Phylogenetic Placement of the Genus *Sternhydrus* (Coleoptera: Dytiscidae: Cybistrini) Based on Larval Morphology, With Description of *S. atratus*

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ABSTRACT The phylogenetic relationships of the Australasian diving beetle genus *Sternhydrus* Brinck, 1945 were studied based on a cladistic analysis of 16 taxa and 112 morphological larval characters. Larvae of *S. atratus* (F., 1801) were described and illustrated in detail with particular emphasis on morphometry and chaetotaxy. Primary chaetotaxy were unknown for the genus. The results supported the inclusion of *Sternhydrus* in a clade of Australian Cybistrini genera along with *Onychohydrus* Schaum and White, 1847 and *Spencerhydrus* Sharp, 1882 based on four synapomorphies: presence of additional pores on antennomere 1, tibial seta TI7 short and spine-like, and urogomphi longer than broad, included in the nonsclerotized ventrodistal area surrounding the anus. A sister group relationship of *Sternhydrus* and *Onychohydrus* is suggested by two synapomorphies: lateral projections of frontoclypeus serrate, and row of additional anteroventral setae on meso- and metatarsus composed of several thin setae of variable length. The genus *Sternhydrus* is characterized by two autapomorphies: presence of both spine-like and hair-like additional setae on the ventral surface of the femur, and absence of primary pores on the urogomphus.

KEY WORDS diving beetle, *Sternhydrus*, phylogeny, chaetotaxy, larva

The dysticid genus *Sternhydrus* Brinck, 1945 is included in the tribe Cybistrini, which comprises some of the largest known aquatic beetles, with the adults reaching almost 50 mm and the larvae 90 mm in body length (Trémouilles 1989, Michat 2006). The name *Sternhydrus* was for a long time recognized as a subgenus of *Onychohydrus* Schaum and White, 1847 (=*Homoeodytes* Régimbart, 1879) until Miller et al. (2007) raised it to generic rank. As presently defined, the genus includes four species, Australian endemic *S. atratus* (F., 1801), *S. gibbosus* (Wilke, 1920) and *S. kolbei* (Wilke, 1920) from New Guinea, and *S. toxopei* (Zimmermann, 1925) described from Maluku (Nilsson 2015). *Sternhydrus atratus*, the type species, occurs along the East coast of Australia south to Brisbane, and in the Darwin area of the Northern Territory (Watts 1964, 1978).

Few studies have explored the phylogenetic relationships of *Sternhydrus* within the Cybistrini, based mainly on adult morphological and molecular characters. Miller (2001) suggested a close phylogenetic relationship of *Sternhydrus* with *Austrodytes* and *Onychohydrus* as part of a broader adult morphology-based phylogenetic analysis of the Dytiscidae, although the resolution in that part of the tree prevented a more conclusive statement. Miller et al. (2007) found *Sternhydrus* to be part of a clade of Australian Cybistrini along with *Austrodytes* Watts, 1978, *Onychohydrus*, and *Spencerhydrus* Sharp, 1882, and within this clade it was resolved either as sister to *Austrodytes* (parsimony analysis) or to *Onychohydrus* (likelihood and Bayesian analyses). In a subsequent analysis, Miller and Bergsten (2014) recovered the same clade with a similar branching pattern, although their study did not include *Austrodytes* and therefore *Sternhydrus* was resolved as sister to *Onychohydrus*.

The larval morphology of *Sternhydrus* is poorly known. The second and third instars of *S. atratus* were described by Watts (1964) under the genus name *Homoeodytes*. These descriptions, however, refer to general morphological characters and did not emphasize chaetotaxy, which has proven to be of great value in providing characters both for diagnosis and for phylogenetic studies within the Cybistrini (Michat 2006, 2010; Alarie et al. 2011). Also, the first instar was not described, so primary chaetotaxy remained unknown for the genus. In this context, the discovery of all instars of *S. atratus* provides an opportunity to study the larval ground plan of *Sternhydrus* and the relative phylogenetic position of the genus within the tribe Cybistrini. The present study, therefore, has the following goals: 1) to describe and illustrate in detail the three larval instars of *S. atratus* with an emphasis on morphometry and chaetotaxy; 2) to recognize the characters useful in distinguishing larvae of *Sternhydrus* from those of other cybistrine genera; and 3) to study the

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phylogenetic relationships of *Sternhydrus* within the Cybistrini based on larval characters.

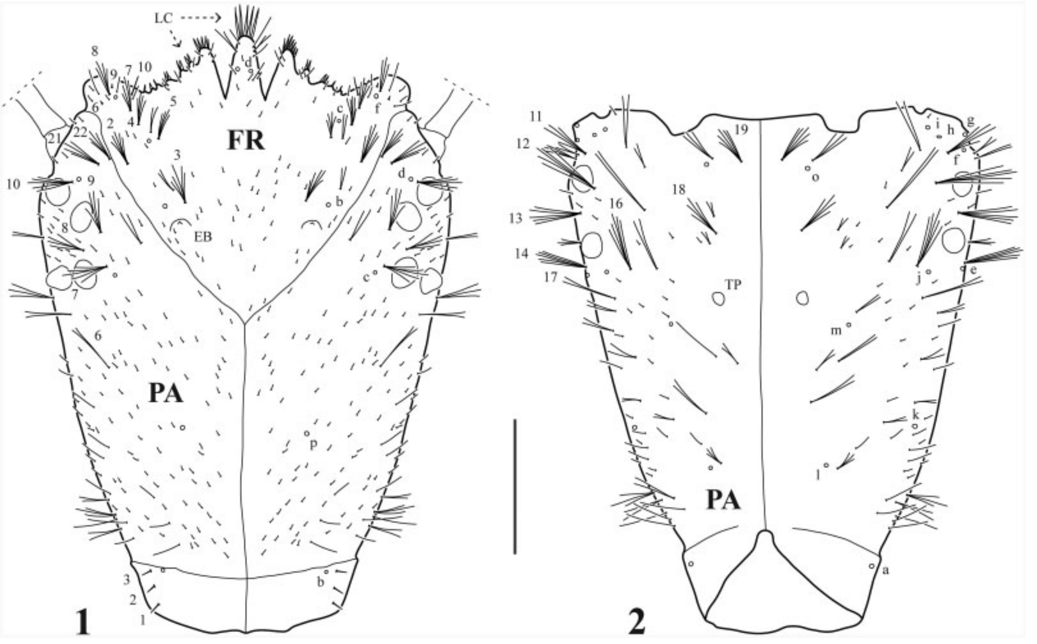
Materials and Methods

Larvae Examination, Preparation, and Description. Eight specimens of instar I, three of instar II, and three of instar III were used for the descriptions. Larvae were collected in association with adults at the following localities: 1) AUSTRALIA, Queensland, Bibohra Storage, 10 km NW Mareeba, 29-I-91; 2) AUSTRALIA, Queensland, 10 km N Bibohra, 4/25-II-91. The association is firm, as *S. atratus* was the only species of Cybistrini collected as adult at those sites and the only other cybistrine species in the region is the much larger *Cybister tripunctatus* (Olivier, 1795) whose larva is known (Watts 1964; Alarie et al. 2011). Larvae were cleared in lactic acid, dissected, and mounted on glass slides in polyvinyl-lacto-glycerol. Microscopic examination at magnifications up to 1,000× and drawings were made using an Olympus CX31 (Olympus Corporation, Japan) compound microscope equipped with a camera lucida. Drawings were scanned and digitally inked using a Genius PenSketch tablet (KYE Corporation, Taiwan). The material is held in the collection of the South Australian Museum.

Morphometric Analysis. 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 the joint of frontal and coronal sutures); occipital foramen width (OCW; maximum width measured along dorsal margin); coronal suture length (COL); length of mandible (MNL; measured from laterobasal angle to apex); width of mandible (MNW; maximum width measured at base); length of palpifer (PPF); length of antenna (A) and maxillary (MP) and labial (LP) palpi were derived by adding the lengths of the individual segments; each segment is denoted by the corresponding letter(s) followed by a number (e.g., A1, first antennomere); A3' is used as an abbreviation for the apical lateroventral process of the antennomere 3; 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, the length of distal portion is included in the femoral length; length of last abdominal segment (LAS; measured dorsally along midline from anterior to posterior margin); length of urogomphus (U). These measurements were used to calculate several ratios that characterize body shape.

Chaetotaxic Analysis. Primary setae and pores were distinguished on the cephalic capsule, head appendages, legs, last abdominal segment, and urogomphus. They 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 lower case letter (pores). The following abbreviations were used: abdominal segment VIII (AB); antenna (AN); coxa (CO); femur (FE); frontoclypeus (FR); labium (LA); mandible (MN); maxilla (MX); parietal (PA); pretarsus (PT); tarsus (TA); tibia (TI); trochanter (TR); urogomphus (UR). Primary sensilla present in *S. atratus* larvae were named by comparison with those of other genera of the tribe Cybistrini for which the primary chaetotaxy was described in detail (Michat 2006, 2010; Alarie et al. 2011). Hypotheses of homology were proposed using the criterion of similarity of position (Wiley 1981), and the ground plan of primary chaetotaxy of the genus was established. Larvae of Cybistrini bear numerous additional sensilla (i.e., those evolved secondarily in the first instar, generally restricted to small groups within the family) that obscure the establishment of homologies with the ancestral systems in other Dytiscinae. Alarie et al. (2011), however, have shown that first instars of Cybistrini are characterized by the presence of multifid setae (i.e., setae that are split into two or more branches beyond the base), which are distributed similarly to the ancestral pattern of primary setae depicted for the subfamily Dytiscinae. The presence of a multifid seta was therefore considered as an additional argument for homology when more than one seta of *S. atratus* was potentially homologous with a given seta of other cybistrine genera. The ancestral chaetotaxy pattern thus established for *Sternhydrus* is in good agreement with that observed in other Dytiscinae genera (Alarie et al. 2011). 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 position of the genus *Sternhydrus* within the tribe Cybistrini was analyzed cladistically using the program TNT (Goloboff et al. 2008) and considering the character set provided by the larval morphology and chaetotaxy. Sixteen species were included in the analysis. The ingroup consisted of 10 Cybistrini species representing five of the seven known genera (*Austrodytes* and *Regimbartina* Chatanay, 1911 were not included because their larvae are unknown). For the outgroup, six species representing all Dytiscinae tribes were included, rooting the tree in Aciliini (*Thermonectus* Dejean, 1833), a morphologically distinct tribe. Although recent studies based on molecular data questioned the traditional placement of Cybistrini within the Dytiscinae (Miller and Bergsten 2014), morphological evidence coming from both adults and larvae strongly suggests a position of Cybistrini within this subfamily (Miller 2000, 2001; Michat 2010; Alarie et al. 2011). Based on the phylogenetic results in Michat (2010), both Dytiscinae and Cybistrini are strongly supported monophyletic groups, not closely related to Laccophilinae as suggested by Miller and Bergsten (2014), which justifies our outgroup selection. All instars of each species were observed for character coding, except *Megadytes robustus* (Aubé, 1838) of which only the third instar was available. All characters were treated as unordered and equally weighted. Multistate characters were treated as



Figs. 1–2. *Sternhydrus atratus*, instar I. (1) Cephalic capsule, dorsal aspect; (2) Cephalic capsule, ventral aspect. EB, egg burster; LC, lamellae clypeales; TP, tentorial pit. Scale bar = 0.40 mm.

nonadditive. An exact solution algorithm (implicit enumeration) was implemented to find the most parsimonious trees. Bremer support values were calculated using the commands "hold 20000," "sub n," and "bsupport," where "n" is the number of extra steps allowed. The process was repeated increasing the length of the suboptimal cladograms by one step, until all Bremer values were obtained (Kitching et al. 1998). Bootstrap values were calculated using the following parameters: "standard (sample with replacement)"; 2,000 replicates.

Results

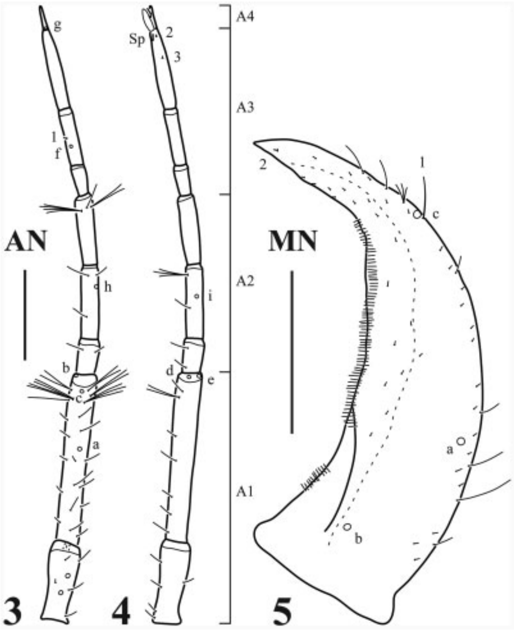
Description of the Larvae of *Sternhydrus atratus*

Diagnosis. Larvae of *Sternhydrus* can be distinguished from those of other Cybistrini genera by the following combination of characters: ratio HL/HW: 1.45–1.51 (instar I), 1.48–1.49 (instar II), 1.40–1.47 (instar III); medial projection of frontoclypeus simple, truncate apically, with several apical setae directed forward (Figs. 1 and 16); lateral projections of frontoclypeus serrate (Figs. 1 and 16); A1 with two additional pores (Fig. 3); distal fourth of MN abruptly narrowed, forming distinct mesal angle, lacking crown of multifid setae (Fig. 5); MP1 subdivided into two articles, MP2 lacking additional setae (Figs. 6 and 7); dorsal surface of prementum lacking additional setae (Fig. 8); median process of prementum short (Figs. 8 and 9); FE bearing both spine-like and hair-like additional setae on ventral surface (Fig. 11); seta TI7 short, spine-like (Fig. 11); natatory anteroventral setae on meso- and metatibia absent (Fig. 10); ventral row of setae on TI and TA formed by setae of different lengths (Fig. 10); urogomphi included in the nonsclerotized ventrodistal area surrounding anus on abdominal segment VIII (Fig. 13), lacking additional pores (Figs. 14 and 15).

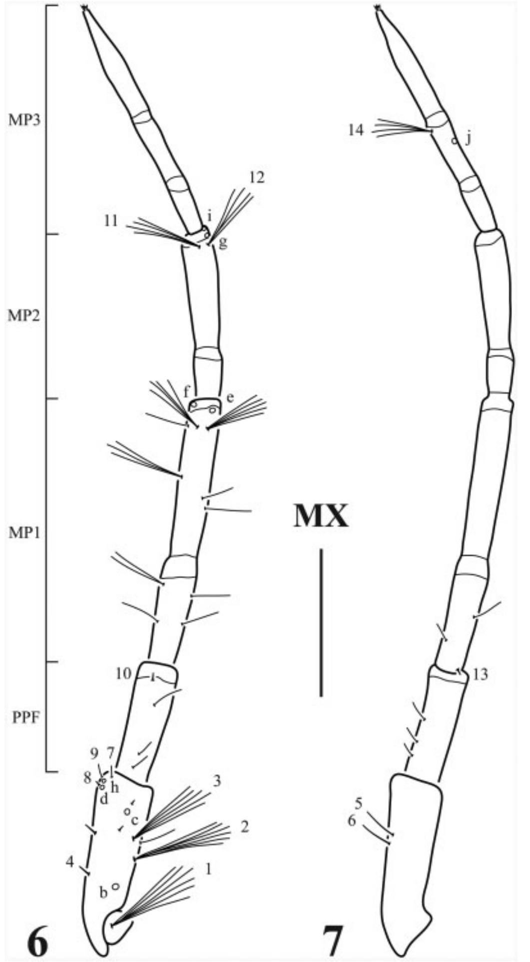
Instar I (Figs. 1–15). *Color.* Cephalic capsule pale yellow except for small, irregular, brown maculae on central portion of FR, on dorsomedial region of PA, and posterior to stemmata (maculae almost absent in some specimens); head appendages pale yellow except for A4 and apices of A3, MP3, and MN light brown; thoracic tergites pale yellow, protergite with some irregular brown maculae on anterolateral surface, meso- and metatergite each with four brown maculae on anterior margin; abdominal tergites I–VII pale yellow, each with four brown maculae on anterior margin (those on tergite VII less evident), abdominal sclerite VIII pale yellow to light brown; membranous parts creamy white; legs pale yellow; U light brown.

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

Head. Cephalic capsule (Figs. 1 and 2). Flattened, subtriangular, longer than broad; maximum width at level of stemmata, slightly constricted at level of occipital region; occipital suture present; ecdysial line well marked, coronal line long; occipital foramen deeply emarginate ventrally; posterior tentorial pits close to well visible ventral midline; FR subtriangular, anterior margin somewhat projected forward, divided into three well developed subtriangular projections: one central, narrow and truncate apically, and two lateral, broad and serrate; anterolateral lobes rounded, not projecting beyond anterior margin; large and rounded egg bursters present laterally close to ecdysial line; four



Figs. 3–5. *Sternhydrus atratus*, instar I. (3) Right antenna, dorsal aspect; (4) Left antenna, ventral aspect; (5) Right mandible, dorsal aspect. SP, spinula. Scale bars = 0.25 mm.

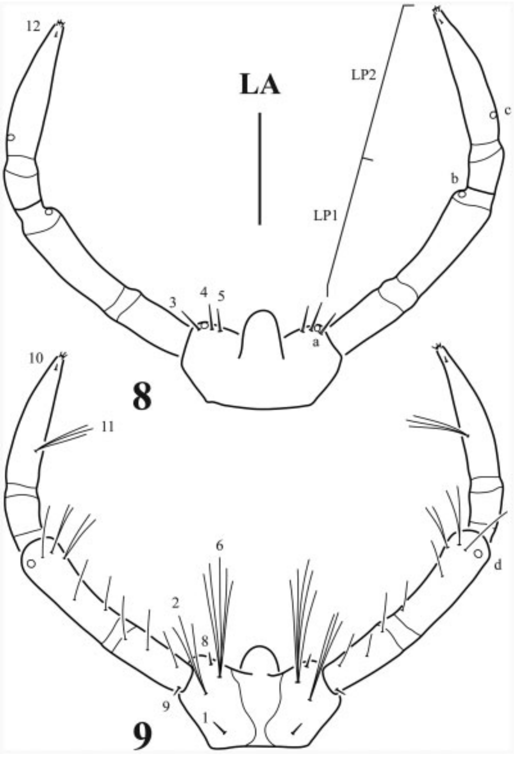


Figs. 6–7. *Sternhydrus atratus*, instar I. (6) Right maxilla, dorsal aspect; (7) Left maxilla, ventral aspect. Scale bar = 0.25 mm.

stemmata on upper side of head and two on underside, arranged in two vertical series. Antenna (Figs. 3 and 4). Elongate, slender, longer than HW, composed of four antennomeres; A1 longest, subdivided into two articles, distal one more than twice longer than basal one; A2 shorter than A1, subdivided into three articles, basal one shortest, the other two subequal; A3 slightly shorter than A2, subdivided into three articles, basal one shortest, distal one longest, bearing a ventroapical spinula; apical lateroventral process of A3 broad, elongate; A4 shortest, with a small lateroventral process on distal third. Mandible (Fig. 5). Strong, falciform, broad at base, acute apically, distal fourth narrow, strongly projected inwards; mandibular channel present. Maxilla (Figs. 6 and 7). Premaxillary lobes strongly developed, projected forward; cardo well developed, subovate; stipes elongate, slender, subcylindrical; galea absent; PPF elongate, palpomere-like; MP elongate, slender, shorter than antenna, composed of three palpomeres, MP1 and MP3 longest, subequal, MP2 shortest; MP1 and MP2 subdivided into two articles, distal one longer than basal one; MP3 subdivided into three articles, basal one shortest, distal one longest. Labium (Figs. 8 and 9). Prementum broader than long, not sclerotized ventromedially; anterior margin broadly emarginate medially, anterodorsal margin projected forward into finger-like median process; LP much shorter than MP, composed of two palpomeres, LP1 slightly longer than LP2; LP1 subdivided into two subequal articles; LP2 subdivided into two articles, distal one about twice longer than basal one.

Thorax. Terga convex, pronotum shorter than subequal meso- and metanotum combined; protergite subrectangular, margins truncate, much more developed than meso- and metatergite; meso- and metatergite small, subrectangular to subtrapezoidal, lacking anterotransverse carina; sagittal line visible on the three tergites; sterna membranous except for two small anterior sclerites on prothorax; spiracles absent. Legs (Figs. 10 and 11). Long, composed of six articles, L1 shortest, L3 longest; CO elongate, TR divided into two parts by an annulus, FE, TI and TA slender, subcylindrical, PT with two long, slender, slightly curved claws, anterior claw slightly longer than posterior claw on L1 and L2, claws subequal on L3; proTA with a row of well-developed ventral spinulae, those on basal third very slender, forming a dense patch (cleaning device).

Abdomen. Eight-segmented; segments I–VI subequal, membranous except for a small anterodorsal sclerite and a minute sclerite on each lateral; tergites I–VI subrectangular, lacking anterotransverse carina, sagittal line well marked, posterior half covered with short spinulae; segment VII narrower, sclerite covering most of dorsal surface, lacking anterotransverse carina, covered



Figs. 8–9. *Sternhydrus atratus*, instar I. (8) Labium, dorsal aspect; (9) Labium, ventral aspect. Scale bar = 0.15 mm.

with short spinulae; spiracles absent on segments I–VII; LAS (Figs. 12 and 13) longest and narrowest, completely sclerotized except ventrodistally around anus, lacking anterotransverse carina, basal third covered with short spinulae; siphon short. Urogomphus (Figs. 14 and 15). Strongly reduced, slightly longer than broad, composed of one urogomphomere.

Chaetotaxy (Figs. 1–15). Similar to that of generalized Cybistrini larva (Alarie et al. 2011) with the following remarks: FR with about 40–50 lamellae clypeales distributed on the apices of the projections, those on medial projection longer than the others; A1 with some additional multifid setae distally and two additional pores proximally; crown of elongate additional (usually multifid) setae on distal fourth of MN absent; MP2 lacking additional setae; seta LA8 present; ventral row on TI and TA formed by setae of different lengths; setae CO7, CO18, TR2, TR3, FE8, FE9, FE10 and pore ABa most likely present but obscured by the presence of additional sensilla; U bearing all ancestral setae, lacking pores; several short additional setae present near urogomphal base.

Instar II. As instar I except for the following features.

Color. Dorsal surface of cephalic capsule covered with numerous small, irregular, brown maculae except on anterior portion of FR and posterior to occipital suture; thoracic tergites (specially protergite) covered with brown maculae except on lateral portions; abdominal tergites I–VI with some brown maculae, abdominal sclerites VII–VIII covered with numerous brown maculae.

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

Head. Occipital constriction more evident; egg bursters absent. Maxilla. MP1 longest.

Thorax. Meso- and metatergite with anterotransverse carina.

Abdomen. Tergites I–VI with anterotransverse carina; sclerites I–VIII lacking spinulae.

Chaetotaxy. FR with about 95–105 lamellae clypeales. Secondary setae most likely present on different body parts, although difficult to evaluate due to large number of additional setae.

Instar III (Fig. 16). As instar II except for the following features.

Color. Somewhat darker in general.

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

Head. (Fig. 16) Antenna. Slightly longer than HW. Labium. LP2 subdivided into two subequal articles.

Thorax. Meso- and metatergite covering most of dorsal surface; well developed spiracles present on mesothorax.

Abdomen. Tergite VII covering the whole dorsal surface; well developed spiracles present on segments I–VII.

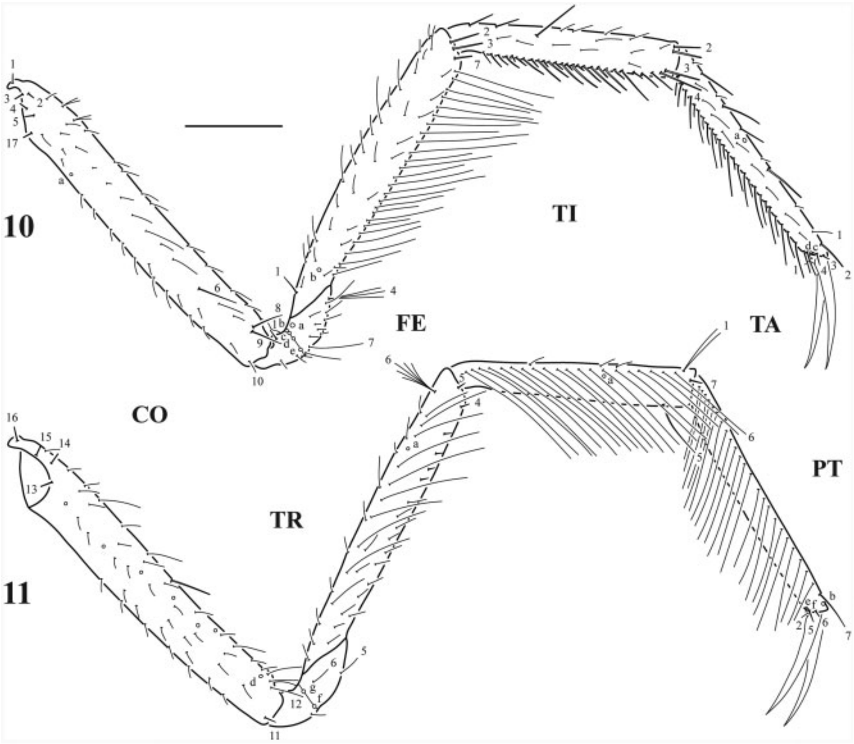
Chaetotaxy. FR with about 140–150 lamellae clypeales.

Character Analysis

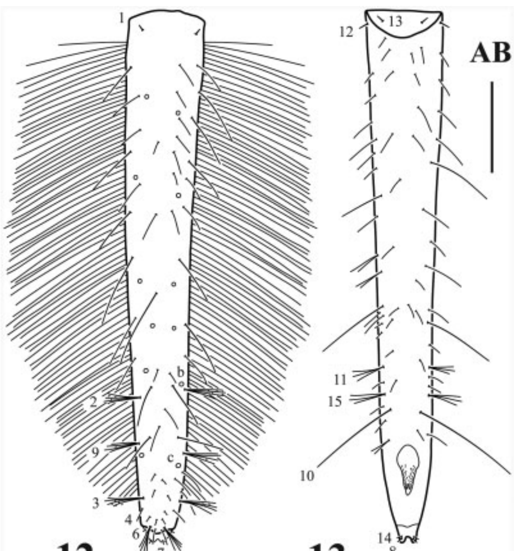
Our sampling resulted in 112 discrete characters that describe the morphology and chaetotaxy of cybistrine larvae. Of these, 85 were coded as binary and 27 as multistate (Table 2). The analysis of the data matrix (Table 3) with TNT resulted in two most parsimonious trees of 195 steps (CI = 0.73; RI = 0.86). These trees differed in the relative position of *Megadytes carcharias* Griffini, 1895, which could be either sister to *Cybister tripunctatus* or in a polytomy along with *Megadytes glaucus* (Brullé, 1837), *Cybister tripunctatus* and the remaining cybistrine genera. The strict consensus was calculated (Fig. 17), in which *Sternhydrus* is resolved as part of a clade of Australian Cybistrini including the genera *Onychohydrus* and *Spencerhydrus*. Within this group, *Sternhydrus* is sister to *Onychohydrus* with robust support (Bremer: 3, Bootstrap: 89). Character state changes are mapped in one of the most parsimonious trees (Fig. 18).

Discussion

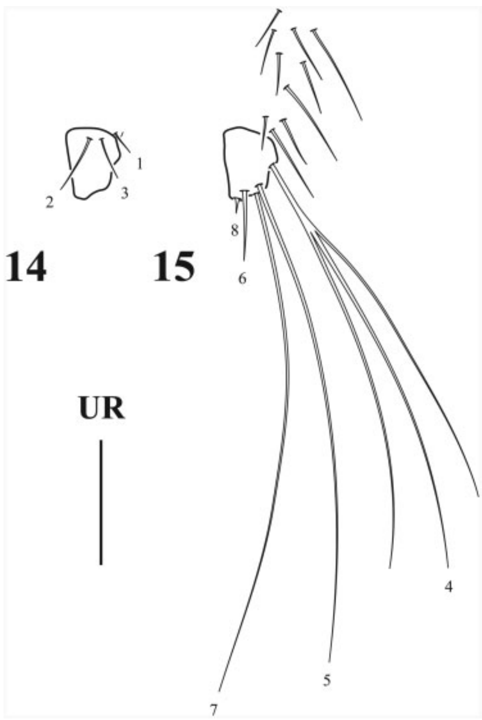
Our study strongly suggests the monophyly of the tribe Cybistrini based on a large number of synapomorphies including anterior margin of frontoclypeus sinuate (character 2.1); antennomeres 2 and 3 subdivided into three articles (characters 22.2, 23.2, and 24.1); premaxillary lobes well developed, projected forward (character 35.1); maxillary palpomere 3 subdivided into



Figs. 10–11. *Sternhydrus atratus*, instar I. (10) Left metathoracic leg, anterior aspect; (11) Right metathoracic leg, posterior aspect. Scale bar = 0.30 mm.



Figs. 12–13. *Sternhydrus atratus*, instar I. (12) Abdominal segment VIII, dorsal aspect; (13) Abdominal segment VIII, ventral aspect. Scale bar = 0.50 mm.



Figs. 14–15. *Sternhydrus atratus*, instar I. (14) Right urogomphus, dorsal aspect; (15) Left urogomphus, ventral aspect. Scale bar = 0.50 mm.

Table 1. Measurements and ratios for the three larval instars of *Sternhydrus atratus*

Measure	Instar I	Instar II	Instar III
TL (mm)	17.00–17.70	24.00–29.20	29.30–34.20
MW (mm)	1.50–1.70	1.90–2.20	2.70–3.40
HL (mm)	1.78–1.80	2.63–2.71	3.53–3.61
HW (mm)	1.18–1.23	1.78–1.83	2.45–2.58
FRL (mm)	0.85–0.89	1.16–1.19	1.40–1.49
OCW (mm)	0.48–0.58	0.85–0.98	1.20–1.35
HL/HW	1.45–1.51	1.48–1.49	1.40–1.47
HW/OCW	2.13–2.58	1.87–2.09	1.91–2.04
COL/HL	0.51–0.52	0.55–0.56	0.59–0.60
FRL/HL	0.48–0.49	0.44–0.45	0.40–0.41
A/HW	1.39–1.46	1.19	1.05–1.08
A2/A1	0.70–0.76	0.64–0.69	0.66–0.68
A3/A1	0.60–0.65	0.52–0.54	0.43–0.50
A4/A3	0.15–0.16	0.12–0.14	0.11–0.12
A3′/A4	0.93–1.00	0.86–1.00	0.86–0.92
MNL/MNW	2.95–3.10	2.79–2.97	2.95–3.05
MNL/HL	0.43–0.44	0.40–0.42	0.41–0.43
PPF/MP1	0.33–0.41	0.43–0.50	0.49–0.56
A/MP	1.43–1.46	1.44–1.48	1.52–1.57
MP1/MP2	1.60–1.70	1.62–1.69	1.75–1.76
MP3/MP2	1.55–1.67	1.38–1.49	1.26–1.34
MP/LP	2.19–2.27	2.06–2.18	2.01–2.05
LP2/LP1	0.93	0.79–0.89	0.71–0.78
L3 (mm)	4.26–4.39	5.75–5.87	7.33–7.81
L3/L1	1.21–1.24	1.20–1.23	1.22–1.24
L3/L2	1.09–1.12	1.10–1.11	1.11–1.13
L3/HW	3.48–3.74	3.19–3.25	2.93–3.04
L3 (CO/FE)	1.03–1.12	1.11–1.13	1.09–1.10
L3 (TI/FE)	0.70–0.74	0.69–0.72	0.70–0.71
L3 (TA/FE)	0.77–0.85	0.67–0.70	0.60–0.65
L3 (CL/TA)	0.44–0.46	0.40–0.41	0.36–0.38
LAS (mm)	2.76–3.00	4.10–4.30	5.53–6.05
LAS/HW	2.26–2.49	2.28–2.36	2.26–2.35
U (mm)	0.02–0.03	0.03	0.02–0.04

Three specimens of each instar were measured.

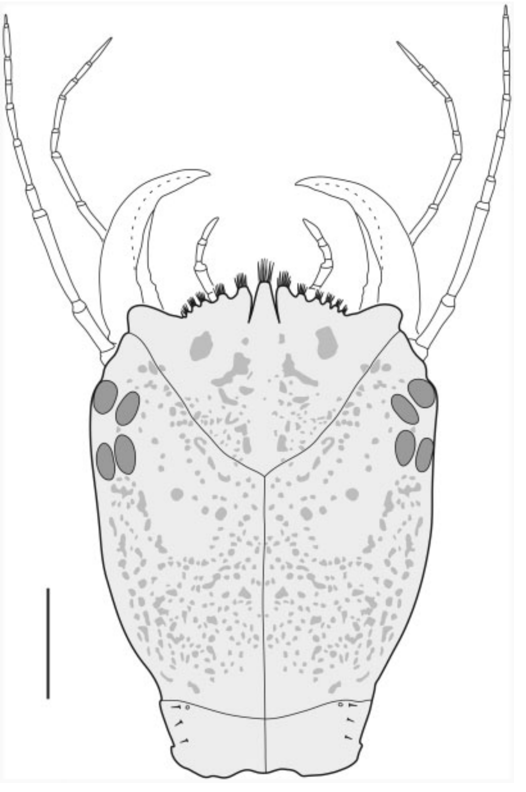


Fig. 16. *Sternhydrus atratus*, instar III. Head, dorsal aspect, showing color pattern on cephalic capsule. Scale bar = 0.80 mm.

three articles (characters 40.2 and 41.1); labial palpomeres 1 and 2 subdivided into two articles in instar I (character 57.1); natatory posterodorsal setae on tarsus present in instar I (character 89.1); and abdominal tergites I–VI reduced (character 93.1) (Figs. 17–18). This result is in line with previous papers based on adult and larval morphology and DNA sequence data (e.g., Miller 2000, Miller et al. 2007, Michat 2010, Alarie et al. 2011).

The inclusion of *Sternhydrus* in a clade of Australian Cybistrini genera along with *Onychohydrus* and *Spencerhydrus* is supported (Fig. 17). Although this clade received moderate support, it is characterized by four synapomorphies: presence of additional pores on antennomere 1 (character 28.1), tibial seta TI7 short and spine-like (character 81.0), urogomphi included in the nonsclerotized ventrodistal area surrounding the anus of abdominal segment VIII (character 97.1), and urogomphus very small but still longer than broad (character 104.1) (Fig. 18). This clade is also characterized by the absence of a crown of multifid setae on the distal fourth of the mandible (character 34.0, shared with members of the subgenus *Trifurcitus* Brinck, 1945), and by the subdivision into two articles of the maxillary palpomere 1 (character 38.1). Our result is consistent with previous findings of Miller (2001), Miller et al. (2007), and Miller and Bergsten (2014) based mainly on adult morphological and molecular characters. The genus *Cybister* Curtis, also present in Australia and represented here by *C. tripunctatus*, appears as more distantly related to the other Australian genera, more closely related to species of the Neotropical genus *Megadytes* Sharp, 1882 (Fig. 17).

Sternhydrus is recovered as sister to *Onychohydrus* with relatively robust support (Fig. 17), also in agreement with Miller and Bergsten (2014) and with the likelihood and Bayesian analyses in Miller et al. (2007). It should be noted, however, that *Austrodytes* was not included in our analysis and therefore the relationships of this genus could not be tested. The clade formed by *Sternhydrus* and *Onychohydrus* is characterized by two synapomorphies: lateral projections of frontoclypeus serrate (character 5.3), and row of additional anteroventral setae on meso- and metatarsus composed of several thin setae of variable length (character 86.2). The first character state was used by Watts (1964) to separate *Sternhydrus* and *Onychohydrus* (then both included under the genus name *Homoeodytes*) from the Australian *Cybister*. Whereas Bertrand (1928, 1934) illustrated some African Cybistrini larvae (most likely *Cybister*) with slightly serrate lateral projections of the frontoclypeus, the serration observed in both *Sternhydrus* and *Onychohydrus* (see Figs. 1 and 16 in

Table 2. Characters and states used for the cladistic analysis

(000)	*Body (instars I–III)*: (0) not gibbous in lateral view; (1) gibbous in lateral view
(001)	*Multifid setae on the body (instar I)*: (0) absent; (1) present
(002)	*Anterior margin of frontoclypeus (instar I)*: (0) rounded; (1) sinuate
(003)	*Medial projection of frontoclypeus (instars I–III)*: (0) absent; (1) simple, sharp apically, bearing few subapical setae; (2) simple, truncate apically, with several apical setae directed forward; (3) bifid, with several apical setae directed forward
(004)	*Lateral projections of frontoclypeus (instars I–III)*: (0) absent; (1) flattened, slightly projected forward; (2) not flattened, well projected forward
(005)	*Lateral projections of frontoclypeus (instars I–III)*: (0) absent; (1) entire; (2) bilobed; (3) serrate
(006)	*Notches between medial and lateral projections of frontoclypeus (instars I–III)*: (0) absent; (1) wide to very wide; (2) very narrow
(007)	*Shape of egg bursters (instar I)*: (0) spiniform; (1) rounded
(008)	*Setae FR3, PA16, PA19 and AB9 (instar I)*: (0) not lanceolate; (1) lanceolate
(009)	*Setae FR9 and FR10 (instar I)*: (0) visible in dorsal view, inserted laterally to lamellae clypeales; (1) not visible in dorsal view, inserted ventrally to lamellae clypeales
(010)	*Pore FRc (instar I)*: (0) not inserted contiguously to frontal suture; (1) inserted contiguously to frontal suture
(011)	*Pore FRe (instar I)*: (0) absent; (1) present
(012)	*Pore FRf (instar I)*: (0) present; (1) absent
(013)	*Additional setae on frontoclypeus (instar I)*: (0) absent; (1) present
(014)	*Lamellae clypeales (instar I)*: (0) stout, rounded, or bluntly pointed; (1) slender, sharp; (2) stout, bifid apically
(015)	*Additional pores on anterior margin of frontoclypeus (instar I)*: (0) absent; (1) present
(016)	*Occipital suture (instar I)*: (0) absent; (1) present
(017)	*Stemmata (instars I–III)*: (0) subequal in size; (1) anterodorsal two strongly developed
(018)	*Additional dorsal setae on parietals (instar I)*: (0) absent; (1) present
(019)	*Additional ventral setae on parietals (instar I)*: (0) absent; (1) a single seta inserted contiguously to seta PA19; (2) numerous
(020)	*Secondary spine-like setae on parietals (instars II–III)*: (0) absent; (1) present
(021)	*Antennomere 1 (instars II–III)*: (0) not subdivided; (1) subdivided into two articles
(022)	*Antennomeres 2 and 3 (instar I)*: (0) not subdivided; (1) subdivided into two articles; (2) subdivided into three articles
(023)	*Antennomeres 2 and 3 (instar II)*: (0) not subdivided; (1) subdivided into two articles; (2) subdivided into three articles
(024)	*Antennomeres 2 and 3 (instar III)*: (0) subdivided into two articles; (1) subdivided into three articles
(025)	*Apical lateroventral process of antennomere 3 (instars I–III)*: (0) protruding (digitiform); (1) protruding (rounded or dentate); (2) not protruding
(026)	*Pore ANi (instar I)*: (0) inserted distally; (1) inserted submedially
(027)	*Additional setae on antennomeres 1 and 2 (instar I)*: (0) absent; (1) present
(028)	*Additional pores on antennomere 1 (instar I)*: (0) absent; (1) present
(029)	*Additional ventroapical pores on antennomere 3 (instar I)*: (0) absent; (1) present
(030)	*Secondary setae on antennomere 2 (instars II–III)*: (0) absent; (1) present
(031)	*Mandible (instars I–III)*: (0) regularly curved and progressively narrowing to apex; (1) distal fourth moderately narrowed, forming weak mesal angle; (2) distal fourth abruptly narrowed, forming distinct mesal angle
(032)	*Seta MN1 (instar I)*: (0) inserted proximally to pore MNc; (1) inserted contiguously to pore MNc; (2) inserted distally to pore MNc
(033)	*Additional setae on mandible (instar I)*: (0) absent; (1) present
(034)	*Crown of multifid setae on distal fourth of mandible (instars I–III)*: (0) absent; (1) present
(035)	*Premaxillary lobes (instars I–III)*: (0) reduced; (1) well developed, projected forward
(036)	*Stipes (instar I)*: (0) narrow, subcylindrical; (1) broad, subtrapezoidal to subtriangular
(037)	*Second galeomere (instars I–III)*: (0) well developed, tronco-conical or subcylindrical; (1) well developed, spiniform; (2) absent, at most a minute bulge
(038)	*Maxillary palpomere 1 (instars I–III)*: (0) not subdivided; (1) subdivided into two articles; (2) subdivided into three articles
(039)	*Maxillary palpomere 2 (instar II)*: (0) not subdivided; (1) subdivided into two articles
(040)	*Maxillary palpomere 3 (instar II)*: (0) not subdivided; (1) subdivided into two articles; (2) subdivided into three articles
(041)	*Maxillary palpomere 3 (instar III)*: (0) subdivided into two articles; (1) subdivided into three articles
(042)	*Lateroventral subapical process of maxillary palpomere 3 (instars I–III)*: (0) absent; (1) present
(043)	*Seta MX6 (instar I)*: (0) present; (1) absent
(044)	*Seta MX8 (instar I)*: (0) inserted subapically on second galeomere; (1) inserted apically on second galeomere; (2) inserted on stipes
(045)	*Seta MX9 (instar I)*: (0) inserted subapically on second galeomere; (1) inserted apically on second galeomere; (2) inserted on stipes
(046)	*Seta MX14 (instar I)*: (0) inserted submedially; (1) inserted at basal third or more basally
(047)	*Pores MXd and MXh (instar I)*: (0) inserted on galea; (1) inserted on stipes
(048)	*Additional setae on stipes (instar I)*: (0) a single seta inserted contiguously to seta MX6; (1) a row of spine-like setae on dorsal surface; (2) several hair-like setae on ventral surface
(049)	*Additional setae on palpifer and maxillary palpomere 1 (instar I)*: (0) absent; (1) present
(050)	*Additional setae on maxillary palpomere 2 (instar I)*: (0) absent; (1) present
(051)	*Additional setae on maxillary palpomere 3 (instar I)*: (0) absent; (1) present
(052)	*Row of long hair-like secondary setae along external margin of stipes (instar III)*: (0) absent; (1) present
(053)	*Secondary setae on maxillary palpomere 1 (instars II–III)*: (0) absent; (1) present
(054)	*Prementum (instar III)*: (0) completely sclerotized ventrally; (1) not sclerotized ventromedially
(055)	*Anterior margin of prementum (instar I)*: (0) straight to slightly emarginate; (1) deeply and widely emarginate; (2) projected forward
(056)	*Median process of prementum (instars I–III)*: (0) absent; (1) single, short; (2) single, strongly developed, covering prementum in dorsal view; (3) bifid from the base, as two separate lobes
(057)	*Labial palpomeres 1 and 2 (instar I)*: (0) not subdivided; (1) subdivided into two articles
(058)	*Labial palpomeres 1 and 2 (instar III)*: (0) not subdivided; (1) subdivided into two articles
(059)	*Seta LA8 (instar I)*: (0) absent; (1) present
(060)	*Additional setae on dorsal surface of prementum (instar I)*: (0) absent; (1) present
(061)	*Additional pore on ventral surface of prementum (instar I)*: (0) absent; (1) present
(062)	*Additional setae on labial palpomere 1 (instar I)*: (0) absent; (1) present
(063)	*Additional setae on labial palpomere 2 (instar I)*: (0) absent; (1) present
(064)	*Secondary setae on prementum (instars II–III)*: (0) absent; (1) present

(*continued*)

Table 2. Continued

(065)	*Setae on median process of prementum (instars I–III)*: (0) absent; (1) present
(066)	*Ventral sclerites on prothorax (instar III)*: (0) one; (1) two
(067)	*Seta CO7 on meso- and metacoxa (instar I)*: (0) inserted submedially to distally; (1) inserted proximally
(068)	*Additional setae on coxa (instar I)*: (0) absent; (1) present
(069)	*Additional pores on anterodorsal surface of coxa (instar I)*: (0) absent; (1) present
(070)	*Additional pores on posterior surface of coxa (instar I)*: (0) absent; (1) present
(071)	*Seta TR2 (instar I)*: (0) present; (1) absent
(072)	*Additional setae on trochanter (instar I)*: (0) absent; (1) present
(073)	*Seta FE4 (instar I)*: (0) present; (1) absent
(074)	*Seta FE5 on meso- and metafemur (instar I)*: (0) present; (1) absent
(075)	*Seta FE6 (instar I)*: (0) present; (1) absent
(076)	*Additional dorsal setae on femur (instar I)*: (0) absent; (1) present
(077)	*Additional ventral setae on femur (excluding natatory setae) (instar I)*: (0) absent; (1) spine-like; (2) hair-like; (3) both
(078)	*Additional pores on posterodorsal surface of femur (instar I)*: (0) absent; (1) present
(079)	*Seta TI5 (instar I)*: (0) spine-like; (1) hair-like
(080)	*Seta TI5 on metatibia (instar I)*: (0) inserted distally; (1) inserted more proximally
(081)	*Seta TI7 (instar I)*: (0) short, spine-like; (1) long, hair-like
(082)	*Additional anterodorsal setae on tibia (instar I)*: (0) absent; (1) present
(083)	*Additional anteroventral setae on tibia (instar I)*: (0) absent; (1) stout, subequal in length; (2) thin, variable in length
(084)	*Additional pores on posterior surface of tibia (instar I)*: (0) absent; (1) present
(085)	*Pore TAa (instar I)*: (0) inserted submedially to distally; (1) inserted proximally
(086)	*Additional anteroventral setae on meso- and metatarsus (instar I)*: (0) absent; (1) several stout setae subequal in length; (2) several thin setae variable in length
(087)	*Natatory anteroventral setae on protarsus (instars II–III)*: (0) absent; (1) present
(088)	*Natatory anteroventral setae on meso- and metatarsus (instars II–III)*: (0) absent; (1) present
(089)	*Natatory posterodorsal setae on tarsus (instar I)*: (0) absent; (1) present
(090)	*Basoventral patch of dense slender spinulae on protarsus (instars II–III)*: (0) absent; (1) present
(091)	*Setae PT1 and PT2 (instar I)*: (0) present; (1) absent
(092)	*Basoventral spinulae on claws (instars I–III)*: (0) absent; (1) present
(093)	*Abdominal tergites I–VI (instars I–III)*: (0) well developed; (1) reduced
(094)	*Abdominal tergites I–VI (instar I)*: (0) with anterotransverse carina; (1) without anterotransverse carina
(095)	*Abdominal segment VII (instar I)*: (0) sclerotized dorsally, membranous ventrally; (1) completely sclerotized except for a narrow longitudinal ventral band; (2) completely sclerotized, ring-like
(096)	*Abdominal segment VII (instar III)*: (0) sclerotized dorsally, membranous ventrally; (1) completely sclerotized, ring-like
(097)	*Nonsclerotized ventrodistal area surrounding the anus on abdominal segment VIII (instars I–III)*: (0) absent; (1) present, including urogomphi; (2) present, not including urogomphi
(098)	*Setae AB10 and AB11 (instar I)*: (0) inserted in a transversal row; (1) inserted in a longitudinal row
(099)	*Setae AB11 and AB15 (instar I)*: (0) inserted far from ventroapical margin; (1) inserted contiguously to ventroapical margin
(100)	*Pore ABc (instar I)*: (0) present; (1) absent
(101)	*Additional setae on abdominal segment VIII (excluding natatory setae) (instar I)*: (0) absent; (1) present
(102)	*Additional pores on dorsal surface of abdominal segment VIII (instar I)*: (0) absent; (1) present
(103)	*Natatory setae on lateral margin of abdominal segment VII (instar I)*: (0) absent; (1) present
(104)	*Urogomphus (instars I–III)*: (0) well developed, much longer than broad; (1) very small, still longer than broad; (2) minute, rounded
(105)	*Number of primary setae on urogomphus (excluding natatory setae) (instar I)*: (0) eight; (1) seven; (2) six
(106)	*Number of primary pores on urogomphus (instar I)*: (0) three or more; (1) one; (2) zero
(107)	*Seta UR5 (instar I)*: (0) long, hair-like; (1) short, spine-like
(108)	*Seta UR6 (instar I)*: (0) long, hair-like; (1) short, spine-like
(109)	*Pore URc (instar I)*: (0) present; (1) absent
(110)	*Additional pores on urogomphus (instar I)*: (0) absent; (1) present
(111)	*Row of natatory setae on urogomphus (instars I–III)*: (0) absent; (1) present

this paper and Fig. 13 in Alarie et al. 2011) is much more distinct, and the shape of the lateral projections differs from those in the African species. For this reason, we think it remains a good character to separate *Sternhydrus* and *Onychohydrus* from other known Cybistrini genera. Other characters provided by Watts (1964), such as the absence of a distal crown of multifid setae on the mandible, and the urogomphi not that reduced and placed much farther forward on the abdominal segment VIII, are proven here to be present also in the genus *Spencerhydrus*, therefore giving information at a more inclusive taxonomic level (see above). Additional characters supporting a close relationship of *Sternhydrus* and *Onychohydrus* are the presence of very narrow notches between the medial and lateral projections of the frontoclypeus (character 6.2, shared with *Cybister tripunctatus* and *Megadytes carcharias*), and the absence of additional setae on the maxillary palpomere 2 (character 50.0).

The genus *Sternhydrus* exhibits two larval autapomorphies in our study: the presence of both spine-like and hair-like additional setae on the ventral surface of the femur (character 77.3), and the absence of primary pores on the urogomphus (character 106.2). It is worth stressing, however, that the presence of urogomphal pores is very difficult to assess owing to the small size of that structure within Cybistrini. *Sternhydrus* is also characteristic among the Australian genera in having rounded egg bursters (character 7.1, shared with some *Cybister* and *Megadytes* species). Miller et al. (2007) elevated *Sternhydrus* to generic rank, calling in question the prominent structural differences between the adults of this taxon, *Austrodytes*, and *Onychohydrus* s.str. These authors, however,

Table 3. Data matrix used for the cladistic analysis

Species	Character										
	00000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000001	11111111111
	00000000001	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667	7777777778	8888888889	9999999990	00000000011
	01234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	12345678901
Thermonectus succinctus	10000000110	0000001001	0000100000	0100011000	0111010100	1100210011	0011100000	1011100011	1000101100	1000110101	00000011000
Notaticus fasciatus	11000000000	0100000011	0010010000	0000001011	0000000000	0000000000	0000001000	0011000000	1000000001	0000210100	00100000000
Dytiscus harrisii	00000001001	1001100010	1110210010	0200000111	0001100000	1010000100	0011001011	0010011110	1111001100	0000000010	01100000011
Hyderodes shuckardi	00000000001	1000110011	1110010010	0200000011	0001110000	1010000111	001?011110	0100011110	1111000101	0100110010	11100000011
Eretes australis	10000000110	0102001011	0000200010	0200011000	0110110100	1110210010	0011101000	1011000011	1000101100	1000110101	00000010000
Hydaticus tuyuensis	00000000000	0000010011	0010010000	0000000011	0001100000	1000230010	0011100000	0011100010	1000000001	0100110100	00100000000
Cybister (Melanectes) sugillatus	01122110000	0011010120	1221011001	2111102212	1002201211	0011111110	0100011101	0100012000	1110010011	0011002100	1112?1??100
Cybister (s.str.) tripunctatus	01122121000	0011010120	1221011001	2111102212	1002201211	0011111110	0100011101	0100012000	1110010011	0011002100	111201??100
Megadytes (s.str.) carcharias	01122121000	0011010120	1221011011	2111102212	1002201211	0011111100	0100011101	0100012000	1110010011	0011002100	111221??100
Megadytes (Paramegadytes) glaucus	01122211000	0011010120	1221011011	2111102212	1002201211	0011111110	0100011101	0100012000	1110010011	0011002100	111211??100
Megadytes (Trifurcitus) fallax	01111111000	0011010120	1221011011	0?10102212	1002201211	0011111110	0100011101	0100012000	1110010011	0011002100	111211??100
Megadytes (Trifurcitus) robustus §	0111111?000	0010010120	1221011011	0?10102212	1002201211	0011111110	0100011101	0100012000	1110010011	0011012100	111211??100
Onychohydrus scutellaris	01122320000	0011010120	1221011101	2?10102112	1002201210	0011111110	0100011101	0100011000	0120020011	0011001100	11110101100
Spencerhydrus latecinctus	01132110000	0011010120	1221011101	1110102112	1002201211	0011?21111	1100001101	0100012000	0120010011	0111001100	11110100100
Spencerhydrus pulchellus	01132110000	0011010120	1221011101	1110102112	1002201211	0011?21111	1100001101	0100012000	0120010011	0111001100	11110100100
Sternhydrus atratus	01122321000	0011010120	1221011101	2110102112	1002201210	0011111110	0100011101	0100013000	0120020011	0011001100	11110201100

The taxon marked with "§" was studied only as instar III.

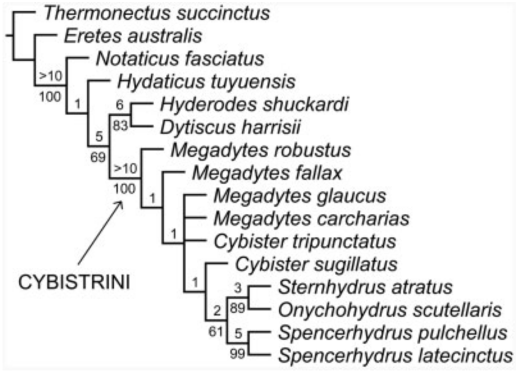


Fig. 17. Strict consensus cladogram obtained from the cladistic analysis of 16 terminal taxa of Dytiscinae, with Bremer support values indicated above branches and Bootstrap values above 50 indicated below branches.

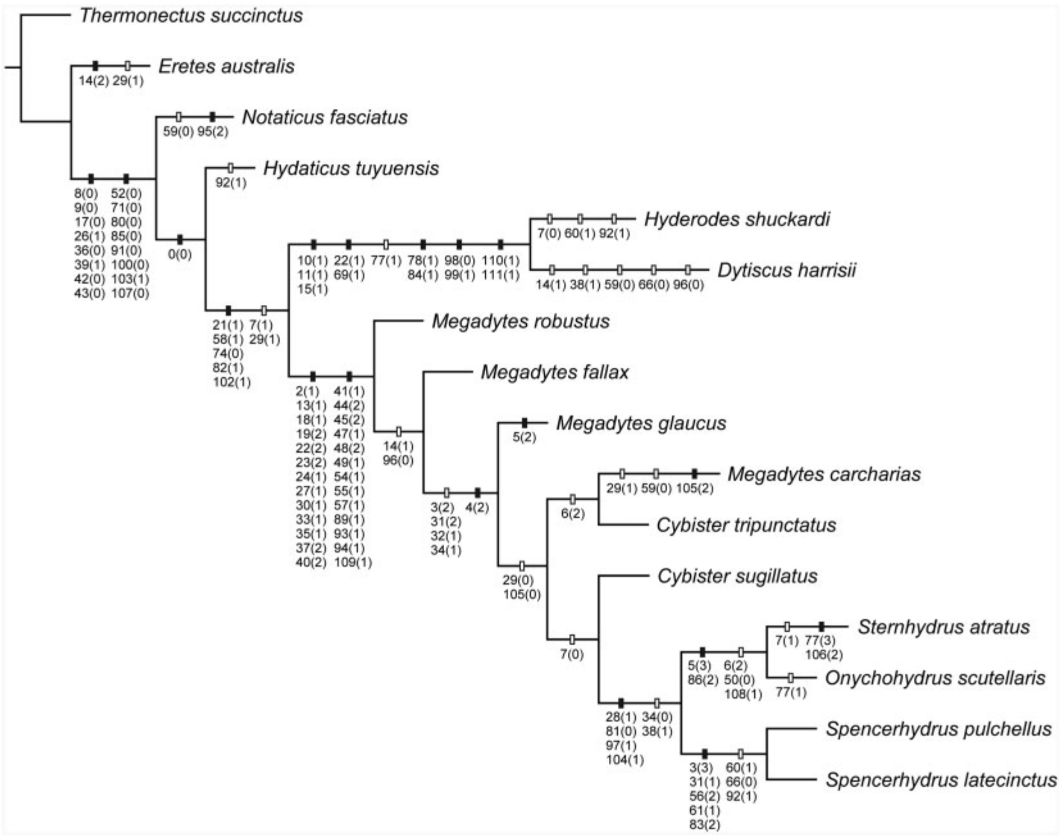


Fig. 18. One of the most parsimonious trees obtained from the cladistic analysis of 16 terminal taxa of Dytiscinae, with selected character changes mapped for each clade (using ACCTRAN optimization). Solid rectangles indicate unique character state transformations; open rectangles indicate homoplasious character state transformations.

recognized the possibility that the three names can be placed as subgenera within *Onychohydrus* s. lat., as suggested by some of their analyses. In absence of larvae of *Austrodytes*, our study does not provide a proper context within which the above mentioned hypotheses can be tested. Whereas the larvae of *Sternhydrus* and *Onychohydrus* share several morphological features (see above) that suggest a close relationship, they also exhibit some differences. Whereas a great effort was placed on obtaining as many taxa and morphological characters as possible for our analysis, a more comprehensive sampling is needed to further resolve the relationships of *Sternhydrus* within the Cybistrini. In particular, the inclusion of larvae of *Austrodytes* would be of the utmost interest. In its present form, our study presents a preliminary framework to revisit the phylogenetic relationships among the Australian cybistrine genera based on larval characters, and provides possible synapomorphies for further investigation.

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