on abdominal segment VIII (excluding natatory setae) (instar I): (0) absent; (1) a single spine-like seta inserted on lateral margin; (2) numerous

(108) Natatory setae on lateral margin of abdominal segment VIII (instar I): (0) absent; (1) present

(109) Natatory setae on lateral margin of abdominal segment VIII (instar III): (0) absent; (1) present

(110) Urogomphus (instars I–III): (0) composed of one urogomphomere; (1) composed of two urogomphomeres

(111) Number of primary setae on urogomphus (excluding natatory setae) (instar I): (0) nine; (1) eight; (2) seven; (3) numerous

(112) Setae UR2, UR3 and UR4 (instar I): (0) inserted contiguously; (1) not inserted contiguously; (2) only UR2 and UR3 contiguous; (3) only UR3 and UR4 contiguous

Table 4 continued

(113) *Seta UR4 (instar I)*: (0) shorter than seta UR2; (1) subequal in length to seta UR2; (2) longer than seta UR2
(114) *Seta UR7 (instar I)*: (0) elongate, hair-like; (1) short, spine-like
(115) *Seta UR8 (instar 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
(116) *Pore URb (instar I)*: (0) proximal to seta UR2; (1) contiguous to seta UR2; (2) distal to seta UR2; (3) absent
(117) *Pore URc (instar I)*: (0) not inserted terminally on urogomphomere 1; (1) inserted terminally on urogomphomere 1
(118) *Secondary setae on urogomphus (instar III)*: (0) absent; (1) present
(119) *Row of natatory setae on urogomphus (instar III)*: (0) absent; (1) present

differs from that of *C. longicornis* in having an occipital suture and five additional anteroventral setae on the femur, whereas in the first-instar larva of *C. longicornis* the occipital suture is absent and there are two additional anteroventral setae on the femur. The third-instar larva of *L. haemorrhoidalis* differs from that described here in the presence of a more marked occipital constriction and 5–8 posterodorsal secondary setae on the coxa, whereas in the third-instar of *C. longicornis,* there is a less marked occipital constriction and 1–5 posterodorsal secondary setae on the coxa.

Character analysis

We included a total of 120 characters derived from the larval morphology and chaetotaxy, of which 89 were coded as binary and 31 as multistate (Table 4). The analysis of the data matrix (Table 5) using TNT resulted in three most parsimonious trees of 361 steps (CI = 0.45; RI = 0.67). The trees differed in the relative positions of the agabine genera *Agabus* Leach, *Ilybius* Erichson, and *Platambus* Thomson, and in the relative positions of the hydroporine genera *Laccornis* Gozis and *Laccornellus* Roughley & Wolfe + *Hydrovatus* Motschulsky. For this reasons, the strict consensus was calculated, in which some Agabinae and Hydroporinae genera collapsed in polytomies (Fig. 20). The genus *Copelatus* was recovered in a relatively basal position within the nonhydroporine clade, as sister group of a large clade composed of members of the subfamilies Lancetinae, Coptotominae, Laccophilinae, Colymbetinae, Matinae, and Dytiscinae, and the agabine genus *Leuronectes* Sharp (Fig. 20). We found no evidence for a sister-group relationship between *Copelatus* and the remaining Dytiscidae, which had previously been suggested by several authors (see Discussion). Given that the evolution of the mandible channel is a major topic of discussion in this article, an alternative analysis was performed in which character 29 (mandible channel) was scored as a simple presence/absence character, assigning the state “absent” to *Copelatus*, *Hydrotrupes* Sharp, and the outgroup, thus favoring the hypothesis of an ancestral condition of *Copelatus* and *Hydrotrupes* within Dytiscidae. However, no changes were observed in the topology with respect to the original analysis. We also ran the analysis excluding character 29, and the topology remained unaltered. As an additional element to evaluate the strength of the arrangement obtained, a constrained analysis was performed in which *Copelatus* was forced to be the sister group of the remaining Dytiscidae. The resulting topologies were five steps longer than the original. Characters were mapped in one of the most parsimonious cladograms (Fig. 21). Derived nodes were generally better supported than nodes supporting larger clades (Fig. 20).

Discussion

Different hypotheses have been proposed regarding the phylogenetic position of *Copelatus* within Dytiscidae. For example, Ruhnau & Brancucci (1984), De Marzo & Nilsson (1986), and Beutel (1994, 1995) considered *Copelatus* sistergroup of the remaining Dytiscidae. Ruhnau & Brancucci (1984) proposed

Table 5 Data matrix of 120 larval characters for 24 terminal taxa of Dytiscidae and Aspidytidae. Missing data coded '?'

Species	Character											
	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0111111111	1111111111
	0000000000	1111111111	2222222222	3333333333	4444444444	5555555555	6666666666	7777777777	8888888888	9999999999	0000000000	1111111111
	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789
Aspidytes niobe	0000030100	0000100110	0010000001	0100010001	1000001000	0102220110	0000110001	0000000101	1000002200	0000?113?0	3??????200	1031002010
Agabus anthracinus	01000?0102	000000?100	0000001000	0000010000	0000001110	010111000?	??00110001	?000??0001	100100000?	??00?00??0	?001001000	1101052000
Hydrotrupes palpalis	0000030102	0000000100	0000001001	0000010000	0000001100	0101110100	1000110001	0000010?01	1000000000	0000000200	0000001000	1130052100
Ilybius vittiger	1100020102	000000?100	0??00?1?00	???0010000	0000001100	0100110?0?	??00110001	10001?0001	1001000001	1100?00??0	0001001000	1100051000
Leuronectes curtulus	1100020102	0000000110	0000000000	0000010000	0001001100	0100010000	1000110001	1000110001	1000000000	0000000000	0000001100	1111050000
Platambus glabrellus	1100020102	000000?100	0100001000	0000010000	0000001000	0101110100	?000110001	1000110001	1000000000	0000000200	0001001000	0101052000
Bunites distigma	1?1?0?0112	000000?111	?100100000	000001??00	000100120?	010111010?	??00100001	10????0100	011?00001?	???10?000?	000000120?	03???5?0??
Rhantus signatus	1111020112	0000000111	0100100000	0000010000	0001001000	0101110101	1000100111	1010110100	0111000011	0111000000	0000001000	0111150011
Copelatus longicornis	1101010102	0000000100	0000001002	1001110001	1101001000	0101110110	1000100001	1000010001	1000001000	0000100010	0000101000	1100010000
Coptotomus interrogatus	01000201?1	000??01?00	0??????000	???0010000	0001001100	010222?1??	?210110001	0000101101	1100001100	0010?000?0	100????001	03???5??11
Thermonectus succinctus	1111030102	0000100210	1100001100	001010010?	0101001320	0101110111	0111101220	0111110?10	0101111001	0010000111	0000112011	0130152000
Dytiscus harrisii	11110301?2	0000000110	1100000100	0010011100	0001001101	010??0000?	1?10100221	1111110?10	0111111001	0010000011	0000112011	0???05??11
Hydaticus tuyuensis	1111030102	0000000100	1010001100	1010011100	0001001120	0101110001	1110101220	0111110?10	0101111001	0011000101	0000112011	0121052000
Amarodytes duponti	0011100002	1111111100	0011011010	1100300010	2211111000	0010001000	0001000001	0000111101	1001001000	0000100210	10?1000000	1111010110
Laccornellus lugubris	0?0???0002	011111?000	?010011?10	111020??00	001110000?	001001100?	??00000001	00????1101	100?00100?	???00?020?	110000200?	12010411??
Hydrovatus caraibus	0000101102	0111111000	0010011010	1010200000	0011100000	2010001000	0001000001	0000011101	1000001000	0001011200	1100000000	1200041100
Hygrotus sayi	0001100002	111111?000	0011011010	?1?0300000	2211110000	00100?1000	0001000002	0000111101	1001001000	1100100210	1001000000	1121021100
Desmopachria concolor	0001100102	1111110100	0011011010	1110300011	2211110000	1011101100	0001000001	0010111101	1101001001	1110111210	1011000000	1101033110
Laccornis latens	0001100002	0111110000	0000011010	11?0200000	0011101000	00100?1000	0000000001	0000011101	1000001000	0000000200	1001000000	1101001100
Celina parallela	0111100002	1101111000	0110011010	1010200000	0001100000	2011101000	0000000001	0000111101	1001001001	1100000200	1021100000	1102001100
Vatellus haagi	0001100102	1101111100	0011010110	1110300010	221111?000	0212221000	0001010001	0000111101	1101001001	1110100210	2011000000	1111001111
Laccophilus obliquatus	0100021101	0000000000	0010011000	1000010000	0001001100	2202220000	1010110001	0000000000	0001001101	0010100010	1001001000	1111150110
Lancetes marginatus	1100030101	0000000101	1110000000	0000011100	0001001001	0100110110	1010110001	0000010000	0011000000	0010100010	0000001000	0111050011
Matus bicarinatus	0011010102	000000?200	0100000100	0000010000	0000001000	0221110001	0000100011	0010111101	0001001101	1110000200	1000001200	0120052010

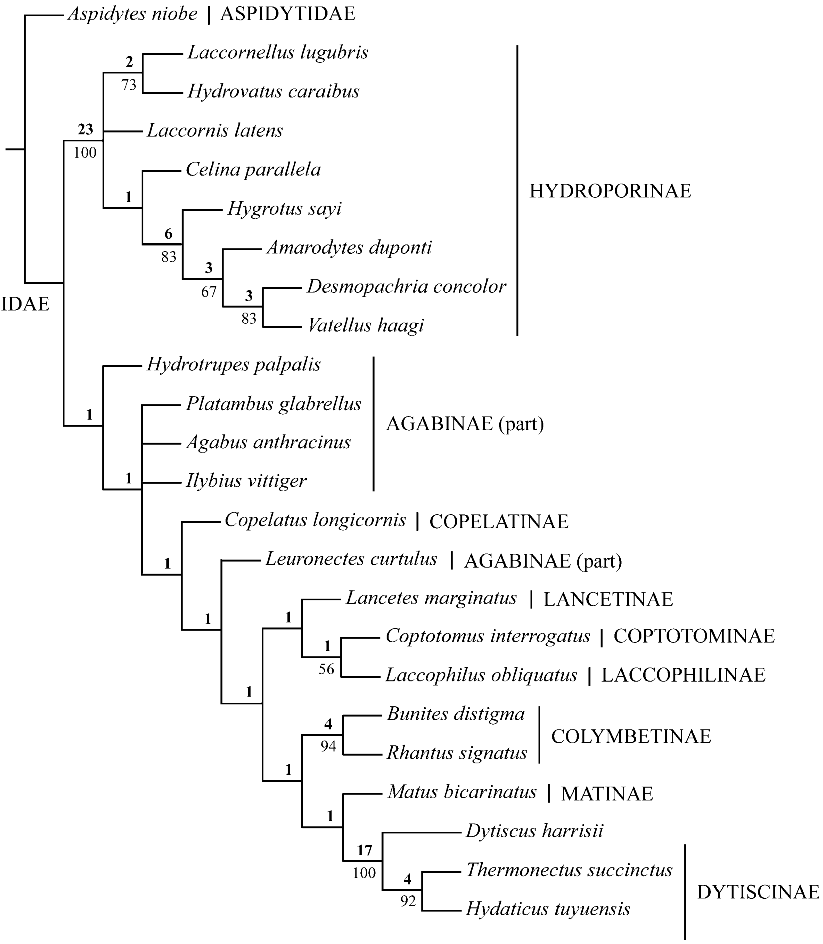


Fig. 20 Strict consensus cladogram of 24 terminal taxa of Dytiscidae and Aspidytidae. Bremer support values are indicated above branches; jackknife values above 50 are indicated below branches

several larval apomorphies for Dytiscidae except *Copelatus* including the presence of a mandibular channel and the absence of a crop. The absence of a channel was considered plesiomorphic within Dytiscidae partly because larvae of Amphizoidae and first instars of Paelobiidae, considered to be close relatives of Dytiscidae (Ruhnau, 1986; Beutel, 1995), lack a channel. Obviously, the evolution of the mandibular channel had been considered of great phylogenetic importance, possibly because of a reversal in this character (i.e., the secondary development of an open channel from a closed channel, or its secondary absence) was considered unlikely (Ruhnau, 1986; Beutel, 1994). Following this interpretation, *Agabetes* Crotch and *Hydrotrupes* were also considered ancestral within Dytiscidae, owing to the presence of widely open channels in these genera (De Marzo & Nilsson, 1986; Beutel, 1994, 1995). None of the studies, however, included a numeric analysis based on an explicit data matrix, thus rendering their conclusions preliminary (see Miller, 2001 for additional criticism). An additional difficulty in assessing this character is continuous morphological variation. As shown by De Marzo & Nilsson (1986), the morphology of the mandibular channel varies from being absent in *Copelatus*, widely open in *Agabetes* (and *Hydrotrupes*, Beutel, 1994), almost closed in Agabinae and *Colymbetes* Clairville, to closed to different degrees in most of the remaining Dytiscidae. This creates difficulties in coding this character. In our study, we coded it as a multistate character in which, among the taxa studied, only *Copelatus* lacks a channel, and only *Hydrotrupes* and the outgroup taxon have widely open channels, with the remaining taxa coded as having a more or less a closed channel. Following this definition of character 29, *Copelatus* is unique within Dytiscidae in lacking a channel, and is here revealed to be in a derived position, in

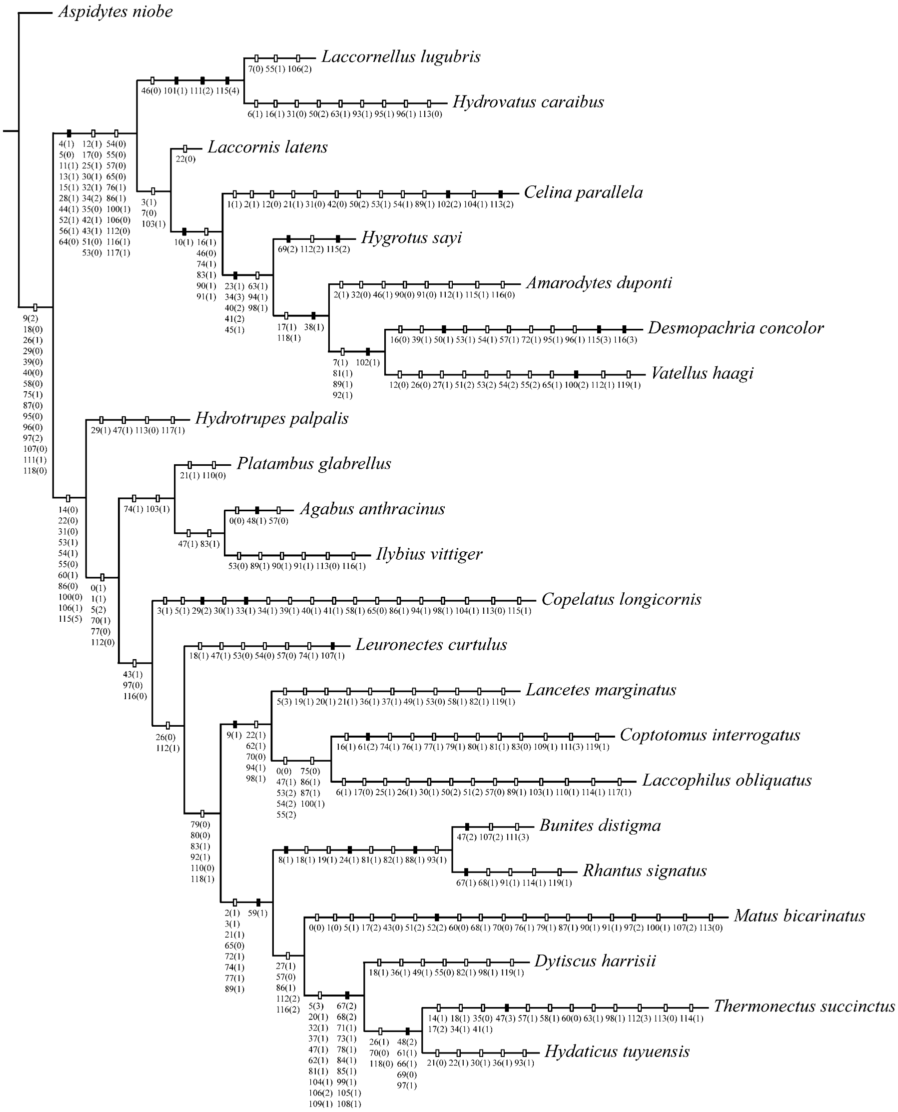


Fig. 21 One of the most parsimonious cladograms of 24 terminal taxa of Dytiscidae and Aspidytidae, with character changes mapped for each clade. *Solid rectangles* indicate unique character state transformations and *open rectangles* indicate homoplastic character state transformations

contradiction to the previous hypotheses (Ruhnau & Brancucci, 1984; De Marzo & Nilsson, 1986; Beutel, 1994, 1995). The alternative analysis in which character 29 was scored as "absent" in *Copelatus*, *Hydrotrupes,* and the outgroup taxon, and "present" in the remaining taxa (favoring the hypothesis of an ancestral condition of *Copelatus* and *Hydrotrupes* within Dytiscidae) also rendered a similar topology, as well as the analysis excluding character 29. This means that when evaluated in the context of a cladistic analysis, the absence of the mandibular channel in *Copelatus* should be considered secondary. *Copelatus* was resolved as part of a large clade comprising all Dytiscidae except Hydroporinae, and within this clade, the genus was the sister group of a clade formed by the subfamilies Lancetinae, Coptotominae, Laccophilinae, Colymbetinae, Matinae, and Dytiscinae, and the agabine genus *Leuronectes* (Fig. 20). This result is more in agreement with those of other authors who suggested a derived position of Copelatinae within Dytiscidae, either as the sister group of the clade Hydrodytinae + Hydroporinae (Miller, 2001), more closely related to Laccophilinae and members of Dytiscinae (Ribera et al., 2002), related to a clade formed by Colymbetinae, Hydroporinae, Dytiscinae and Lancetinae (Balke et al., 2004), or to a clade formed by Hydroporinae, Laccophilinae and Cybistrini (Ribera et al., 2008). The differences among the above results, however, suggest that the position of *Copelatus* and Copelatinae within Dytiscidae is not well understood, and that additional effort is needed based on an increased taxon sample and new character systems.

Our analysis reveals three apomorphies for *Copelatus*: mandibular channel absent (character 29.2), internal margin of the stipes with three robust spinulae (character 33.1), and seta MX8 inserted subapically on the galea (character 40.1) (Fig. 21). The presence of strong denticles on the internal margin of the mandible (not coded in this study) is another distinctive feature of *Copelatus* larvae. This last character and others not included in this study were proposed as apomorphies for *Liopterus* by Beutel (1994).

As stated above, the larval morphology of members of Copelatinae is poorly known, with only two (*Copelatus* and *Liopterus*) out of seven genera having described larvae. Also, the larvae of few species of the large genus *Copelatus* have been described so far. This interferes considerably with the study of the phylogenetic relationships of (and within) the subfamily based on larval characters, and emphasizes the preliminary nature of our study. Nevertheless, our study has the advantage of being the first in gathering a considerable amount of larval information and analyzing it comprehensively using numerical techniques, with the objective of studying the systematic placement of a member of Copelatinae. Detailed descriptions (including chaetotaxy) of the members of *Agaporomorphus*, *Aglymbus*, *Exocelina*, *Lacconectus*, and *Madaglymbus* as well as of more species of *Copelatus* are much needed, to gain confidence in the hypotheses of relationships presented here.

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