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# Phylogeny and classification of diving beetles in the tribe Cybistrini (Coleoptera, Dytiscidae, Dytiscinae)

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Miller, K. B., Bergsten, J. & Whiting, M. F. (2007). Phylogeny and classification of diving beetles in the tribe Cybistrini (Coleoptera, Dytiscidae, Dytiscinae). — *Zoologica Scripta*, 36, 41–59. Phylogenetic relationships among members of the diving beetle tribe Cybistrini (Coleoptera: Dytiscidae) were inferred from analysis of 47 adult and larval morphological characters and sequences from portions of the genes *cytochrome oxidase I* (COI) and *II* (COII), *histone III* (H3) and *wingless*. Thirty-three species of Cybistrini were included, representing all genus-groups except *Regimbartina* Chatanay and *Megadytes* (*Bifurcitus*) Brinck, and most historically recognized species groups and subgenera used in the tribe. Outgroups include six species from other tribes within Dytiscinae and Lancetinae. Analyses included parsimony analysis of the combined data, likelihood analysis of combined molecular data and partitioned Bayesian analysis of the combined data. Results indicate that Cybistrini is well supported as a monophyletic group. Within the tribe, all currently recognized genus groups were found to be monophyletic with the exception of *Onychobydrus* Schaum, which is paraphyletic with respect to *Austrodytes* Watts in the parsimony analysis, but monophyletic in the likelihood and Bayesian analyses, and *Cybister sensu stricto*, which is paraphyletic with respect to *C. (Melanectes)* Brinck and *C. (Scaphinectes)* Ádám in the parsimony analysis or only the latter in the likelihood and Bayesian analyses. Results also suggest that some, but not all, historically recognized species groups or subgenera in the large genus *Cybister* Curtis are monophyletic, and this is discussed and compared. To improve the classification, the name *Sternhydrus* Brinck is elevated from subgenus to genus rank (**new status**). Four subgenera in the genus *Cybister* are recognized: *C. (Melanectes)* Brinck, *C. (Megadytoides)* Brinck (**resurrected**), *C. (Neocybister)* Miller, Bergsten and Whiting (**new subgenus**) and *C. (Cybister)* Curtis. The following new synonyms are established: *Trochalus* Dejean (**new synonym**), and *Scaphinectes* Ádám = *Cybister* (*Cybister*) (**new synonym**). The Neotropical species *Cybister parvus* Trémouilles (not examined) apparently does not fit any historical or currently recognized genus-group diagnosis in Cybistrini, so it is retained in *Cybister* but *incertae sedis* with respect to subgenus. In addition to classification, the evolution of the unique character combinations present in cybistrines are discussed. A key to the adults of genera and subgenera is presented.

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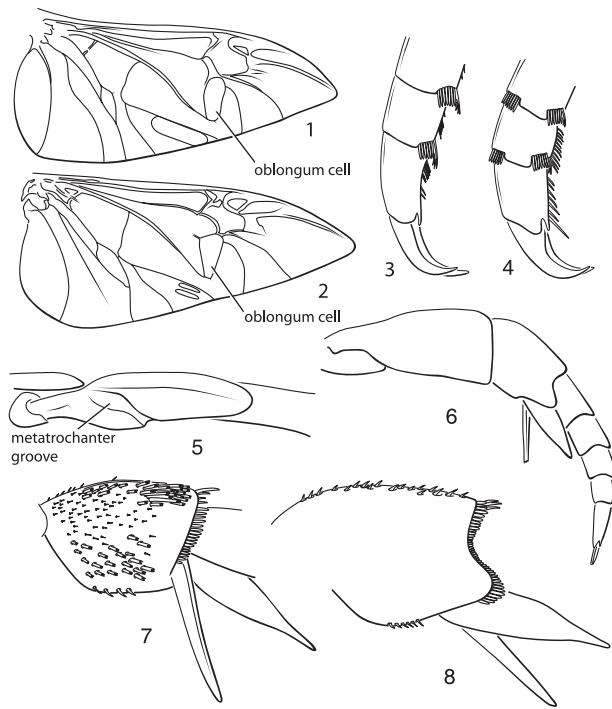
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## Introduction

Members of the tribe Cybistrini (Dytiscidae: Dytiscinae) are conspicuous components of the water beetle fauna throughout low latitudes of the world. Among the 135 included species (Nilsson 2001) are many exceptionally large aquatic beetles. For example, several South American species such as *Megadytes giganteus* (Laporte), with a name reflecting its remarkable size, approach 50 mm in length as adults and 90 mm as larvae.

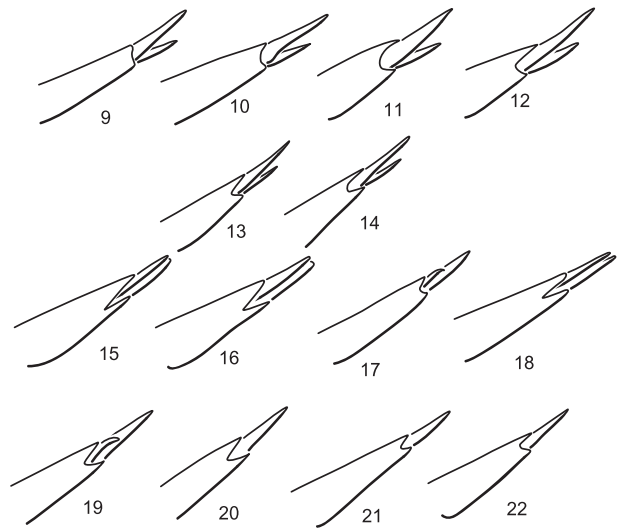
The only aquatic beetles that approach the size of large Cybistrini are members of the related dytiscine genus *Dytiscus* Linnaeus and species of Hydrophilinae (*Hydrophilus* Müller). Although not small by comparison to other Dytiscidae, Cybistrini also includes more modest sized species, with several only 12–14 mm in length. Most cybistrines occur in lentic habitats with extensive emergent vegetation, though some are common in turbid pools or those with mineral substrates.



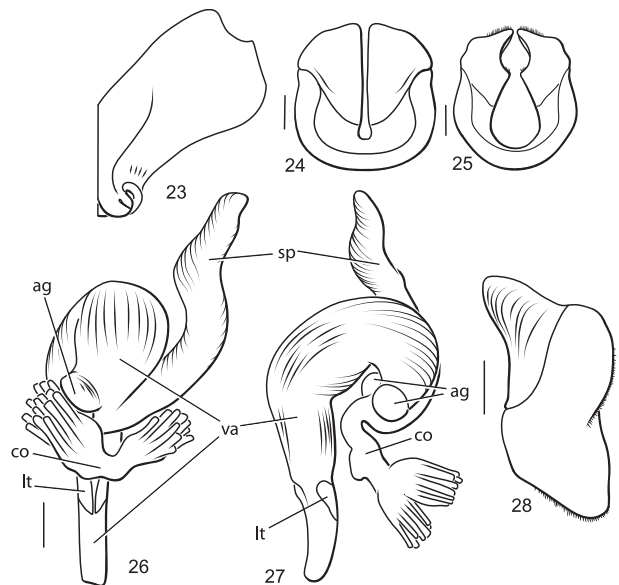
**Figs 1–8** Figs 1, 2, Right metathoracic wing: —1. *Dytiscus marginalis*; —2. *Cybister* (*Cybister*) *tripunctatus*. Figs 3, 4. Cybistrini species, left mesotarsomeres III–V, posterior aspect: —3. *Megadytes* (*Megadytes*) *fraternus*; —4. *Cybister* (*Cybister*) *tripunctatus*. Fig. 5. *Megadytes* (*Trifurcitus*) *robustus*, left metatrochanter and portions of metacoxa and metafemur, ventral aspect, showing oblique metatrochanteric groove. Fig. 6. *Megadytes* (*Trifurcitus*) *robustus*, left metaleg, anterior aspect. Fig. 7. *Onychobydrus scutellaris*, right metatibia, posterior aspect. Fig. 8. *Cybister* (*Cybister*) *tripunctatus*, left metatibia, anterior aspect.

Larvae feed on other large insects, small fish and anuran larvae. Adults feed on similar prey items or scavenge on freshly dead animals.

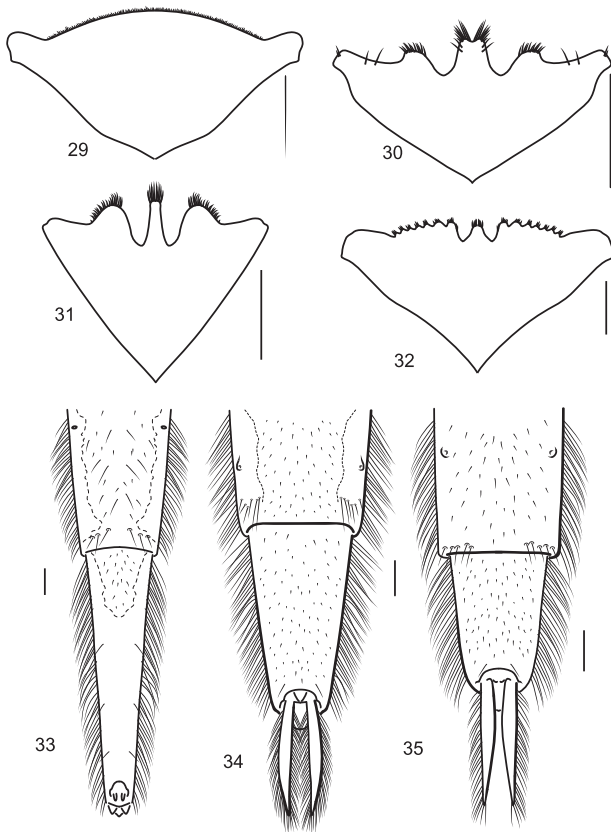
Cybistrini is one of the most well defined groups in all of Dytiscidae. The tribe exhibits numerous unambiguous adult and larval synapomorphies including: (adults): (1) metafemur and metatibia very broad and short (Figs 6–8); (2) metatibial spurs different in size and shape, with the anterior spur acuminate and broader than the posterior one (Figs 6–8); (3) posteroapical cluster of bifid setae on the metatibia (Fig. 7); (4) cluster of stiff apicoventral elytral setae; (5) female with two glands near the base of the common oviduct (Figs 26, 27); (6) female with extensive muscles surrounding the vagina (Figs 26, 27); (7) male with adhesive setae on mesotarsomeres apically simple (when present); (8) natatory setae present along the dorsal margin of metafemur, (larvae); (9) anterior margin of the clypeus prominently dentate (Figs 30–32); (10) abdominal tergites reduced (though see Discussion of *Megadytes* below); (11) egg bursters absent in instar I (Larson



**Figs 9–22** Cybistrini species, left metatarsomere V and metatarsal claws. Fig. 9. *Spencerbydrus latecinctus*. Fig. 10. *Onychobydrus scutellaris*. Figs 11, 12. *Austrodytes plateni*: —11. female; —12. male. Figs 13, 14. *Sternbydrus atratus*: —13. female; —14. male. Figs 15, 16. *Megadytes* (*Trifurcitus*) *robustus*: —15. female; —16. male. Figs 17, 18. *Megadytes* (*Megadytes*) *fraternus*: —17. female; —18. male. Figs 19, 20. *Cybister* (*Megadytoides*) *marginicollis*: —19. female; —20. male. Figs 21, 22. *Cybister* (*Cybister*) *gschwendtneri*: —21. female; —22. male.



**Figs 23–28** Fig. 23. *Cybister* (*Cybister*) *fimbriolatus*, left metacoxa, ventral aspect showing stridulatory device. Figs 24, 25. Male sternum VIII, ventral aspect: —24. *Megadytes* (*Megadytes*) *fraternus*; —25. *Cybister* (*Cybister*) *tripunctatus*. Figs 26, 27. *Megadytes* (*Megadytes*) *marginithorax*, female internal genitalia, uncleared: —26. ventral aspect; —27. left lateral aspect. Abbreviations: ag, accessory gland; co, common oviduct; va, vagina; sp, spermatheca; lt, laterotergite. Fig. 28. *Megadytes* (*Paramegadytes*) *glaucus*, right gonocoxosternite, ventral aspect. Scale bars = 1 mm.



**Figs 29–35** Figs 29–32. Dytiscinae species, larval clypeus, instar III: —29. *Hyderodes shuckardi*; —30. *Spencerhydrus latecinctus*; —31. *Onychobydrus scutellaris*; —32. *Cybister (Cybister) tripunctatus*. Figs 33–35. Dytiscinae species, larval abdominal segments VII and VIII and cerci, ventral aspect: —33. *Cybister (Cybister) tripunctatus*; —34. *Dytiscus marginalis*; —35. *Hyderodes shuckardi*. Scale bars = 1 mm.

*et al.* 2000) (though see Discussion of *Megadytes* below); (12) anterior margin of the prementum with a distinct lobe without spinous setae; (13) antennae, maxillary palpi and labial palpi subdivided in all instars, and (14) cerci very short or absent (Fig. 33).

Cybistrini is the sister-group to a clade containing the rest of the Dytiscinae (Miller 2000, 2003). The phylogeny within the tribe has received little attention, though the classification has been substantially modified over the years. The species classification remains in a poor state, partly because many of the species are relatively rare inhabitants of remote tropical regions. Prior to this analysis there were 12 recognized genus groups (six genera and several genera with subgenera). Although some of these groups appear clearly monophyletic, others may not be, and relationships between the genus groups have not been comprehensively investigated.

The earliest comprehensive work on the group was by Sharp (1882) who erected the tribe Cybistrini to include four

genera (*Spencerhydrus* Sharp, *Homoeodytes* Régimbart (= *Onychobydrus* Schaum), *Megadytes* Sharp and *Cybister* Curtis) and about 72 species. He based the grouping on a number of characters, many of which remain unambiguously characteristic of the tribe as currently recognized (see above). He found particularly compelling the dramatic development of the cybistrine swimming legs, writing: ‘It is in this group of the Dytiscidae that the swimming legs attain their greatest and most admirable development ...’ (Sharp 1882: 943). Cybistrines are among the aquatic beetles best adapted for strong, swift swimming as adults.

The next major contribution to cybistrine literature was by Wilke (1920) who placed all species under the genus name *Cybister*, with the subgenera *Spencerhydrus*, *Onychobydrus*, *Megadytes*, *Régimbartina* and *Cybister*. The most extensive contribution, however, to cybistrine taxonomy was by Brinck (1945), who erected no less than nine new genus group names within Cybistrini. These were largely applied to *Cybister* as subgenera. Most of these genus groups were based on informal groups established by Sharp (1882) and Zimmermann (1919). Brinck’s (1945) classification was not generally followed, and the most recent classification of *Cybister* (Nilsson 2001; Nilsson *et al.* 1989) recognized only three subgenera, *Cybister s.s.*, *Melanectes* Brinck and *Scaphinectes* Ádám. This classification (species content of subgenera) is essentially the one established by Guignot (1961), though nomenclatural rules have resulted in some changes to which name applies to which group (Nilsson *et al.* 1989). Informal groups of species within the *Cybister* of Africa (where the greatest diversity in the genus resides) were established by Guignot (1961) and Omer-Cooper (1967). Within the broader Cybistrini, the most recent contribution to classification of the genera includes recognition of the new taxa *Austrodytes* Watts, 1978, and *Megadytes (Paramegadytes)* Trémouilles & Bachmann, 1980. Finally, a recent study by Michat (in press) presents a review of the several distinctive larval characters in the tribe in comparison with other members of Dytiscinae, first descriptions of larvae of several *Megadytes*, and an analysis testing the monophyly of *Megadytes* based on larval characters.

Because of their large size and conspicuous presence, cybistrines have been the subjects of an exceptional number of studies of histology, chemistry, ecology and behaviour. Their phylogeny has received less attention, however. The goals of this project are to present a phylogenetic analysis of the tribe to test the current classification of the genus groups and relationships among them and to improve the classification based on its phylogeny.

## Materials and methods

### Taxon sampling

*Ingroup.* Thirty-three species of Cybistrini were included in this analysis (see Table 1), including members of each currently recognized genus group except *Régimbartina* and

**Table 1** Taxa used in analysis including locality data, UNM voucher numbers and GenBank accession numbers for DNA sequences.

| Tribe      | Genus                                 | Species              | Collection data  | UNM voucher # | COI      | COII     | H3       | Wnt      |
|------------|---------------------------------------|----------------------|--|---------------|----------|----------|----------|----------|
| Lancetini  | <i>Lancetes</i>                       | <i>lanceolatus</i>   | AUSTRALIA: Vic: Sheepwash Lagoon 10 km E Yea, 37°10'16"S 145°31'44"E, 08 Nov 2000, KB Miller     | KBMLcla91     | DQ813695 | DQ813797 | DQ813761 | AF392032 |
| Lancetini  | <i>Lancetes</i>                       | <i>varius</i>        | CHILE: Rio Hollenberg, 25 km S Puerto Natales, 14 Nov 2001, L Ström                              | KBMLcva196    | DQ813696 | DQ813798 | DQ813762 | DQ813726 |
| Dytiscini  | <i>Dytiscus</i>                       | <i>marginalis</i>    | SWEDEN: Västerbotten, Täfteåhalvön, 31 May 2003, J Bergsten                                      | KBMDymg168    | DQ813691 | DQ813793 | DQ813757 | DQ813725 |
| Dytiscini  | <i>Dytiscus</i>                       | <i>verticalis</i>    | USA: New York: Tompkins Co., Ithaca, 26 May 2000, KB Miller                                      | KBMDyve24     | DQ813692 | DQ813794 | DQ813758 | AF392012 |
| Hyderodini | <i>Hyderodes</i>                      | <i>shuckardi</i>     | AUSTRALIA: Vic: roadside pool ~20 km W Cowwarr, 38°00'52"S 146°32'03"E, 07 Nov 2000, KB Miller   | KBMHdsh104    | DQ813694 | DQ813796 | DQ813760 | AF392018 |
| Aciliini   | <i>Graphoderus</i>                    | <i>liberus</i>       | USA: New York: Tompkins Co., Ringwood Pres., 19 Sep 2000, KB Miller                              | KBMGrl69      | DQ813693 | DQ813795 | DQ813759 | AF392016 |
| Cybistrini | <i>Austrodytes</i>                    | <i>plateni</i>       | AUSTRALIA: WA: Weano Gorge, Karrigini NP Pibarrez, Aug 2002, R Leys                              | KBMAupl237    | DQ813668 |          |          | DQ813707 |
| Cybistrini | <i>Sternhydrus</i>                    | <i>atratus</i>       | AUSTRALIA: QL: Townsville, 27–30 Nov 2001, G. Svenson  | KBMStat165    | DQ813703 | DQ813804 | DQ813769 | DQ813732 |
| Cybistrini | <i>Onychohydrus</i>                   | <i>scutellaris</i>   | AUSTRALIA: Vic: marsh 18.6 km W Casterton, 37°3 5'57"S 141°09'45"E, 11 Nov 2000, KB Miller       | KBMOncs107    | DQ813704 | DQ813805 | DQ813770 | DQ813733 |
| Cybistrini | <i>Spencerhydrus</i>                  | <i>latecinctus</i>   | AUSTRALIA: Vic: 25km W Casterton, 6 Oct 2000, CHS Watts  | KBMSpla123    | DQ813705 | DQ813806 | DQ813771 | AF392043 |
| Cybistrini | <i>Spencerhydrus</i>                  | <i>pulchellus</i>    | AUSTRALIA: WA: Byenup Lagoon, Oct 2000, CHS Watts  | KBMSppu108    | DQ813706 | DQ813807 | DQ813772 | DQ813734 |
| Cybistrini | <i>Megadytes</i> ( <i>Megadytes</i> ) | <i>carcharias</i>    | PERU: Madre de Dios: boat landing ~20 km S Infierno, nr Puerto Maldonado, 14 Dec 2003, KB Miller | KBMMeca255    | DQ813697 | DQ813799 | DQ813763 | DQ813727 |
| Cybistrini | <i>M. (Megadytes)</i>                 | <i>fraternus</i>     | PARAGUAY: Dpt. Alto Paraguay: Ea. Choroveca, 29 Nov 2002   | KBMMefr300    | DQ813698 | DQ813800 | DQ813764 | DQ813728 |
| Cybistrini | <i>M. (Megadytes)</i>                 | <i>laevigatus</i>    | BOLIVIA: Sta Cruz Prov.: San Ignacio, 13 Jan 2004, G Svenson                                     | KBMMela352    | DQ813700 | DQ813802 | DQ813766 | —        |
| Cybistrini | <i>M. (Megadytes)</i>                 | <i>marginithorax</i> | PERU: Madre de Dios: boat landing ~20 km S Infierno, nr Puerto Maldonado, 14 Dec 2003, KB Miller | KBMMema266    | DQ813701 | —        | DQ813767 | DQ813730 |
| Cybistrini | <i>M. (Paramegadytes)</i>             | <i>glaucus</i>       | ARGENTINA: Buenos Aires, Mar 2002  | KBMMegl305    | DQ813699 | DQ813801 | DQ813765 | DQ813729 |
| Cybistrini | <i>M. (Trifurcitus)</i>               | <i>robustus</i>      | PERU: Madre de Dios: boat landing ~20 km S Infierno, nr Puerto Maldonado, 14 Dec 2003, KB Miller | KBMMero254    | DQ813702 | DQ813803 | DQ813768 | DQ813731 |
| Cybistrini | <i>Cybister</i> ( <i>Cybister</i> )   | <i>cardoni</i>       | INDIA: Karnataka, Siddapur, 14°20,307'N 74°5 2.881'E, 07 Oct 2004, KB Miller                     | KBMCycd329    | DQ813671 | DQ813774 | DQ813737 | DQ813710 |
| Cybistrini | <i>C. (Cybister)</i>                  | <i>cinctus</i>       | MADAGASCAR: Mangily, 12 Jan 2005, J. Bergsten  | KBMCyct394    | DQ813674 | DQ813777 | DQ813740 | DQ813713 |
| Cybistrini | <i>C. (Cybister)</i>                  | <i>cognatus</i>      | INDIA: Karnataka, Udipi, 03 Oct 2004, KB Miller  | KBMCycg325    | DQ813672 | DQ813775 | DQ813738 | DQ813711 |
| Cybistrini | <i>C. (Cybister)</i>                  | <i>convexus</i>      | CHINA: Yunnan, 4 km S Shizong, 11 Sep 2000, J Bergsten   | KBMCyco172    | DQ813673 | DQ813776 | DQ813739 | DQ813712 |
| Cybistrini | <i>C. (Cybister)</i>                  | <i>explanatus</i>    | USA: California: Modoc Co., Hwy299, ~5 km E Cedarville, Surprise Valley, 22 Sep 2002, J Bergsten | KBMCyex183    | DQ813675 | DQ813778 | DQ813741 | DQ813714 |

Table 1 *Continued.*

| Tribe      | Genus                    | Species                   | Collection data  | UNM voucher # | COI      | COII     | H3       | Wnt      |
|------------|--------------------------|---------------------------|--|---------------|----------|----------|----------|----------|
| Cybistrini | <i>C. (Cybister)</i>     | <i>fimbriolatus</i>       | USA: Florida: Collier Co., Fakahatchee Strand Pres. St. Pk., 22–24 Mar 2000                            | KBMCyfi131    | DQ813676 | DQ813779 | DQ813742 | AF392008 |
| Cybistrini | <i>C. (Cybister)</i>     | <i>gschwendtneri</i>      | TANZANIA: Amani I Usambarabergen, 29 Mar 2003, Å Granberg  | KBMCygs311    | DQ813677 | DQ813780 | DQ813743 | DQ813715 |
| Cybistrini | <i>C. (Cybister)</i>     | <i>guerini</i>            | INDIA: Karnataka, 5mi E Jog Falls, 7 Oct 2004, 16°13.492'N 074°52.542'E, KB Miller                     | KBMCygu351    | DQ813678 | DQ813781 | DQ813744 | DQ813716 |
| Cybistrini | <i>C. (Cybister)</i>     | <i>japonicus</i>          | JAPAN: Hokkaido: Ebelsnak, Echigo-numa pond, 23 Aug 2000, J Bergsten                                   | KBMCyja173    | DQ813680 | DQ813783 | DQ813746 | DQ813718 |
| Cybistrini | <i>C. (Cybister)</i>     | <i>lateralimarginalis</i> | RUSSIA: Volgograd Oblast: Artyedinsko Donskie Peski, 3 May 2001, J Bergsten                            | KBMCyla178    | DQ813681 | DQ813784 | DQ813747 | —        |
| Cybistrini | <i>C. (Cybister)</i>     | <i>senegalensis</i>       | NAMIBIA: Skeleton Coast NP, Okau Spring, 18°18.845'S 12°05.161'E, 13 May 2004, KB Miller               | KBMCyse314    | DQ813685 | DQ813787 | DQ813751 | DQ813721 |
| Cybistrini | <i>C. (Cybister)</i>     | <i>tripunctatus</i>       | AUSTRALIA: Vic: 10 km E Corryong, 36°10'41"S 148°02'52"E, 05 Nov 2000, K.B. Miller                     | KBMCytr102    | DQ813687 | DQ813789 | DQ813753 | AF392010 |
| Cybistrini | <i>C. (Cybister)</i>     | <i>ventralis</i>          | INDIA: Karnataka, 13°40.807'N 74°41.172'E, 6 Oct 2004, KB Miller                                       | KBMCyve322    | DQ813688 | DQ813790 | DQ813754 | —        |
| Cybistrini | <i>C. (Megadytoides)</i> | <i>marginicollis</i>      | GHANA: Volta Reg., rd btwn Nkwanta and Odumase, 08°15'32.2"N 000°26'33.7"E, 15–17 Jun 2005, KB Miller  | KBMCyma416    | DQ813682 | DQ813785 | DQ813748 | DQ813719 |
| Cybistrini | <i>C. (Melanectes)</i>   | <i>brevis</i>             | JAPAN: Honshu: Aichi Pref., Ichinomiya, Samiyaki 27 Aug 2000, J Bergsten & Y Utsenomyia                | KBMCybr202    | DQ813669 | DQ813773 | DQ813735 | DQ813708 |
| Cybistrini | <i>C. (Melanectes)</i>   | <i>burgeoni</i>           | NAMIBIA: Etosha NP, Devilwater Spr., 18°59.266'S 015°15.593'E, 18 May 2004, KB Miller & GW Wolfe       | KBMCybu313    | DQ813670 | —        | DQ813736 | DQ813709 |
| Cybistrini | <i>C. (Melanectes)</i>   | <i>immarginatus</i>       | GHANA: Volta Reg., rd btwn Nkwanta and Odumase, 08°15'32.2"N 000°26'33.7"E, 15–17 Jun 2005, KB Miller  | KBMCyim418    | DQ813679 | DQ813782 | DQ813745 | DQ813717 |
| Cybistrini | <i>C. (Melanectes)</i>   | <i>posticus</i>           | INDIA: Maharashtra, Pond nr Pune, 29 Sep 2004, KB Miller   | KBMCypo327    | DQ813683 | DQ813786 | DQ813749 | DQ813720 |
| Cybistrini | <i>C. (Melanectes)</i>   | <i>sugillatus</i>         | HONG KONG: Lantau Island: Mui Wo, 30 Sep 2000, J Bergsten  | KBMCysu194    | DQ813686 | DQ813788 | DQ813752 | DQ813722 |
| Cybistrini | <i>C. (Melanectes)</i>   | <i>vicinus</i>            | SOUTH AFRICA: E Cape Prov.: Dwesa Nature Res., 32°18.443'S 28°48.551'E, 24 Jan 2005, J Bergsten        | KBMCyvi396    | DQ813689 | DQ813791 | DQ813755 | DQ813723 |
| Cybistrini | <i>C. (Melanectes)</i>   | <i>vulneratus</i>         | SOUTH AFRICA: Silaka, E Cape Prov.: Silaka Nat. Res., 31°38.862'S 29°30.551'E, 27 Jan 2005, J Bergsten | KBMCyvu395    | DQ813690 | DQ813792 | DQ813756 | DQ813724 |
| Cybistrini | <i>C. (Neocybister)</i>  | <i>puncticollis</i>       | BOLIVIA: Beni: Prov. Cercado, 9.5km N Trinidad, 14°46'34"S 64°58'00"W 17 Jun 1999, K.B. Miller         | KBMCypu110    | DQ813684 | —        | DQ813750 | AF392009 |

*Megadytes* (*Bifurcitus*) Brinck. *Regimbartina* includes a single rarely collected species from western Africa and *M. (Bifurcitus)* includes species from South America that are widespread, but not predictably collectable. Of the genus groups that have been variously recognized historically, but not currently, only *Cybister* (*Alocomerus*) Brinck is not represented. An attempt was made to include as many species from historical classifications of *Cybister* as possible (see Table 6). Species were identified by the first two authors.

**Outgroup.** Six outgroup taxa were included from other tribes within Dytiscinae and Lancetinae (see Table 1). The cladogram was rooted with *Lancetes* Sharp, a group well outside the Cybistrini (Miller 2001) and the sister group to or near relative of Dytiscinae (Alarie *et al.* 2002; Miller 2001, 2003; Ruhnnau & Brancucci 1984).

### DNA

DNAs were extracted using Qiagen DNEasy kit (Valencia, CA, USA) and the protocol for animal tissue. For each specimen an incision was made along the lateral margin of the thorax using a sharp razor and thoracic muscle tissue was removed with fine forceps and placed in buffer. Specimens of Cybistrini are very large and seemingly impervious to ethanol. Because of this, some extractions, even from relatively recently collected specimens, appear to have a decreased yield because of compromised DNA. To avoid this problem, many specimens were dissected immediately after collecting in the field, with thoracic muscle tissue placed in ethanol. This tissue was then extracted and used for PCR. Tissue treated in this way appeared to have better DNA extraction. Vouchers are deposited in at the Museum of South-western Biology, University of New Mexico (KBM).

Four genes were used in the analysis: *cytochrome oxidase I* (COI, 1294 bp), *cytochrome oxidase II* (COII, 678 bp), *bistone III* (H3, 328 bp) and *wingless* (wnt, 453 bp). Primers used for amplification and sequencing were derived from several sources (see Table 2). For most taxa, but not all, COI and COII were amplified and sequenced together using several combinations of primers and the intervening ~60 bp leucine-coding tRNA sequence (which was length variable in many taxa) was excised. In some cases, COI and COII were amplified and sequenced separately without the intervening tRNA

**Table 2** Primers used for amplification and sequencing.

| Gene | Primer                           | Direction | Sequence (5'–3')                   |
|------|----------------------------------|-----------|------------------------------------|
| COI  | C1-J-1718 ('Mtd6') <sup>1</sup>  | For       | GGA GGA TTT GGA AAT TGA TTA GTT CC |
| COI  | C1-J-2183 ('Jerry') <sup>1</sup> | Rev       | CAA CAT TTA TTT TGA TTT TTT GG     |
| COI  | TL2-N-3014 ('Pat') <sup>1</sup>  | Rev       | TCC AAT GCA CTA ATC TGC CAT ATT A  |
| COII | F-lue <sup>2</sup>               | For       | TCT AAT ATG GCA GAT TAG TGC        |
| COII | 9b <sup>2</sup>                  | Rev       | GTA CTT GCT TTC AGT CAT CTW ATG    |
| COII | R-lys <sup>2</sup>               | Rev       | GAG ACC AGT ACT TGC TTT CAG TCA TC |
| H3   | Hal <sup>3</sup>                 | For       | ATG GCT CGT ACC AAG CAG ACG GC     |
| H3   | Hal <sup>3</sup>                 | Rev       | ATA TCC TTG GGC ATG ATG GTG AC     |
| Wnt  | LepWg1 <sup>4</sup>              | For       | GAR TGY AAR TGY CAY GGY ATG TCT GG |
| Wnt  | LepWg2a <sup>4</sup>             | Rev       | ACT ICG CAR CAC CAR TGG AAT GTR CA |
| Wnt  | WgDytf1 <sup>5</sup>             | For       | CGY CTT CCW TCW TTC CGW GTY ATC    |
| Wnt  | WgDytr1 <sup>5</sup>             | Rev       | CCG TGG ATR CTG TTV GCH AGA TG     |

<sup>1</sup>Simon *et al.* (1994).

<sup>2</sup>Whiting (2002).

<sup>3</sup>Colgan *et al.* (1998).

<sup>4</sup>Brower & Egan (1997).

<sup>5</sup>Miller (2003).

sequence. *Wingless* was sometimes amplified by an initial PCR and a reamplification with gel purified product from the initial PCR.

DNA fragments were amplified using PCR with either Taq Gold (Applied Biosystems, Foster City, CA, USA) or Platinum (Invitrogen, Carlsbad, CA, USA) on a DNA Engine DYAD Peltier Thermal Cycler. Amplification conditions are presented in Table 3. Contamination was mediated using negative controls, and fragments produced from PCR were examined using gel electrophoresis. Products were purified using Montage PCR<sub>96</sub> Cleanup Kit (Millipore, Billerica, MA, USA) and cycle sequenced using ABI Prism Big Dye (version 3, Fairfax, VA, USA) using the same primers used to amplify (or nested primers in the case of long amplifications of COI + COII). Sequencing reaction products were purified using Sephadex G-50 Medium and sequenced using an ABI 3730xl DNA analyser (DNA Sequencing Center, BYU). Gene regions were sequenced in both directions. Resulting sequence data were examined and edited using the program Sequencher (Genecodes 1999).

### Morphology

The morphological characters analysed in the cladistic analysis of Cybistrini are described in Appendix 1. Many of these

**Table 3** Amplification conditions used in PCR reactions.

|           | Hot start                | Denature      | Anneal         | Extension     | Cycles |
|-----------|--------------------------|---------------|----------------|---------------|--------|
| H3        | 95° (12 min)             | 94° (0.5 min) | 48–50° (1 min) | 70° (1 min)   | 40     |
| COI, COII | 95° (12 min)             | 94° (1 min)   | 50–52° (1 min) | 64° (1.5 min) | 40     |
| Wnt       | 95° (12 min) Taq Gold    |               |                |               |        |
|           | 95° (2 min) Taq Platinum | 94° (1 min)   | 46–54° (1 min) | 70° (1 min)   | 40     |

characters have been used extensively in the classification of Dytiscidae, particularly Dytiscinae and Cybistrini, and descriptions of these can be found in general treatments of the family including Sharp (1882), Balfour-Browne (1950), Guignot (1961), and Larson *et al.* (2000). Characters specific to Cybistrini used here have been described in several treatments including Brinck (1945), Guignot (1961), Sharp (1882), Trémouilles & Bachmann (1980), Wilke (1920), Mouchamps (1957) and Watts (1978). Several of the characters have been described and tested in phylogenetic analyses (Miller 2000, 2001, 2003) and those analyses and character numbers are cited in Table 4, since these papers include more thorough descriptions of many characters. Characters presented here as new or tested in a cladistic analysis for the first time are discussed in greater detail. Coded states are presented in Table 5.

### Analysis

Sequence alignment was done within Sequencher (GeneCodes 1999), since the sequences are not length variable in these taxa and alignment is unambiguous. Data were analysed using parsimony and NONA (Goloboff 1995), implemented within WinClada (Nixon 1999–2002), with the ‘heuristics’ option and the commands set to hold 5000 trees total (‘h 5000’), 50 replications (‘mu\*50’), 40 trees held per replication (‘h/40’), and multiple TBR + TBR (‘max\*’). Characters 39, 41 and 47 were treated as additive. Trees were examined and analysed under different optimizations and character distributions on the resulting topologies were examined using WinClada.

The extent to which the data support branches was measured using bootstrap values, Bremer supports (Bremer 1994), and partitioned Bremer supports (Baker & DeSalle 1997), which were examined for relative contribution of several partitions to a combined analysis and compared with the number of informative characters provided by each partition. Bootstrap values were calculated in WinClada using 1000 replications, 10 search reps, one starting tree per rep, ‘don’t do max\*(TBR)’, and saving the consensus of each replication. Total and partitioned Bremer support values were calculated in PAUP\* (Swofford 2000) using a batch file generated by TreeRot (Sorenson 1999).

Likelihood (combined molecular data) and partitioned Bayesian analyses (combined morphological and molecular data) were also conducted. The model GTR + G + I was determined to be the most justified model using ModelTest (Posada & Crandall 1998). Likelihood analysis was accomplished using Treefinder (Jobb 2005). Partitioned Bayesian analysis was conducted using MrBayes ver. 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) with mixed model settings. A GTR + G + I model was assigned to each of the six data partitions defined as 1st, 2nd and 3rd codon positions of COI + COII and the same for H3+wingless. The

**Table 4** Character correspondence between this analysis and previous published analyses incorporating them. Numbers refer to character numbers in the analyses, those marked with ‘—’ were not included in the prior analysis.

| Character | Miller (2000) | Miller (2001) | Miller (2003) |
|-----------|---------------|---------------|---------------|
| 1         | 1             | 34            | 3             |
| 2         | 2             | 35            | 1             |
| 3         | —             | 48            | 13            |
| 4         | —             | —             | —             |
| 5         | 7             | 59            | 15            |
| 6         | 8             | 71            | 8             |
| 7         | 9             | 72            | 9             |
| 8         | —             | —             | —             |
| 9         | —             | —             | —             |
| 10        | —             | —             | —             |
| 11        | 12            | 76            | 17            |
| 12        | 13            | 74            | 18            |
| 13        | 15            | —             | 22            |
| 14        | 17            | 80            | 25            |
| 15        | 18            | —             | 26            |
| 16        | 22            | —             | 29            |
| 17        | 23            | —             | 30            |
| 18        | 19            | 79            | 31            |
| 19        | 6             | 89            | 32            |
| 20        | 24            | 90            | 33            |
| 21        | 27            | 92            | 34            |
| 22        | 31            | 94            | 41            |
| 23        | 29            | 82            | 44            |
| 24        | 36            | 98            | 51            |
| 25        | 35            | 101           | 52            |
| 26        | —             | —             | —             |
| 27        | —             | 85            | —             |
| 28        | 38            | —             | 53            |
| 29        | 39            | —             | —             |
| 30        | —             | —             | 56            |
| 31        | 37            | 62            | 54            |
| 32        | 45            | 1             | 57            |
| 33        | —             | 32            | 67            |
| 34        | —             | 33            | 68            |
| 35        | —             | 14            | —             |
| 36        | 41            | 2             | 64            |
| 37        | 44            | —             | 65            |
| 38        | —             | —             | 70            |
| 39        | —             | —             | 75            |
| 40        | —             | —             | 77            |
| 41        | —             | —             | 79            |
| 42        | —             | —             | 80            |
| 43        | —             | —             | 82            |
| 44        | —             | —             | 85            |
| 45        | —             | —             | 86            |
| 46        | —             | —             | 88            |
| 47        | —             | —             | 89            |

substitution- and state frequency parameters were linked across the nuclear and mitochondrial partitions, respectively. Likewise, the gamma shape parameter was linked across the 1st and 2nd nuclear and mitochondrial codon partitions,

**Table 5** Data matrix of assigned states of 47 morphological characters for 39 species of Dytiscidae. Characters marked with '+' are additive. Characters coded as '—' are inapplicable. Characters coded with '?' are unobserved. Characters coded with '\$' are polymorphic and equal states 3 and 4.

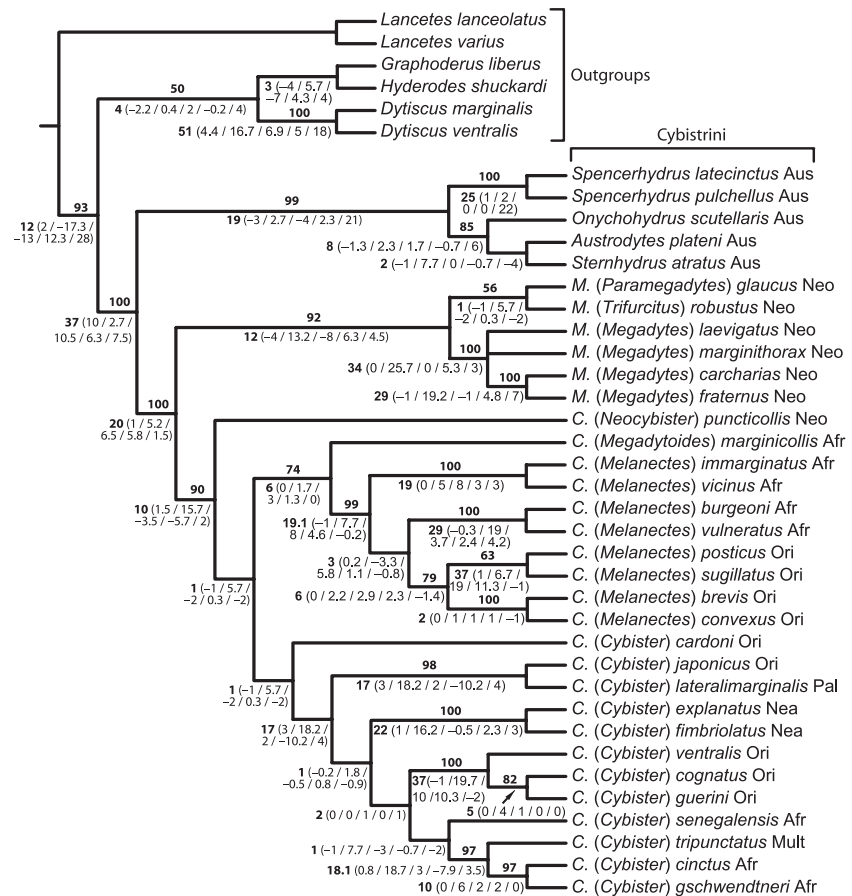
|  | 000000001<br>1234567890 | 111111112<br>1234567890 | 222222223<br>1234567890 | 333333334<br>1234567890 | 4444444<br>1234567 |
|--|-------------------------|-------------------------|-------------------------|-------------------------|--------------------|
| <i>Lancetes lanceolatus</i>            | 0100010000              | 1001011000              | 0001001000              | 00000010-1              | -000110            |
| <i>L. varius</i>                       | 0100010000              | 1001011000              | 0001001000              | 0000001???              | ???????            |
| <i>Dytiscus marginalis</i>             | 1001111010              | 1101112010              | 0000101101              | 1100010011              | 1010112            |
| <i>D. ventralis</i>                    | 1001111010              | 1101112010              | 0000101101              | 1100010011              | 1010112            |
| <i>Hyderodes shuckardi</i>             | 100010-000              | 0100012010              | 0000101000              | 1100011011              | 1010111            |
| <i>Graphoderus liberus</i>             | 100000-000              | 0111012000              | 0001001000              | 1100011001              | 0200110            |
| <i>Austrodytes plateni</i>             | 0001012011              | 100100-000              | 1110001100              | 1111010???              | ???????            |
| <i>Sternhydrus atratus</i>             | 0011012011              | 1001010000              | 1110000100              | 1111010???              | ???????            |
| <i>Onychohydus scutellaris</i>         | 0001012011              | 1001110000              | 1110000100              | 1111010120              | 2111000            |
| <i>Spencerhydrus latecinctus</i>       | 0011012011              | 101000-000              | 1110001100              | 1111010110              | 1111000            |
| <i>Spencerhydrus pulchellus</i>        | 0011012011              | 101000-000              | 1110001100              | 1111010110              | 1111000            |
| <i>M. (Megadytes) carcharias</i>       | 0001012101              | 1001010001              | 1110201100              | 1111110???              | ???????            |
| <i>M. (M.) fraternus</i>               | 0000012101              | 1001010001              | 1110201100              | 1111110120              | 3111000            |
| <i>M. (M.) laevigatus</i>              | 0000012101              | 1001010001              | 1110201100              | 1111110???              | ???????            |
| <i>M. (M.) marginithorax</i>           | 0001012101              | 1001010001              | 1110201100              | 1111110???              | ???????            |
| <i>M. (Paramegadytes) glaucus</i>      | 0000012001              | 1001010001              | 1110201100              | 1111110???              | ???????            |
| <i>M. (Trifurcitus) robustus</i>       | 0001012111              | 1001010001              | 1110101100              | 1111010???              | ???????            |
| <i>C. (Melanectes) brevis</i>          | 0000012001              | 1011010101              | 1110301110              | 1111010???              | ???????            |
| <i>C. (M.) burgeoni</i>                | 0000012001              | 1011010101              | 1110301110              | 1111010???              | ???????            |
| <i>C. (M.) convexus</i>                | 0000012001              | 1011010101              | 1110301110              | 1111010???              | ???????            |
| <i>C. (M.) immarginatus</i>            | 0000012001              | 1011010101              | 1110301110              | 1111010???              | ???????            |
| <i>C. (M.) posticus</i>                | 0000012001              | 1011010101              | 1110301110              | 1111010???              | ???????            |
| <i>C. (M.) sugillatus</i>              | 0000012001              | 1011010101              | 1110301110              | 1111010???              | ???????            |
| <i>C. (M.) vicinus</i>                 | 0000012001              | 1011010101              | 1110301110              | 1111010???              | ???????            |
| <i>C. (M.) vulneratus</i>              | 0000012201              | 1011010101              | 1110301110              | 1111010???              | ???????            |
| <i>C. (Megadytoides) marginicollis</i> | 0001012001              | 1011010101              | 1110301110              | 1111010???              | ???????            |
| <i>C. (Neocybister) puncticollis</i>   | 0001012011              | 1011010101              | 1110301110              | 1111010???              | ???????            |
| <i>C. (Cybister) explanatus</i>        | 0001012211              | 1011010101              | 1110\$11110             | 1111010???              | ???????            |
| <i>C. (C.) fimbriolatus</i>            | 0001012211              | 1011010101              | 1110\$11110             | 1111010120              | 3111000            |
| <i>C. (C.) japonicus</i>               | 0001012211              | 101100-101              | 1111401110              | 1111010???              | ???????            |
| <i>C. (C.) lateralmarginalis</i>       | 0001012211              | 101100-101              | 1111401110              | 1111010120              | 3111000            |
| <i>C. (C.) cognatus</i>                | 0001012211              | 1011010101              | 1110401110              | 1111010???              | ???????            |
| <i>C. (C.) guerini</i>                 | 0001012211              | 1011010101              | 1110401110              | 1111010???              | ???????            |
| <i>C. (C.) ventralis</i>               | 0001012211              | 1011010101              | 1110401110              | 1111010???              | ???????            |
| <i>C. (C.) cardoni</i>                 | 0001012011              | 1011010101              | 1110401110              | 1111010???              | ???????            |
| <i>C. (C.) senegalensis</i>            | 0001012011              | 1011010101              | 1110401110              | 1111010???              | ???????            |
| <i>C. (C.) tripunctatus</i>            | 0001012011              | 1011010101              | 1110401110              | 1111010120              | 3111000            |
| <i>C. (C.) cinctus</i>                 | 0001012011              | 1011010101              | 1110401110              | 1111010???              | ???????            |
| <i>C. (C.) gschwendtneri</i>           | 0001012011              | 1011010101              | 1110401110              | 1111010???              | ???????            |

respectively. The proportion of invariable sites was included as a separate parameter for all six partitions.

For the morphological matrix a Markov k model + G was assigned (Lewis 2001), and we accounted for the fact that only parsimony informative characters were included in the matrix. As with the parsimony analysis, characters 39, 41 and 47 were treated as additive. Branch lengths were estimated separately in the model for each of the seven partitions. Default prior and proposal settings were used, except for branch length with multiplier, which was changed from a default proposal rate of 0–5. Two Markov Chain Monte

Carlo runs, each with one cold and three incrementally heated chains, were performed with 5 million generations sampled every 1000th generation. After an initial run chain mixing was found to be inadequate so the incremental temperature parameter for the heated chains was reduced from 0.2 to 0.075. The first 2.5 million generations were discarded in each run as burn-in and the last 2500 sampled trees were pooled from the two runs and summarized to identify the topology with highest posterior probability and to calculate clade support values as the frequency of each clade among the pooled trees. Chain mixing, acceptance probabilities, the log





**Fig 36** Consensus cladogram of three most parsimonious trees from cladistic analysis of combined morphology and four genes (length of trees = 5118, CI = 33, RI = 50). Numbers above branches are bootstrap values. Numbers below branches are total Bremer support value (in bold) followed by partitioned Bremer support values for five data partitions in the order: morphology/COI/COII/H3/wingless. Taxon names are followed by abbreviations of zoogeographical regions: Aus, Australian; Afr, Afrotropical; Neo, Nearctic; Ori, Oriental; Pal, Palearctic; Mult, multiple regions (Palearctic + Afrotropical + Oriental + Australian).

likelihood through generation plot, average standard deviation of split frequencies between the two runs and convergence diagnostics values were examined in each run to ensure that the sampling was long enough and had reached stationarity.

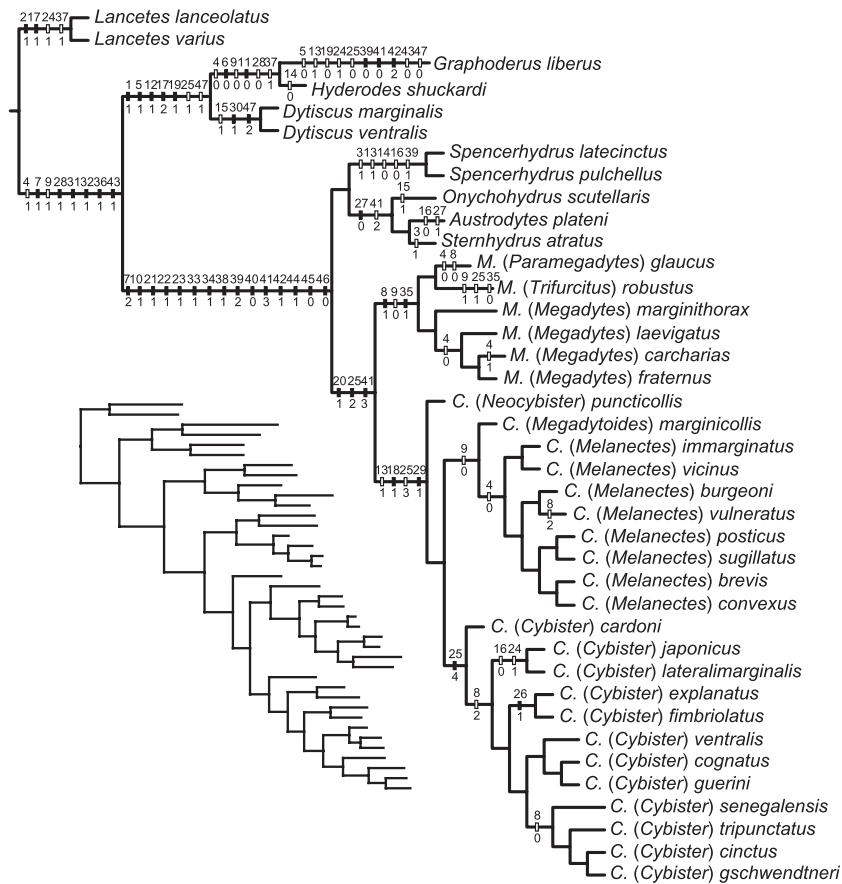
## Results

The combined parsimony analysis resulted in three equally parsimonious cladograms ( $L = 5118$ ,  $CI = 33$ ,  $RI = 50$ ), with the consensus shown in Fig. 36. High support for this topology is evenly distributed throughout the tree (Fig. 36), with relatively high support for most groups with the notable exception of several groups within *Cybister* (Fig. 36) which also have short parsimony branches (Fig. 37). Total Bremer support ranges from 1 to 51. Several data partitions contributed to the resulting topology roughly proportionately to the proportion of informative characters contributed to the analysis (Table 7). An exception was COII, which contributed about 25% of the informative characters in the analysis, but less than 10% of the total support (8.5% of the support normalized for the number of informative characters provided by the partition). Similarly, morphology provided about 5% of the informative characters but only 0.4% of the total support

(1.7% of the normalized support). Morphology primarily supports relationships among outgroups with relatively few characters grouping taxa within Cybistrini (Fig. 37).

In this topology, the following recognized and named groups are found to be monophyletic: (1) Dytiscinae, (2) *Dytiscus*, (3) Cybistrini, (4) *Spencerhydrus*, (5) *Megadytes sensu lato*, (6) *Megadytes* (*Megadytes*), (7) *Cybister*, (8) *C. (Scaphinectes)*, and (9) *C. (Melanectes)*. Two larger monophyletic groups within Cybistrini are indicated, the Australian *Spencerhydrus* + *Onychohydrus* (*Onychohydrus*) + *O. (Sternhydrus)* + *Austrodytes* and the Neotropical *Megadytes s.l.* + the circumtropical *Cybister*. Within *Megadytes*, *M. (Megadytes)* is monophyletic, with *M. (Trifurcitus)* and *M. (Paramegadytes)* together forming a sister clade. Within *Cybister*, the currently recognized subgenera *C. (Melanectes)* and *C. (Scaphinectes)* are monophyletic, but the subgenus *C. (Cybister)* is not.

The likelihood and Bayesian analyses resulted in similar topologies, with similar support (Fig. 38). The primary disagreements with the parsimony analysis are: (1) a different relationship among the outgroups; (2) a rearrangement between *Austrodytes*, *Onychohydrus* (*Sternhydrus*) and *Onychohydrus s.s.*; (3) *Cybister puncticollis* nested well within *Cybister*,



**Fig. 37** One of three most parsimonious cladograms from combined parsimony analysis with morphological characters mapped using 'fast' optimization in WinClada. Black hash marks indicate unambiguous changes, white hash marks indicate homoplasious changes or reversals. Numbers above hash marks are character numbers, those below hash marks are state numbers. Dendrogram in lower left depicts branch lengths of the same tree based on number of changes (all changes in combined parsimony analysis including morphology and DNA sequence data) mapped using 'fast' optimization in WinClada.

rather than sister to all other species of *Cybister*, and (4) the relative placement of the Nearctic *Cybister* clade (*C. fimbriolatus* + *C. explanatus*) within *Cybister*. The only difference between the likelihood and Bayesian analyses is the relative placement of *C. cardoni* (Fig. 38) within the *Cybister* clade. Other portions of the topology are identical. The differences in arrangement between different methods of tree reconstruction are in areas of the topology with long-terminal branches subtended by short internal branches that are weakly supported, suggesting that these areas may require additional data and analysis before firm conclusions can be made about their relationships.

## Discussion

### Classification

Our analysis provides convincing support for a comprehensive phylogeny of Cybistrini. Corroborating previous results (Miller 2001, 2003), monophyly of Cybistrini has some of the highest support for any clade in the analysis (bootstrap = 100, Bremer = 37). In addition, each of the genera is monophyletic with the exception of *Onychohydrus* in which *Austrodytes* is nested (in the parsimony analysis). Support for a close

relationship between *Onychohydrus* and *Austrodytes* is strong (bootstrap = 85, Bremer = 8). *Onychohydrus* has been historically divided into two subgenera, *O. (Sternhydrus)* Brinck and *Onychohydrus s.s.* These two groups exhibit numerous prominent morphological differences including the presence in *O. (Sternhydrus)* of a laterally carinate prosternal process. In addition, *O. (Sternhydrus)* are much smaller than the very large *Onychohydrus s.s.* In the parsimony analysis, *Austrodytes* is resolved as sister to *O. (S.) atratus*. However, this association is not strong (bootstrap < 50, Bremer = 2) and the likelihood and Bayesian analyses contradict it (Fig. 38).

Based on this there are several ways to remedy the classification. One is to simply maintain the status quo and leave *O. (Sternhydrus)* as a subgenus of *Onychohydrus*, as suggested by the likelihood and Bayesian results. It may also be reasonable to synonymize *Austrodytes* with *Onychohydrus* or relegate it to subgeneric status within the genus. However, *Austrodytes*, *O. (Sternhydrus)* and *Onychohydrus s.s.* are each substantially different from each other in prominent structural characters. For this reason, we propose elevating *Sternhydrus* to genus rank (**new status**), continue to recognize *Austrodytes* and recognize *Onychohydrus* as a taxon exclusive of *Sternhydrus*.

**Table 6** Correspondence between major historical classifications of *Cybister* species groups and subgenera and representative taxa included in analysis from each group. Since Guignot's (1961) informal species groups apply only to African species, they are not included in this synopsis and only his subgenera classification is presented here.

| Nilsson (2001);<br>Nilsson et al. (1989) | Guignot (1961)    | Brinck (1945)             | Sharp (1882)     | Taxa included in analysis  |
|--|-------------------|---------------------------|------------------|--|
| <i>Scaphinectes</i>                      | <i>Cybister</i>   | <i>Cybister</i>           | Group 6          | <sup>3</sup> <i>japonicus</i><br><sup>1</sup> <i>lateralimarginalis</i>  |
| <i>Melanectes</i>                        | <i>Melanectes</i> | <i>Melanectes</i> (SecI)  | Group 2          | <sup>3</sup> <i>brevius</i><br><sup>1,2</sup> <i>burgeoni</i><br><sup>3</sup> <i>convexus</i><br><sup>3</sup> <i>posticus</i><br><sup>3</sup> <i>sugillatus vulneratus</i> |
|  |                   | <i>Melanectes</i> (SecII) | Group 3          | <i>immarginatus</i><br><sup>1</sup> <i>vicinus</i>   |
| <i>Cybister</i>                          | <i>Meganectes</i> | <i>Meganectes</i>         | Group 5 (part)   | <sup>3</sup> <i>cognatus</i><br><sup>3</sup> <i>guerini</i><br><sup>3</sup> <i>ventralis</i><br><i>marginicollis</i>   |
|  |                   | <i>Megadytoides</i>       | Group 1          | <sup>3</sup> <i>fimbriolatus</i>   |
|  |                   | <i>Nealocomerus</i>       | Group 4 (part)   | <sup>3</sup> <i>explanatus</i>   |
|  |                   | <i>Alocomerus</i>         | Group 5 (part)   | none   |
|  |                   | <i>Gschwendtnerhydrus</i> | Group 4 (part)   | <sup>1,3</sup> <i>cardoni cinctus</i><br><sup>1</sup> <i>gschwendtneri senegalensis tripunctatus</i>   |
|  |                   | <i>Megadytes</i>          | <i>Megadytes</i> | <sup>3</sup> <i>puncticollis</i>   |

<sup>1</sup>Not included in Sharp (1882).

<sup>2</sup>Not included in Brinck (1945).

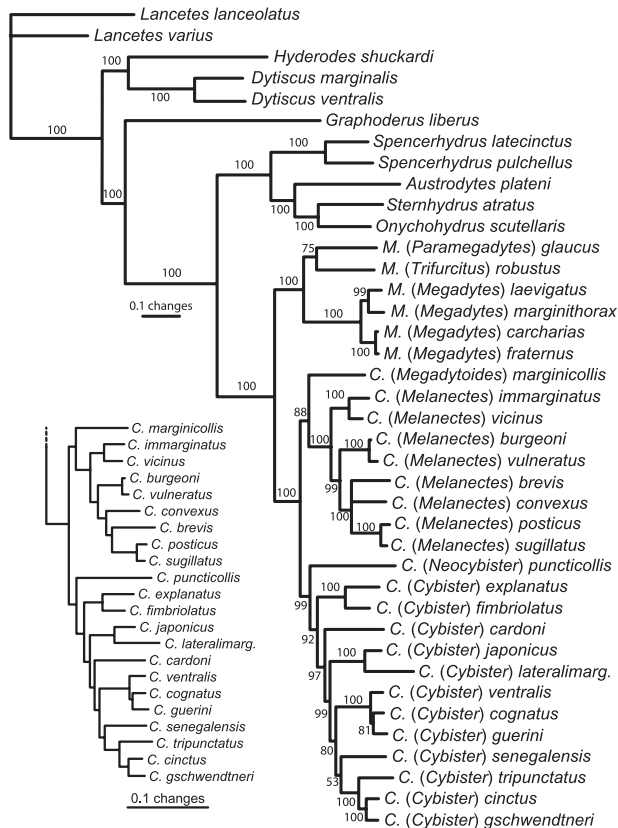
<sup>3</sup>Not included in Guignot (1961).

**Table 7** Results of partitioned Bremer analysis. Normalized support is the proportion of the total support divided by the number of informative characters provided by each partition.

|            | Support | # aligned<br>base pairs | # informative<br>characters | % informative<br>characters | % total<br>support | Support/# informative<br>characters | Normalized<br>support |
|------------|---------|-------------------------|-----------------------------|-----------------------------|--------------------|-------------------------------------|-----------------------|
| morphology | 1.9     | —                       | 46                          | 4.9%                        | 0.4%               | 0.041                               | 1.7%                  |
| COI        | 257     | 1294                    | 416                         | 43.2%                       | 51.0%              | 0.618                               | 25.9%                 |
| COII       | 49.5    | 678                     | 244                         | 25.3%                       | 9.8%               | 0.203                               | 8.5%                  |
| H3         | 69.9    | 328                     | 92                          | 9.6%                        | 13.9%              | 0.760                               | 31.8%                 |
| Wnt        | 125.9   | 453                     | 164                         | 17.0%                       | 25.0%              | 0.768                               | 32.1%                 |
| Total      | 504.2   | 2753                    | 962                         |                             |                    | 2.39                                |                       |

Naturally, this configuration is weakened in that we did not include the several other species of *Onychobydrus*, *Sternbydrus* or *Austrodytes* in the analysis, but based on published descriptions and examination of pinned specimens of many of the species by the first author, these taxa appear to be similar to those included, including similarity in morphological characters that support the topology discovered here. Even if *Onychobydrus s.l.* is monophyletic (as is indicated by the likelihood and Bayesian analyses) we do not think elevation of the subgeneric names to genus rank is a significant disruption in the classification, and it demonstrates the compelling differences between the groups and obviates the use of unwieldy subgeneric names.

The Neotropical genus *Megadytes* is monophyletic (bootstrap = 92, Bremer = 12). *Megadytes* includes four subgenera, one of which, *M. (Bifurcitus)*, was not included in the analysis. *Megadytes s.s.* includes the largest number of taxa, and this group is monophyletic based on the included taxa (Figs 36–38). *Megadytes (Paramegadytes)* includes two species, one of which, *M. (P.) glaucus*, was included in our analysis. The other species is very similar to the included one (Trémouilles & Bachmann 1980). *Megadytes (Bifurcitus)* and *M. (Trifurcitus)* each include a few species, but there is little doubt that they are monophyletic, and probably sister groups, based on the bi- or trifurcation of the posterior metatibial spur and other less discrete characters such as extreme size, similar general shape of



**Fig. 38** Majority-rule consensus tree derived from combined (partitioned) Bayesian analysis of morphology and four genes and *Cybister* clade derived from likelihood analysis of four genes (lower left); topology of other portions of likelihood tree is identical to Bayesian tree. Numbers on branches of Bayesian tree are posterior probability values. Branch lengths are the average over the separately estimated branch lengths for the seven partitions. The topology with highest posterior probability ( $P = 0.092$ ) had the unresolved clade resolved as (((*C. posticus* + *C. sugillatus*) *C. brevis*) *C. convexus*).

the metatarsal claws, etc. We propose continued recognition of four subgenera in *Megadytes* — *M. (Megadytes)*, *M. (Paramegadytes)*, *M. (Bifurcitus)*, and *M. (Trifurcitus)* — since each of these taxa is structurally unique and seemingly monophyletic, yet recognition of this prominent Neotropical clade with a more inclusive name is also useful and historically consistent.

It should be noted that a recent study of the genus based on larval characters (Michat in press) came to a somewhat different conclusion. Results from that project found that *Megadytes* is not monophyletic, with *M. (Trifurcitus)* resolved outside the genus (or with *Onychohydus* and *Cybister* nested within the genus). *Megadytes (Trifurcitus)* shares a unique combination of characters (Michat in press; pers. comm.) making it dissimilar to other cybistrines, and future analyses incorporating these

characters with a greater sampling of larval characters and a more complete understanding of their distribution throughout Dytiscinae will likely clarify relationships within this interesting part of the Cybistrini.

The genus *Cybister* is very clearly monophyletic, with considerable support from this analysis (bootstrap = 90, Bremer = 10) and several convincing morphological synapomorphies, including the presence of the posteroapical marginal setae on the mesotibia in a continuous line (char. 13), the presence of a series of setae along the posteroventral apical margin of the mesotarsomeres of males and pro- and mesotarsomeres of females (char. 18, Fig. 4), males with a single metatarsal claw (char. 25, Figs 20–22), and the emarginate medial margin of the lobes of the male eighth abdominal sternum (char. 29, Fig. 25). A comparison of several historical classification schemes for *Cybister* with the results of the parsimony analysis is presented in Fig. 39. Within *Cybister*, the currently recognized subgenera *C. (Melanectes)* and *C. (Scaphinectes)* are monophyletic (Figs 36–38). The other subgenus *C. (Cybister)* is not, however, under any optimality criterion (Figs 36–38).

Based on this, it might be reasonable to suggest recognition of multiple subgenera, perhaps based on Brinck's (1945) classification. His subgenera, however, are also not all monophyletic (Fig. 39). Another possibility is the recognition of two subgenera, *Cybister s.s.* and *Cybister (Melanectes)*, with *C. (Scaphinectes)* a synonym of the former. This arrangement has the support of the likelihood and Bayesian analyses (Fig. 38). However, this arrangement is not supported by the parsimony analysis, which places the Neotropical species *C. puncticollis* as sister to all other *Cybister* (Fig. 36). This species represents a phylogenetically intermediate entity in our analysis (Figs 36–38). Clearly, there is utility in recognizing the large clade *Cybister* with its own name, but there is also a substantial argument for formally recognizing subgenera within this large genus (> 100 species) based on monophyletic groups supported in our analysis, relative character support for those groups and historical classifications. Based on this we propose recognition of four subgenera according to the following classification:

*Cybister (Cybister)* Curtis, 1827

syn *Alocomerus* Brinck, 1945

syn *Cybisteter* Bedel, 1881

syn *Gschwendtnerhydus* Brinck, 1945

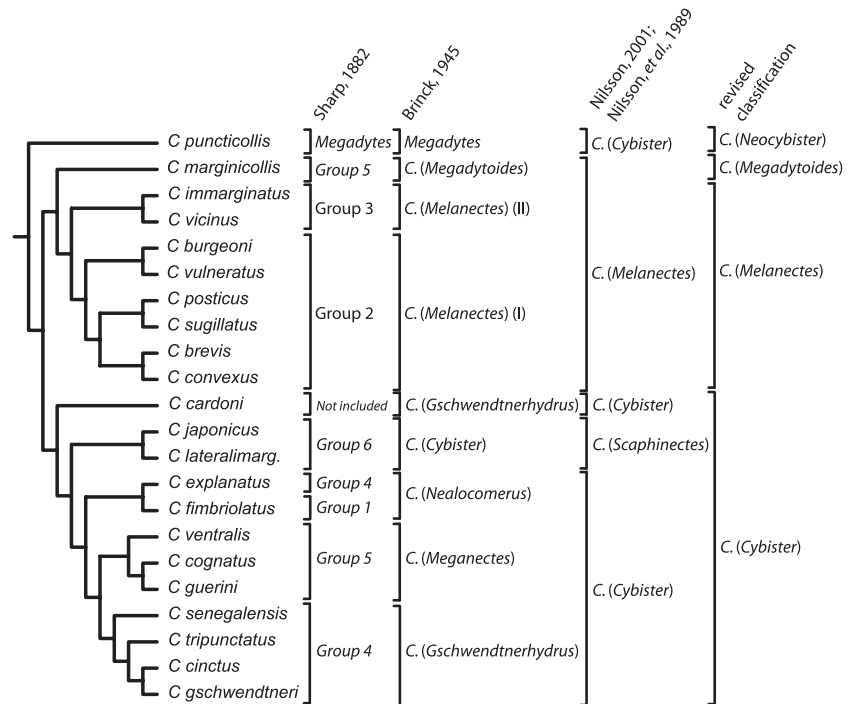
syn *Meganectes* Brinck, 1945

syn *Nealocomerus* Brinck, 1945

syn *Trogus* Leach, 1817, preoccupied syn *Scaphinectes* Ádám, 1993, new synonym

syn *Trochalus* Dejean, 1833, new synonym

*Type species.* *Dytiscus lateralis* Fabricius, 1798 (= *Dytiscus tripunctatus* Olivier, 1795).



**Fig. 39** *Cybister* clade derived from combined parsimony analyses comparing three main historical classification schemes (Sharp 1882; Brinck 1945; Nilsson *et al.* 1989/Nilsson 2001) and a new classification system based on this analysis.

**Diagnosis.** *Cybister* species with distinct lateral yellow margins on pronotum and elytra; males with a single metatarsal claw, females of most species with a single metatarsal claw, females of some North and Central American species with females dimorphic, some with a single metatarsal claw, others also with a rudimentary posterior claw.

**Taxon content.** All *Cybister* species historically placed in *Cybister s.s. sensu* Nilsson (2001) and *Scaphinectes* Ádám, 1993 *sensu* Nilsson (2001) (new synonym) except *C. puncticollis* (Brullé), *C. festae* Griffini and *C. parvus* Trémouilles (see below).

**Discussion.** This subgenus includes a diverse, but monophyletic, assemblage of taxa (Figs 36–38). There are several distinctive monophyletic groups within the subgenus including those taxa with male stridulatory devices on the metacoxae and females with dimorphic metatarsal claws (some with a single, others with a second rudimentary claw) (Figs 36–38). These taxa were placed in the subgenera *C. (Alocomerus)* and *C. (Nealocomerus)* by Brinck (1945). Also monophyletic in the analysis is a clade containing the two closely related species, *C. japonicus* Sharp and *C. lateralimarginalis* (DeGeer). These have generally been placed in their own subgenus (most recently *C. (Scaphinectes)* Ádám (Nilsson 2001; Nilsson *et al.* 1989)). The taxa are united by both males and females with natatory setae on the dorsal and ventral margins of the metatibia and metatarsomeres.

This subgenus could, conceivably, be divided into multiple additional subgenera based on available names (requiring a new subgenus for the unique *C. cardoni*; Figs 36–38). Support for relationships among these clades is not strong, however, and it seems likely that additional taxon sampling within this diverse group will eventually result in changes. The subgenus is reasonably well characterized as we have defined it here and includes about 60 species, comparable to the other large subgenus in the group, *C. (Melanectes)*.

#### ***Cybister (Megadytoides)* Brinck, 1945**

**Type species.** *Cybister marginicollis* Boheman, 1848.

**Diagnosis.** *Cybister* species with yellow on the lateral margins of the pronotum, but not laterally on the elytron; males with a single metatarsal claw, females always with a rudimentary posterior metatarsal claw.

**Taxon content.** A single species, *Cybister marginicollis* Boheman.

**Discussion.** This subgenus includes a single Afrotropical species, but it is morphologically unique and phylogenetically intermediate. It is sister to *C. (Melanectes)* (Figs 36–38). *Cybister marginicollis* has females with a rudimentary posterior claw and the lateral pronotal margins distinctly marked with yellow, but the lateral elytral margins are not marked. In

addition, this is one of the smallest species of *Cybister*, with specimens generally less than 20 mm in length.

***Cybister (Melanectes)* Brinck, 1945**

*Type species.* *Cybister owas* Laporte, 1835.

*Diagnosis.* *Cybister* species without distinct lateral yellow margins on pronotum and elytra; males with a single metatarsal claw, females always with a rudimentary posterior metatarsal claw.

*Taxon content.* All *Cybister* species historically placed in *C. (Melanectes)* *sensu* Nilsson (2001).

*Discussion.* This group has a long history of recognition dating back to Sharp's (1882) *Cybister* Groups 2 and 3. Brinck (1945) erected the subgenus and also recognized two subgroups: *C. (Melanectes)* Section I and *C. (Melanectes)* Section II. Each of these sections is also monophyletic based on this analysis (Figs 36, 38). However, as a whole, the subgenus is relatively homogeneous in morphological features. This is one of two large subdivisions within *Cybister*, with over 40 included species.

***Cybister (Neocybister)* Miller, Bergsten and Whiting, new subgenus**

*Type species.* *Dytiscus puncticollis* Brullé, 1838.

*Diagnosis.* *Cybister* species with distinct lateral yellow margins on pronotum and elytra; males with a single metatarsal claw, females always with a rudimentary, sinuate posterior metatarsal claw.

*Taxon content.* *Cybister puncticollis* (Brullé) and *Cybister festae* Griffini.

*Etymology.* Named using a combination of the Greek *neo*, meaning 'new' and *cybister*, meaning 'diver', because it is a new name in the genus *Cybister*; also, all known species in the subgenus are from the Neotropical region.

*Discussion.* This subgenus is erected to include two species, *C. puncticollis* (with a synonym, *C. kemneri* Brinck) and *C. festae*. The second species was not examined, but it appears from descriptions to be similar to *C. puncticollis*. This group represents a unique Neotropical lineage that is phylogenetically intermediate (Figs 36–38). Females apparently always have a rudimentary second posterior claw and specimens are green with lateral yellow margins on the pronotum and elytra. The species do not appear to be closely allied with the other New World species, which occur in North and Central America (Figs 36–38).

An additional species of *Cybister*, *C. parvus* Trémouilles, requires some discussion. This small Neotropical species has two metatarsal claws with the posterior claw rudimentary in both males and females. This diagnostic combination does not fit any currently recognized genera within Cybistrini, and appears to be intermediate between *Megadytes* and *Cybister*. However, we have not examined this species and are hesitant to make changes to its classification without examining it. For this reason, we leave *C. parvus* in *Cybister*, but *incerta sedis* with respect to subgenus.

In our analysis Cybistrini is divided into two distinct monophyletic groups. One includes *Austrodytes*, *Sternhydrus*, *Onychohydus* and *Spencerhydrus*, the other *Megadytes* and *Cybister*. The first group includes Australian taxa whereas the second includes South American taxa along with *Cybister*, a pantropical group. It seems highly likely that *M. (Bifurcitus)* will be found to be closely related to *M. (Trifurcitus)* (see above). The unusual genus *Regimbartina*, from west Africa, is, however, more enigmatic. Morphological characters, including the shape and number of metatarsal claws, strongly suggest a close relationship with the Australian clade.

**Character evolution**

Cybistrines retain a number of plesiomorphic features within the Dytiscinae, including adhesive setae on the male protarsi with apices that are elongate and bilaterally symmetrical (char. 12) and mandibles with a continuous line of setae from mesal margin curved along the apicoventral surface (char. 1). They exhibit an exceptional number of structural apomorphies, however (Fig. 37), making them among the most distinctive groups of diving beetles.

An important historical adult character for groups within Cybistrini is the number and type of metatarsal claws (char. 25). The plesiomorphic condition within the group is asymmetrical claws in both sexes, with the anterior claw shorter than the posterior (Figs 9–14). This is a common condition within Dytiscidae including Lancetinae and Colymbetinae, close relatives of Dytiscinae (Ruhnau & Brancucci 1984; Miller 2001; Ribera *et al.* 2002) and other Dytiscinae such as Hydaticini, Eretini, Aubehydrini and Aciliini (Miller 2000, 2001, 2003). Equal-length metatarsal claws (at least in males) are derived in the clade containing *Megadytes*. *Megadytes (Trifurcitus)* and *M. (Bifurcitus)* have equal-length claws in both males and females, whereas *M. (Megadytes)* and *M. (Paramegadytes)* have females with a small, rudimentary posterior claw. Although coded the same in the analysis, the equal-length claws in these taxa differ from those of *Dytiscus* and *Hyderodes* (and other Dytiscidae such as Copelatinae and Hydroporinae) in that they are straight. In other dytiscids, the equal-length claws are curved. Members of *Cybister* have the claws reduced with males of all species having only a single claw (except, perhaps, the Neotropical *C. parvus* (see Discussion above)).



Many species have females with a single claw, and many have females with a rudimentary posterior claw, as in species of *M. (Megadytes)* and *M. (Paramegadytes)*. This appears to be dimorphic in many species.

Coloration has also been an important historical character for grouping taxa within the tribe (chars 4, 9). The plesiomorphic condition for cybistrines is a dark green coloration with yellow lateral margins on the pronotum and elytron. This coloration is also present in *Dytiscus*. Within Cybistrini the yellow markings are reduced in several lineages including species of *M. (Megadytes)*, *M. (Paramegadytes)* and *Cybister*. *Cybister (Megadytoides) marginicollis* is phylogenetically intermediate (Fig. 36), with a yellow pronotal margin and entirely dark elytra. Within *Cybister*, absence of yellow bands was used in part to characterize the subgenus *C. (Melanectes)*. Even in specimens with reduced yellow markings, however, the margins of the elytra and pronotum are often ferruginous or testaceous.

Two species within *Cybister*, *C. lateralimarginalis* and *C. japonicus*, have historically been placed in their own subgenus based in large part on the presence in both males and females of natatory setae along the dorsal and ventral margins of the metatibia and metatarsus (char. 24). Other Cybistrini have only males with this condition, whereas females have natatory setae only along the dorsal margins. Within Dytiscidae, similar independent evolution of this character occurred in several lineages including another clade of Dytiscinae uniting Aubehydrini, Hydaticini, Eretini, and Aciliini (Miller 2000, 2001, 2003).

Males of several members of *Cybister* have a stridulatory apparatus in the form of several parallel grooves at the base of the metacoxa which apparently interfaces with the posterior surface of the metatrochanter (Fig. 23, char. 26). This includes all the species in North and Central America and two species in Africa placed by Brinck (1945) in the subgenera *C. (Nealocomerus)* and *C. (Alocomerus)*. To date, there have been no observations of any sound made using this apparatus.

Cybistrine larvae exhibit a large number of unique morphological synapomorphies including the dentate anterior margin of the clypeus (char. 28, Figs 30–32), the absence of a galea (char. 40), the presence of a ligula on the prementum in the form of a single, medial lobe (char. 42), the abdominal tergites reduced to a small, anteriorly located plate on each segment (char. 44), the ventral surface of abdominal segment VII entirely membranous (char. 45) (except in *Megadytes (Trifurcitus)*; see below), and the cerci extremely small or apparently absent (char. 46, Fig. 33). Although it is generally reported that first instar larvae have egg bursters absent in Cybistrini (e.g. Larson *et al.* 2000), recent evidence suggests that they are present in, at least, *Megadytes (Trifurcitus)* (Michat in press; pers. comm.). This character was not included since it has not been observed for the taxa included

in the analysis. Additional recent evidence presented by Michat (in press; pers. comm.) also indicates that members of *Megadytes (Trifurcitus)* have the ventral surface of abdominal segment VII sclerotized in contrast to other members of the tribe.

Sexual conflict has been implicated as a model of evolution, explaining the evolution of certain sexually dimorphic characters in both males and females of Dytiscinae (Bergsten *et al.* 2001; Miller 2003; Härdling & Bergsten in press). Although cybistrines lack the circular sucker discs present in other tribes of Dytiscinae, they do have adhesive setae in the form of large fields of protarsal setae resembling elongate, flattened structures (char. 12) and tufts of simple setae on the ventral surfaces of the mesotarsomeres (char. 17). Females of many Cybistrini are known to have modified pronota and elytra in various forms, including aciculate striae (e.g. *M. (Megadytes)*, *M. (Trifurcitus)*; char. 8) or more extensive, anastomosing lines impressed in the cuticle (e.g. *Cybister*; char. 8). The extent to which the evolution of these characters was influenced by sexual conflict has not been explored. To date, no observations have been made of the mating behaviour of cybistrines to determine whether females exhibit active resistance to male mating attempts, nor has mating been observed to determine how males are using their legs prior to or during the mating event. Nevertheless, this group may be another example of a group of organisms influenced by sexual conflict as in other Dytiscines (Bergsten *et al.* 2001; Miller 2003).

## Conclusion

Cybistrines are a group of insects remarkable for their extreme size and unique character combinations in adults and larvae. A cladistic phylogeny of the group has been previously unavailable, but the one provided here represents comprehensive taxon and character sampling and is well supported. Although the analysis solves some longstanding classification problems within the group, additional problems remain. Of the genera in the group, only *Spencerhydrus* (with two species) has been revised during modern times (Watts 1978). The large groups *Megadytes* and *Cybister* are in particular need of monographic treatments. Several unusual taxa were not included in this analysis including *Regimbartina*, *Cybister buqueti* and *C. dytiscoides* (subgenus *C. (Alocomerus)* *sensu* Brinck, 1945), and *Cybister parvus*, an unusually small *Cybister* from Brazil with several characters unusual for the genus (see above and Trémouilles 1984). Despite these missing taxa, this phylogenetic analysis forms a firm foundation for future additional research on relationships and evolution within this interesting group.

## Revised classification of the genera of Cybistrini

For full details regarding taxon names, refer to Nilsson (2001).

*Austrodytes* Watts, 1978*Regimbartina* Chatanay, 1911*Sternhydrus* Brinck, 1945, **new status***Onychohydrus* Schaum, 1847Syn *Homoeodytes* Régimbart, 1878*Spencerhydrus* Sharp, 1882*Megadytes* Sharp, 1882*Megadytes sensu stricto**M. (Bifurcitus)* Brinck, 1945*M. (Trifurcitus)* Brinck, 1945*M. (Paramegadytes)* Trémouilles & Bachmann, 1980*Cybister* Curtis, 1827Syn *Cybister sensu stricto*Syn *Alocomerus* Brinck, 1945Syn *Cybisteter* Bedel, 1881Syn *Gschwendtnerhydrus* Brinck, 1945Syn *Meganectes* Brinck, 1945Syn *Nealocomerus* Brinck, 1945Syn *Scaphinectes* Ádám, 1993, **new synonym**Syn *Trochalis* Dejean, 1833, **new synonym**Syn *Trogus* Leach, 1817*C. (Megadytoides)* Brinck, 1945*C. (Melanectes)* Brinck, 1945*C. (Neocybister)* Miller, Bergsten and Whiting, **new subgenus****Key to the genera and subgenera of Cybistrini**

- 1 Posterior metatibial spur bi- or trifurcate (Fig. 6); Neotropical.....*Megadytes* Sharp (in part) 2
- 1' Posterior metatibial spur simple (Figs 7, 8); distribution various..... 3
- 2 Posterior metatibial spur bifurcate .....*M. (Bifurcitus)* Brinck
- 2' Posterior metatibial spur trifurcate (Fig. 6).....*M. (Trifurcitus)* Brinck
- 3 Prosternal process with prominent longitudinal sulcus; Australian ..... 4
- 3' Prosternal process without prominent longitudinal sulcus though lateral margins may be variously bordered and anterior portion may be shallowly sulcate; distribution various..... 5
- 4 Metacoxal lines absent ..... *Sternhydrus* Brinck
- 4' Metacoxal lines present..... *Spencerhydrus* Sharp
- 5 Metacoxal lines absent; Australian..... *Onychohydrus* Schaum
- 5' Metacoxal lines present; distribution various ..... 6
- 6 Male with a single metatarsal claw, female either with one claw or with an additional, small posterior rudimentary claw (Figs 19–22); with posteroventral series of setae near apical margin of mesotarsomeres of males and pro- and mesotarsomeres of females (Fig. 4).....*Cybister* Curtis 7
- 6' Male and female with two metatarsal claws, though female may have posterior claw rudimentary (Figs 11,12,17,18); without posteroventral series of setae on pro- and mesotarsomeres (Fig. 3)..... 10
- 7 Elytron without distinct yellow lateral margins; female always with a second, rudimentary posterior metatarsal claw..... 8
- 7' Elytron with distinct yellow lateral margins; female with either a single metatarsal claw or, sometimes, with a second rudimentary posterior claw ..... 9
- 8 Pronotum with distinct yellow lateral margins ..... *C. (Megadytoides)* Brinck
- 8' Pronotum without distinct yellow lateral margins..... *C. (Melanectes)* Brinck
- 9 Female with a single metatarsal claw or, in some species, dimorphic with some specimens with a second, rudimentary posterior claw; distribution including North and Central America, Africa, Eurasia and Australia, absent from Neotropical region..... *C. (Cybister)* Curtis
- 9' Female always with two metatarsal claws, posterior claw short and sinuate; Neotropical species from Panama to southern South America..... *C. (Neocybister)* Miller, Bergsten and Whiting
- 10 Male with two metatarsal claws, subequal in length, female with two metatarsal claws but with posterior claw shorter (Figs 17, 18); Neotropical .....*Megadytes* Sharp (in part) 11
- 10' Male and female with claws similar, anterior claw shorter than posterior (Figs 11, 12); Australian or Ethiopian..... 12
- 11 Size large, TL > 27 mm; metasternal wings relatively short, about half length of metasternum medially.....  
.....*M. (Paramegadytes)* Trémouilles and Bachmann
- 11' Size smaller, TL < 24 mm; metasternal wings relatively long, nearly length of metasternum medially .....  
.....*M. (Megadytes)* Sharp
- 12 Dorsal surface light green with sparsely distributed, small black dots; lateral margins of prosternal process not demarcated by distinct ridge; central Ethiopian..... *Regimbartina* Chatanay
- 12' Dorsal surface dark green without black dots; lateral margins of prosternal process distinctly ridged; Australian.....  
..... *Austrodytes* Watts



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## Appendix 1

Morphological characters analysed in the cladistic analysis of Cybistrini

### Head

- 1 *Mandible, mesal line of setae*: (0) discontinuous, not extending along apicoventral surface, with an isolated patch of setae medially on ventral surface; (1) with continuous line of setae from mesal margin in curve along apicoventral surface.
- 2 *Eyes, anterior margin*: (0) not emarginate; (1) emarginate.

### Prothorax

- 3 *Prosternal process*: (0) without longitudinal groove; (1) with longitudinal groove.
- 4 *Pronotal color*: (0) without lateral yellow margin; (1) with lateral yellow margin. The presence or absence of a lateral band of yellow on the pronotum and elytron (see char. 9) has been used extensively to characterize larger groups of Cybistrini, especially within *Cybister*. However, these colour characters are not easily coded. Most dark coloured diving beetles have the lateral margins of the pronotum and elytron lighter in colour. Many *Cybister*, in particular, have these margins ferruginous or rufous. We have coded these as lacking a lateral yellow margin, however, since taxa which possess a yellow lateral margin have it very distinct. We have also chosen to divide the character into pronotal and elytral coloration since at least one species, *C. (Megadytoides) marginicollis*, has the pronotum laterally yellow with the elytron entirely black, suggesting the characteristics should be treated independently.

### Abdomen

- 5 *Series of transverse carinae dorsally on abdominal segment II*: (0) absent; (1) present.

### Elytron

- 6 *Apicoventral setal patch on elytron*: (0) absent; (1) present.
- 7 *Apicoventral setal patch on elytron*: (0) a line of short stiff setae along margin; (1) a field of short, fine setae; (2) a field of short, stiff setae.
- 8 *Female sexual sculpture on pronotum and elytron*: (0) not comprised of slender lines or striae; (1) comprised of short, aciculate striae; (2) comprised of dense, extensive anastomosing lines. Since the unique modifications to the pronotum and elytron present in *Dytiscus marginalis* and *Hyderodes shuckardi* are apomorphic we have not coded them. Within Cybistrini there appear to be two modifications to female cuticle. In species of *M. (Megadytes)* and *M. (Trifurcitus)* the modifications include a field of fine, short scratches on the pronotum and base of the elytron. In *Cybister*, the modifications are more extensive and include dense fields of long, anastomosing lines. Because of differences in form we have coded these as not homologous. The modifications appear to be variable in many species with some females modified and others not modified, or with some females more extensively modified than in others.
- 9 *Elytral color*: (0) without lateral yellow margin; (1) with lateral yellow margin. See the discussion under Character 4.

### Metathoracic wing

- 10 *Oblongum cell*: (0) oval (Fig. 1); (1) lateral margins convergent posteriorly, posterior margin short (Fig. 2). This character has not previously been compared cladistically. In members of Cybistrini the oblongum cell is narrowed posteriorly with the sides approximately straight (Fig. 2). Other diving beetles have a more oval oblongum cell with the sides curved (Fig. 1).

### Prolegs

- 11 *Male posteroapical protarsal spur*: (0) absent; (1) present.
- 12 *Apex of male ventral protarsal adhesive setae*: (0) oval or elongate, apex bilaterally symmetrical; (1) round, sucker-shaped, radially symmetrical.

### Mesolegs

- 13 *Posteroapical marginal setae on mesotibia*: (0) absent medially; (1) present, continuous line.
- 14 *Posterodorsal series of setae on mesotibia*: (0) simple; (1) bifid.
- 15 *Posteroventral series of setae on mesotibia*: (0) simple; (1) bifid.
- 16 *Male ventral adhesive setae on mesotarsomere*: (0) absent; (1) present.
- 17 *Apex of male ventral adhesive setae on mesotarsomeres*: (0) simple; (1) oval or elongate, apex bilaterally symmetrical; (2) round, sucker-shaped, radially symmetrical.

### Metalegs

- 18 *Posteroventral series of setae near apical margin of mesotarsomeres of males and pro- and mesotarsomeres of females*: (0) absent (Fig. 3); (1) present (Fig. 4).

**19** *Metacoxal process and medial portion of metacoxa*: (0) not deeply concave laterally, metacoxa entirely visible near base of metafemur; (1) deeply concave laterally such that a portion of metacoxa not visible when viewed in ventral aspect.

**20** *Oblique groove across posterior surface of metatrochanter*: (0) absent; (1) present (Fig. 5).

**21** *Natatory setae on dorsal margin of metafemur*: (0) absent; (1) present.

**22** *Anterior metatibial spur*: (0) similar to posterior spur, unmodified; (1) apically acuminate, much broader than posterior spur (Figs 6–8).

**23** *Posterodorsal series of setae on metatibia*: (0) a linear series; (1) a dense cluster (Fig. 7).

**24** *Natatory setae on posteroventral margin of metatibia and metatarsomeres of female*: (0) absent; (1) present.

**25** *Metatarsal claws*: (0) different lengths in both sexes, anterior shorter than posterior (Figs 9–14); (1) same length in both sexes (Figs 15, 16); (2) male, same length, female with posterior shorter than anterior (Figs 17, 18); (3) male with single claw, female with posterior shorter than anterior (Figs 19, 20); (4) with single claw in both sexes (Figs 21, 22). The primary difficulty with this character is the possibility of dimorphism in females of some *Cybister* species which sometimes have a rudimentary second metatarsal claw, and sometimes only a single claw. This is easily observed in some North American species such as *C. (C.) fimbriolatus*. However, in some *Cybister* species there may or may not be dimorphism. Examination of series of females convincingly attributable to particular species will help to clarify whether and to what extent this dimorphism occurs.

**26** *Male stridulatory device on metacoxa*: (0) absent; (1) present, series of close grooves on metacoxal surface that apparently interfaces with the posterodorsal surface of the metatrochanter (Fig. 23).

**27** *Metacoxal lines*: (0) absent; (1) present.

#### **Male genitalia**

**28** *Series of long setae along ventral margin of lateral lobe*: (0) absent; (1) present.

**29** *Medial margin of lobes of male eighth abdominal sternum*: (0) not emarginate (Fig. 24); (1) emarginate (Fig. 25).

**30** *Setae along apicoventral margin of male median lobe*: (0) absent; (1) present.

**31** *Median lobe of aedeagus*: (0) asymmetrical; (1) symmetrical.

#### **Female genitalia**

**32** *Female genitalia configuration*: (0) ‘hydroporine type’; (1) ‘dytiscine type’ (Miller 2001).

**33** *Accessory glands on each side of base of common oviduct*: (0) absent; (1) present (Figs 26, 27).

**34** *Thick muscles surrounding vagina*: (0) absent; (1) present (Figs 26, 27).

**35** *Series of short, stiff setae along medial margin of gonocoxosternite*: (0) absent; (1) present (Fig. 28).

**36** *Gonocoxae*: (0) fused; (1) not fused.

**37** *Subapical setal brush on gonocoxae*: (0) absent; (1) present.

#### **Larva head**

**38** *Anterior margin of clypeus*: (0) evenly curved (Fig. 29); (1) conspicuously dentate or excavated (Figs 30–32).

**39** *Number of antennal segments (of subdivided ones)*: (0) six; (1) eight; (2) nine.

**40** *Galea*: (0) absent; (1) present.

**41** *Number of maxillary palpomeres (of subdivided ones, and not including palpiger)*: (0) four; (1) six; (2) seven; (3) eight.

**42** *Apicomedial margin of labial prementum*: (0) unmodified; (1) with rounded projection; (2) bilobed, with two projections; (3) with a single, elongate, generally spinous projection.

**43** *Labial palpomeres*: (0) not secondarily subdivided; (1) secondarily subdivided.

#### **Larva abdomen**

**44** *Abdominal terga*: (0) large, covering dorsum of each segment; (1) reduced to a small sclerite located anteriorly on dorsum of segment.

**45** *Venter of abdominal segment VII*: (0) sclerotized; (1) not sclerotized.

**46** *Cerci*: (0) extremely small (Fig. 33); (1) present, elongate (Figs 34, 35).

**47** *Fringe of natatory setae on cerci*: (0) absent; (1) present along lateral margins (Fig. 35); (2) present along lateral and medial margins (Fig. 34). Additive.