

Phylogenetic placement of North American subterranean diving beetles (Insecta: Coleoptera: Dytiscidae)

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Abstract

A phylogenetic analysis of Hydroporinae (Coleoptera: Dytiscidae) is conducted with emphasis on placement of the North American subterranean diving beetles *Psychopompopus felipi* Jean, Telles & Miller, *Ereboporus naturaconservatus* Miller, Gibson & Alarie, and *Haedeoporus texanus* Young & Longley. Analyses include 49 species of Hydroporinae, representing each currently recognized tribe except Carabhydrini Watts. Data include 21 characters from adult morphology and sequences from seven genes, *12S rRNA*, *16S rRNA*, *cytochrome c oxidase I*, *cytochrome c oxidase II*, *histone III*, *elongation factor 1a*, and *wingless*. The combined data were analyzed using parsimony and mixed-model Bayesian tree estimation, and the combined molecular data were analyzed using maximum likelihood. Less inclusive branches (genera, genus-groups, and tribes) are well-supported under each criterion, but relationships among tribes are not. These branches are short, poorly supported and in conflict among the estimation methods. Because of certain anomalous relationships in the parsimony and likelihood results, and greater consistency of the Bayesian results with morphological data, this is our preferred phylogenetic estimate. *Haedeoporus* Young & Longley is related to *Neoporus* Guignot, *Heterosternuta* Guignot, and other members of the *Hydroporus*-group of genera of Hydroporini *sensu lato*. *Psychopompopus* Jean, Telles & Miller and *Ereboporus* Miller, Gibson & Alarie are nested within the *Graptodytes*-group of genera of Hydroporini *sensu lato*, and are the only North American members of this otherwise Mediterranean group. Congruent results between optimality criteria indicate that Hydroporini *sensu lato* is conspicuously non-monophyletic.

Key words

Subterranean, stygobitic, aquifer, phylogeny, water beetles.

1. Introduction

Diving beetles (Coleoptera: Dytiscidae) represent a heterogeneous and fascinating assembly of species associated with a wide range of different water habitats, including the generally considered ‘atypical’ groundwater domain. Most of the obligate dytiscid inhabitants of such environments have a characteristic, convergent morphology including reduced or absent eyes, wings, flight musculature,

and pigment as well as fused elytra and typically a strong constriction between the pronotum and elytra. Such morphology makes it easy to distinguish them from any other epigeal counterparts, regardless of the taxonomic lineage. Until recently, groundwater adapted (= stygobitic) Dytiscidae were sporadically known (GUIGNOT 1925; MILLER et al. 2009b; ORDISH 1976; PESCHET 1932; SAN-

FILIPPO 1958; YOUNG & LONGLEY 1976). The recent discovery of more than 100 species in Australia (LEYS & WATTS 2008; WATTS et al. 2007; WATTS & HUMPHREYS 1999, 2000, 2004, 2006) suggests, however, that stygobitic Dytiscidae may be more diverse and widespread than previously thought.

In North America, five subterranean species of Dytiscidae are recognized: *Ereboporus naturaconservatus* Miller, Gibson & Alarie, *Haedeoporus texanus* Young & Longley, *Stygoporus oregonensis* Larson, *Psychopompus felipi* Jean, Telles & Miller and *Comaldessus stygius* Spangler & Barr (MILLER et al. 2009b). Whereas *Stygoporus oregonensis* is found in Oregon, all others appear to be restricted to the Edwards-Trinity Aquifer of Texas (MILLER et al. 2009b). These stygobitic dytiscids belong to the large and diverse subfamily Hydroporinae, a heterogeneous grouping of minute- to moderate-sized beetles composed of nine tribes worldwide (Laccornini, Methlini, Bidessini, Hydrovatini, Hyphyrini, Vatellini, Hydroporini, Hygrotini and Carabhydrini; NILSSON 2010). Except for *C. stygius*, which clearly belongs to the tribe Bidessini (SPANGLER & BARR 1995), all North American subterranean dytiscids have been placed in the poorly-defined tribe Hydroporini, which has historically been a kind of taxonomic amalgamation of numerous genera that do not fit into other more clearly-defined tribes. Moreover, since the diagnostic features used historically in taxonomic treatments of the Hydroporini appear to be all plesiomorphic (MILLER 2001), placement of the North American subterranean hydroporine species remains questionable because of the extreme modifications consistent with their lifestyle in addition to the morphological similarities and reductions in other important characters.

This study aims at reviewing and investigating the taxonomic placement of the North America endemic stygobitic *E. naturaconservatus*, *H. texanus*, and *P. felipi* within the subfamily Hydroporinae using molecular data and adult morphological features. Since the three investigated species appear to belong to the Hydroporini *sensu lato*, a second objective of this study is to clarify relationships among taxa included in that tribe. The most comprehensive analyses of the Hydroporini published to date included those by MILLER (2001), based on morphology, and RIBERA et al. (2008), based on four molecular markers. This study is more comprehensive since both adult morphological characters and molecular data from seven markers are used to investigate Hydroporinae phylogeny.

2. Material and methods

2.1. Taxon sampling

Forty-nine species of Hydroporinae were included in this analysis from each major tribe. Particular emphasis

was placed on more thorough sampling within Hydroporini and 22 of the 37 currently recognized genera were sampled (~60%), including three subterranean diving beetles: *E. naturaconservatus*, *H. texanus*, and *P. felipi*. Included taxa are listed in Table 1. Resulting trees were rooted using *Laccornis difformis*, a member of a group (Laccornini) that has been resolved as the sister to the rest of Hydroporinae in several analyses (MILLER 2001; WOLFE 1985, 1988).

2.2. Data sampling

2.2.1. DNA

DNA was extracted using Qiagen DNEasy kit (Valencia, California, USA) and the protocol for animal tissue. Hydroporines are relatively small, and DNA was extracted by removing the abdomen at its juncture with the metathorax and placing the remaining portion of the specimen in a buffer solution. The portions of the specimens remaining after extraction and the abdomen were retained for vouchering. Vouchers and DNA are deposited in the Division of Arthropods, Museum of Southwestern Biology, University of New Mexico (MSBA, K.B. Miller) except for *Canthyporus parvus* Omer-Cooper and *Laccornellus lugubris* (Aubé), for which data were derived from GenBank (Table 1). Not all gene fragments were available for these two taxa (Table 1).

Seven gene fragments were used in the analysis, *12S rRNA* (12S, 364 aligned nucleotides), *16S rRNA* (16S, 535 aligned nucleotides), *cytochrome c oxidase I* (COI, 1256 aligned nucleotides), *cytochrome c oxidase II* (COII, 674 aligned nucleotides), *histone III* (H3, 328 aligned nucleotides), *elongation factor 1 alpha* (Ef1 α , 348 aligned nucleotides), and *wingless* (wnt, 460 aligned nucleotides). Primers used for amplification and sequencing were derived from several sources (Table 2). The 3' end of the amplified fragment of COI and the 5' and 3' ends of the fragment of COII include partial leucine and lysine tRNA coding regions. These were trimmed off because of considerable ambiguity in alignment. Data are missing for some included taxa because of difficulty in amplification or sequencing (Table 1).

DNA fragments were amplified using PCR with TaKaRa Amplitaq (Applied Biosystems, Foster City, CA, USA) on an Eppendorf Mastercycler ep gradient S Thermal Cycler (Eppendorf, Hamburg, Germany). Amplification conditions (Table 3) were similar to those used by MILLER et al. (2007, 2009a). Contamination was investigated using negative controls, and fragments produced from PCR were examined using gel electrophoresis. Products were purified using ExoSAP-IT (USB-Affymetrix, Cleveland, OH, USA) and cycle sequenced using ABI Prism Big Dye (version 3.1, Fairfax, VA, USA) using the same primers used to amplify (Table 2). Sequencing reaction products were purified using Sephadex G-50

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

Species	Code	Locality	12S 16S	COI COII	H3 Ef1 α	wnt
<i>Laccornis difformis</i> (LeConte, 1855)	KBMC Ladi16	USA, New York, St Lawrence Co., Macomb Twp, Fish Cr Marsh, 44°28.333' N 75°33.8' W, 23 May 2000, KB Miller leg., KBM2305001	KF575591 KF575544	KF575484 KF575441	KF575363 KF575406	—
<i>Methles cribratellus</i> (Fairmaire, 1880)	KBMC Mtrc459	Ghana, Volta Region, road between Nkwanta and Odumase, 8°15.537' N 0°26.562' E, 210 m, 15 Jun 2005, KB Miller leg., KBM1506051	KF575594 KF575547	KF575487 KF575444	KF575366 —	—
<i>Celina hubbelli</i> Young, 1979	KBMC Cehu442	USA, New York, Tompkins Co. Jennings Pond. 1 km SW Danby, 370 m, 42°20.825' N 76°29.233' W, 3 Sep 2002, KB Miller leg.	KF575581 KF575534	KF575475 KF575432	KF575353 KF575398	—
<i>Celina imitatrix</i> Young, 1979	KBMC Ceim533	USA, Alabama, Monroe Co., 3 km E Owassa, 31°29.791' N 86°51.184' W, 99 m, 11 May 2006, KB Miller leg., KBM1105064	KF575611 KF575563	— KF575460	KF575382 —	—
<i>Canthyporus parvus</i> Omer-Cooper, 1955	NHM- IR616	NCBI GenBank	— EF056668	EF056596 —	EF056553 —	—
<i>Laccornellus lugubris</i> (Aubé, 1838)	BMNH # 681715	NCBI GenBank	— AJ850421	AJ850668 —	EF670227 —	—
<i>Vatellus bifenestratus</i> Zimmermann, 1921	KBMC Vabi452	Peru, Madre de Dios, Explorers Inn, 12°50.208' S 69°17.603' W, 10 Dec 2003, KB Miller leg., KBM1012031	KF585004 KF585005	KF585006 KF585007	KF585003 —	—
<i>Coelambus semivittatus</i> Fall, 1919	KBMC Cosv732	USA, Wyoming, Natrona Co., ~ 12 km S Midwest, salt pool, 43°17.633' N 106°16.375' W, 10 Aug 2010, KB Miller leg.	KF575621 KF575573	KF575509 KF575469	KF575392 KF575429	KF575530
<i>Herophydrus inquinatus</i> (Boheman, 1848)	KBMC Hpin578	Namibia, Skeleton Coast NP, Uniab River, pools, 20°13.017' S 13°12.603' E, 14 May 2004, KB Miller leg., KBM1405041	KF575620 KF575572	KF575508 KF575468	KF575391 KF575428	KF575529
<i>Hygrotus acaroides</i> (LeConte, 1855)	KBMC Htac370	USA, Alabama, Conecuh Co., 13 km E Evergreen along Hwy 31, Old Town Cr., 31°27.037' N 86°49.81' W, 53 m, 11 May 2006, KB Miller leg., KBM1105061	KF575589 KF575542	KF575482 KF575439	KF575361 KF575405	KF575516
<i>Hydrovatus pustulatus</i> (Melsheimer, 1844)	KBMC Hvpu446	USA, New York, Tompkins Co., pond 1 km SW Danby, 370 m, 42° 20.825' N 26°29.233' W, 23 Sep 2002, KB Miller leg.	KF575590 KF575543	KF575483 KF575440	KF575362 —	—
<i>Queda youngi</i> Biström, 1990	KBMC Quyo455	Peru, Madre de Dios, boat landing ~20 km S Infierno, nr Puerto Maldonado, 14 Dec 2003, KB Miller leg.	KF575600 KF575553	KF575493 KF575450	KF575371 KF575412	—
<i>Microdytes svensoni</i> Miller & Wewalka, 2010	KBMC Mysv765	India, Karnataka, forest stream 30 km E Udipi, 04 Oct 2004, KB Miller leg.	KF575578 KF575531	— —	KF575350 KF575397	—
<i>Desmopachria convexa</i> (Aubé, 1838)	KBMC Dpc081	USA, New York, St Lawrence Co., Macomb Twp, Fish Cr Marsh, 44°28.333' N 75°33.8' W, 23 May 2000, KB Miller leg., KBM2305001	KF575584 KF575537	KF575477 KF575435	KF575356 —	—
<i>Hyphydrus elegans</i> (Montrouzier, 1860)	KBMC Hpel440	Australia, Northern Territory, 5km S Alice Springs, 23°44.74' S 133°52.048' E, 8 Oct 2002, KB Miller leg., KBM0810022	KF575587 KF575540	KF575480 KF575437	KF575359 KF575403	—
<i>Hyphydrus excoffieri</i> Régimbart, 1899	KBMC Hpex441	China: Yunnan, 4 km S Shisong, veg. rich lake, 11 Sep 2000, J Bergsten leg.	KF575588 KF575541	KF575481 KF575438	KF575360 KF575404	—
<i>Peschetius quadricostatus</i> (Aubé, 1838)	KBMC Pequ345	India, Maharashtra, 16°34.992' N 73°35.221' E, 1 Oct 2004, KB Miller leg., KBM0110041	KF575599 KF575552	KF575492 KF575449	— —	—
<i>Amarodytes</i> sp.	KBMC Arsp282	Peru, Madre de Dios, Explorers Inn, 12°50.208' S 69°17.603' W, 10 Dec 2003, KB Miller leg., KBM1012031	KF575580 KF575533	KF575474 KF575431	KF575352 —	—
<i>Liodes affinis</i> (Say, 1825)	KBMC Liaf132	USA, New York, Tompkins Co., Ringwood Preserve, 10 May 2001, KB Miller leg., KBM1005011	KF575592 KF575545	KF575485 KF575442	KF575364 —	—
<i>Uvarus baoulicus</i> (Guignot, 1939)	KBMC Uvba432	Ghana, Volta Region, road between Nkwanta and Odumase, 8°15.537' N 0°26.562' E, 210 m, 15 Jun 2005, KB Miller leg., KBM1506051	KF575604 KF575556	KF575496 KF575454	KF575375 —	—
<i>Graptodytes ignotus</i> (Mulsant & Rey, 1861)	KBMC Gpig575	Italy, Sardinia, Sassari Prov., 8 km N Aggius, 40°56.271' N 9°1.113' E, 521 m, 15 Apr 2006 2006, KB Miller leg., KBM1504064	KF575619 KF575571	KF575507 KF575467	KF575390 KF575427	KF575528
<i>Rhithrodytes sexguttatus</i> (Aubé, 1838)	KBMC Rrse508	Italy, Sardinia, Nuoru Prov., 4.5 km SE Bottidda, 40°20.332' N 9°1.785' E, 200 m, 16 Apr 2006, KB Miller leg., KBM1604063	KF575605 KF575557	KF575497 KF575455	KF575376 KF575415	KF575521
<i>Stictonectes rufulus</i> (Aubé, 1838)	KBMC Scru511	Italy, Sardinia, Nuoru Prov., river upstream from Lake Flumendosa, 39°58.943' N 9°24.722' E, 785 m, 18 Apr 2006, KB Miller leg., KBM1804063	KF575606 KF575558	— KF575456	KF575377 KF575416	—
<i>Stictonectes optatus</i> (Seidlitz, 1887)	KBMC Scop512	Italy, Sardinia, Nuoru Prov., river upstream from Lake Flumendosa, 39°58.943' N 9°24.722' E, 785 m, 18 Apr 2006, KB Miller leg., KBM1804063	KF575607 KF575559	KF575498 KF575457	KF575378 KF575417	KF575522

Table 1 continued.

Species	Code	Locality	12S 16S	COI COII	H3 Ef1 α	wnt
<i>Suphrodytes dorsalis</i> (Fabricius, 1787)	KBMC Sudo554	Sweden, Övre Tälningan, 2 Jul 2000, J Bergsten leg.	KF575625 KF575577	KF575507 KF575472	KF575396 —	—
<i>Hydrocolus paugus</i> (Fall, 1923)	KBMC Hlpa133	Zambia, Northwestern Province, Nkunya military base, stream, 11°48.79' S 24°22.01' E, 7 Nov 2007, KB Miller leg., KBM07110704	KF575586 KF575539	KF575479 KF575436	KF575358 KF575402	KF575515
<i>Sanfilippodytes</i> sp.	KBMC Sasp78	USA, Arizona, Santa Cruz Co., Santa Cruz River nr Beyerville, 31°25.233' N 100°55.25' W, 27 Apr 2000, KB Miller leg., KBM2704002	KF575601 KF575554	KF575494 KF575451	KF575372 KF575413	KF575520
<i>Neoporus mellitus</i> (LeConte, 1855)	KBMC Npme532	USA, Alabama, Conecuh Co., 23 km SE Evergreen along CR42, 31°20.059' N 86°47.641' W, 62 m, 12 May 2006, KB Miller leg., KBM1205063	KF575610 KF575562	KF575501 KF575459	KF575381 KF575420	KF575524
<i>Heterosternuta pulcher</i> (LeConte, 1855)	KBMC Hepu136	USA, New York, Tompkins Co. Fall Cr., Ithaca, 15 Sep 1999, K.B. Miller leg.	KF575585 KF575538	KF575478 KF575472	KF575357 KF575401	—
<i>Hydroporus palustris</i> (Linnaeus, 1761)	KBMC Hypa548	Sweden, Härjedalen: Rogen: Hundviken, 30 Jun 2001, J Bergsten leg.	KF575623 KF575575	KF575510 KF575470	KF575394 —	—
<i>Hydroporus angustatus</i> Sturm, 1835	KBMC Hyan549	Russia, Volgograd Obl., Krasnoslobodsk, pond, 15 May 2001, J Bergsten leg.	KF575624 KF575576	KF575511 KF575471	KF575395 —	—
<i>Nebrioporus clarkii</i> (Wollaston, 1862)	KBMC Nbcl513	Italy, Sardinia, Nuoru Prov., river upstream from Lake Flumendosa, 39°58.943' N 9°24.722' E, 785 m, 18 Apr 2006, KB Miller leg., KBM1804063	KF575608 KF575560	KF575499 —	KF575379 KF575418	KF575523
<i>Nebrioporus rotundatus</i> (LeConte, 1863)	KBMC Nbro563	USA, New York: Tompkins Co., Ithaca, 21 Jun 2001, KB Miller leg.	KF575618 KF575570	KF575506 —	KF575389 KF575426	—
<i>Stictotarsus roffii</i> (Clark, 1862)	KBMC Stro26	USA, Arizona, Santa Cruz Co., Santa Cruz River nr Beyerville, 31°25.233' N 100°55.25' W, 27 Apr 2000, KB Miller leg., KBM2704002	KF575603 KF575555	KF575495 KF575453	KF575374 KF575414	—
<i>Oreodytes scitulus</i> (LeConte, 1855)	KBMC Orsc63	USA, Colorado, Pitkin Co., Crystal River nr Marble, 39°4.25' N 107°12.4' W, 1315 m, 2 Aug 2000, KB Miller leg., KBM0208001	KF575598 KF575551	KF575491 KF575448	KF575370 KF575411	—
<i>Oreodytes congruus</i> (LeConte, 1878)	KBMC Orco62	USA, Colorado, Pitkin Co., Crystal River nr Marble, 39°4.25' N 107°12.4' W, 1315 m, 2 Aug 2000, KB Miller leg., KBM0208001	KF575597 KF575550	KF575490 KF575447	KF575369 KF575410	KF575519
<i>Oreodytes quadrimaculatus</i> (Horn, 1883)	KBMC Orqu543	USA, Oregon, Lane Co., Heceta Head, Devil's Elbow, Cape Cr. 01 Sep 2001, KB Miller leg.	KF575615 KF575567	— KF575464	KF575386 KF575424	KF575527
<i>Megaporus hamatus</i> (Clark, 1862)	KBMC Mpha96	Australia, New South Wales, 6 km SW Braidwood, 35°29.867' S 149°44.15' E, 2 Nov 2000, KB Miller leg., KBM0211001	KF575593 KF575546	KF575486 KF575443	KF575365 KF575407	KF575517
<i>Megaporus howittii</i> (Clark, 1862)	KBMC Meho547	Australia, Victoria, pond ~ 25 km S Casterton, Bottletrap, 37°39.289' S 141°14.380' E, 3 Oct 2002, KB Miller leg.	KF575617 KF575569	KF575505 KF575466	KF575388 —	—
<i>Necterosoma susanna</i> Zwick, 1979	KBMC Ntsu443	Australia, New South Wales, Deva River, 20 km NW Moruya, 35°53.046' S 144°58.756' E, 1 Oct 2002, KB Miller leg., KBM0110021	KF575595 KF575548	KF575488 KF575445	KF575367 KF575408	KF575518
<i>Necterosoma undecimlineatum</i> (Babington, 1841)	KBMC Ntwa95	Australia, New South Wales, nr Brogo Dam, 36°32.067' S 149°46.7' E, 1 Nov 2000, KB Miller leg., KBM0111001	KF575596 KF575549	KF575489 KF575446	KF575368 KF575409	—
<i>Chostonectes gigas</i> (Boheman, 1858)	KBMC Chgi444	Australia, Victoria, nr Carranballac, 37°42.758' S 142°45.868' E, 3 Oct 2002, KB Miller leg., KBM0310021	KF575582 KF575535	KF575476 KF575433	KF575354 KF575399	KF575513
<i>Chostonectes nebulosus</i> (MacLeay, 1871)	KBMC Chne545	Australia, South Australia, Victoria R. at Williamstown, 12 Oct 2002, 34°40.372' S 138°53.448' E, KB Miller leg.	KF575616 KF575568	KF575504 KF575465	KF575387 KF575425	—
<i>Sternopriscus tasmanicus</i> Sharp, 1882	KBMC Snta99	Australia, Victoria, nr Won Wron, 38°24.55' S 146°45.22' E, 30 Oct 2000, KB Miller leg., KBM3010001	KF575602 —	— KF575452	KF575373 —	—
<i>Antiporus femoralis</i> (Boheman, 1858)	KBMC Anfe100	Australia, New South Wales, 6 km SW Braidwood, 35°29.867' S 149°44.15' E, 2 Nov 2000, KB Miller leg., KBM0211001	KF575579 KF575532	KF575473 KF575430	KF575351 —	KF575512
<i>Antiporus blakei</i> (Clark, 1862)	KBMC Anbl541	Australia, Victoria, grassy roadside pool nr Carranballac, 3 Oct 2002, 37°42.758' S 142°45.868' E, KB Miller leg.	KF575614 KF575566	KF575503 KF575463	KF575385 KF575423	KF575526
<i>Psychopompus felipi</i> Jean et al., 2012	KBMC Hysp731	USA, Texas, Val Verde Co., San Felipe Springs, Del Rio, 15 Aug 2008, Foley leg.	KF575622 KF575574	— —	KF575393 —	—
<i>Ereboporus naturaconservatus</i> Miller et al., 2009	KBMC Ebna538	USA, Texas, Terrell Co., Caroline Sp., 19 May 2007, Denton and Gibson leg.	KF575613 KF575565	KF575502 KF575462	KF575384 KF575422	KF575525
<i>Haideoporus texanus</i> Young & Longley, 1976	KBMC Hite536	USA, Texas, Comal Co., Comal Springs Run 3, 8 Nov 2006, Gibson leg.	KF575612 KF575564	— KF575461	KF575383 KF575421	—

Table 2. Primers used for amplification and sequencing.

Gene	Primer	Direction	Sequence (5'–3')
12S	12Sai ¹	For	AAA CTA CGA TTA GAT ACC CTA TTA T
	12Sbi ¹	Rev	AAG AGC GAC GGG CGA TGT GT
16S	16S A ¹	For	CGC CTG TTT ATC AAA AAC AT
	16S B ¹	Rev	CTC CGG TTT GAA CTC AGA TCA
COI	C1-J-1718 ("Mtd6") ²	For	GGA GGA TTT GGA AAT TGA TTA GTT CC
	C1-J-1751 ("Ron") ²	For	GGA TCA CCT GAT ATA GCA TTC CC
	C1-J-2183 ("Jerry") ²	Rev	CAA CAT TTA TTT TGA TTT TTT GG
	C1-N-2191 ("Nancy") ²	Rev	CCC GGT AAA ATT AAA ATA TAA ACT TC
	Emb-COIF1 ³	For	GTW ATA CCM ATY ATA ATT GGW GG
	Emb-COIR3 ³	Rev	RGT WGC TGA WGT RAA RTA RGC TC
	TL2-N-3014 ("Pat") ²	Rev	TCC AAT GCA CTA ATC TGC CAT ATT A
COII	F-lue ⁴	For	TCT AAT ATG GCA GAT TAG TGC
	R-lys ⁴	Rev	GAG ACC AGT ACT TGC TTT CAG TCA TC
	9b ⁴	Rev	GTA CTT GCT TTC AGT CAT CTW ATG
Ef1α	For3 ⁵	For	GGY GAC AAY GTT GGT TTY AAY
	Cho10 ⁵	Rev	ACR GCV ACK GTY TGH CKC ATG TC
H3	Haf ⁶	For	ATG GCT CGT ACC AAG CAG ACG GC
	Har ⁶	Rev	ATA TCC TTG GGC ATG ATG GTG AC
Wnt	Wnt1 ⁷	For	GAR TGY AAR TGY CAY GGY ATG TCT GG
	Wnt2a ⁷	Rev	ACT ICG CAR CAC CAR TGG AAT GTR CA
	WgDytF1 ⁸	For	CGY CTT CCW TCW TTC CGW GTY ATC
	WgDytR1 ⁸	Rev	CCG TGG ATR CTG TTV GCH AGA TG

¹SVENSON & WHITING (2004); ²SIMON et al. (1994); ³MILLER & EDGERLY (2008); ⁴WHITING (2002); ⁵DANFORTH et al. (1999); ⁶COLGAN et al. (1998); ⁷BROWER & EGAN (1997); ⁸MILLER (2003)

Table 3. Amplification conditions used in PCR reactions.

Gene	Step	Denature/hotstart	Anneal	Extension	Cycles
H3	1	94°C (1 min)	48–50°C (1 min)	70°C (1.5 min)	40
COI, COII	1	94°C (1 min)	54–58°C (0.5 min)	60°C (1.5 min)	5
	2		50–52°C (0.5 min)		5
	3		45°C (0.5 min)		30
12S, 16S, Ef1α, wnt	1	94°C (1 min)	46–54°C (1 min)	70°C (1.5 min)	40

Fine or Medium (GE Healthcare, Uppsala, Sweden) and sequenced using an ABI 3130xl Genetic analyzer (Applied Biosystems, Foster City, CA, USA) in the Molecular Biology Facility at the University of New Mexico. Gene regions were sequenced in both directions. Resulting sequence data were examined and edited using the program Sequencher (GENECODES 1999), and sequences are deposited in GenBank (Table 1).

Sequence alignment was done using the program MUSCLE (EDGAR 2004) and subsequently examined for potential alignment ambiguities. Ef1α, COI, COII, and H3 are length-invariant in the sampled taxa and alignment was unambiguous. Wingless exhibits length variation that comprises three nucleotide (at position 143 in the aligned data) and six nucleotide (at position 188) indels, and the MUSCLE alignment required slight manual adjustment to conserve the reading frame. 12S and 16S each exhibit limited length variability, but these ambiguities are seemingly minor. Gaps were treated as missing data.

2.2.2. Morphology

Brief character descriptions are presented in the Appendix. The morphological character matrix is presented in Table 4. Characters coded for this analysis were derived from previous analyses (MILLER 2001; MILLER et al. 2006). There was no intention to make this morphological data set comprehensive. Instead, the focus is on resolving relationships among Hydroporini and morphological features emphasizing that tribe. One character (Char. 11) was treated as additive (see Table 4 and Appendix).

2.3. Phylogenetic analysis

2.3.1. Parsimony (P)

Data were organized using WinClada (NIXON 2002). Combined molecular and morphological data were ana-

Table 4. Data matrix of assigned states of 21 morphological characters for 49 species of Dytiscidae. Characters marked with “+” are additive. Characters coded with “?” are unobserved.

Character	00000	00001	11111	11112	2
	12345	67890	12345	67890	1
	+				
<i>Laccornis difformis</i>	00000	00000	20010	01000	0
<i>Methles cribratellus</i>	00000	01010	20010	01000	0
<i>Celina hubbelsi</i>	01000	01010	20010	01000	0
<i>Celina imitatrix</i>	01000	01010	20010	01000	0
<i>Canthyporus parvus</i>	00000	00000	20010	00000	0
<i>Laccornellus lugubris</i>	00000	00000	20010	00000	0
<i>Vatellus bifenestratus</i>	00000	00000	11010	00100	0
<i>Coelambus semivittatus</i>	00000	00100	10010	00100	0
<i>Herophydrus inquinatus</i>	10000	00100	10010	00001	0
<i>Hygrotus acaroides</i>	10000	00100	10010	00001	0
<i>Hydrovatus pustulatus</i>	10001	00110	10110	00000	0
<i>Queda youngi</i>	10001	00100	10110	00000	0
<i>Microdytes svensoni</i>	10000	00100	00000	00100	0
<i>Desmopachria convexa</i>	10000	10100	00000	00100	0
<i>Hyphydrus elegans</i>	10000	00100	00000	00100	0
<i>Hyphydrus excoffieri</i>	10000	00100	00000	00100	0
<i>Amarodytes</i> sp.	00010	10000	10011	00110	0
<i>Peschetius quadricostatus</i>	00000	10000	10011	00110	0
<i>Liodessus affinis</i>	00010	10001	10011	00110	1
<i>Uvarus baoulicus</i>	00010	10001	10011	00110	1
<i>Graptodytes ignotus</i>	00100	00000	11010	00100	0
<i>Rhithrodytes sexguttatus</i>	00100	00000	11010	10100	0
<i>Stictonectes rufulus</i>	00000	00000	11010	10100	0
<i>Stictonectes optatus</i>	00000	00000	11010	10100	0
<i>Suphrodytes dorsalis</i>	00000	00000	11010	00100	0
<i>Hydrocolus paugus</i>	00000	00000	10010	00100	0
<i>Sanfilippodytes</i> sp.	00000	00000	10010	00100	0
<i>Neoporus mellitus</i>	00000	00000	11010	00100	0
<i>Heterosternuta pulcher</i>	00000	00000	11010	00100	0
<i>Hydroporus palustris</i>	00000	00000	11010	00100	0
<i>Hydroporus angustatus</i>	00000	00000	11010	00100	0
<i>Nebrioporus clarki</i>	00000	00000	11010	00100	0
<i>Nebrioporus rotundatus</i>	00000	00000	11010	00100	0
<i>Stictotarsus roffi</i>	00000	00000	11010	00100	0
<i>Oreodytes scitulus</i>	00100	00000	11010	00100	0
<i>Oreodytes congruus</i>	00100	00000	11010	00100	0
<i>Oreodytes quadrimaculatus</i>	00100	00000	11010	00100	0
<i>Megaporus hamatus</i>	00000	00000	11010	00100	0
<i>Megaporus howitti</i>	00000	00000	11010	00100	0
<i>Necterosoma suzannae</i>	00000	00000	11010	00100	0
<i>Necterosoma wallastoni</i>	00000	00000	11010	00100	0
<i>Chostonectes gigas</i>	00000	00000	11010	00100	0
<i>Chostonectes nebulosus</i>	00000	00000	11010	00100	0
<i>Sternopriscus tasmanicus</i>	00000	00000	11010	00100	0
<i>Antiporus femoralis</i>	00000	00000	11010	00100	0
<i>Antiporus blakei</i>	00000	00000	11010	00100	0
<i>Psychopompus felipi</i>	00000	00000	11010	?????	0
<i>Ereboporus naturaconservatus</i>	00000	00000	11010	10100	0
<i>Haideoporus texanus</i>	00000	00000	11010	00100	0

lysed using parsimony in the program NONA (GOLOBOFF 1995), as implemented from within WinClada with the ‘heuristics’ option and the commands set to hold 10000 trees total (‘h 10000’), 100 replications (‘mu*100’), 50 trees held per replication (‘h/50’), and multiple TBR + TBR (‘max*’). Support for branches was measured us-

ing bootstrap values. These were calculated in NONA as implemented by WinClada using 1000 replications, 10 search reps, one starting tree per rep, ‘don’t do max*(TBR)’, and saving the consensus of each replication. Results were examined under different optimizations using WinClada (NIXON 2002) and Mesquite (MADDISON & MADDISON 2004).

2.3.2. Maximum Likelihood (ML)

A bootstrap likelihood analysis was conducted using RaxML v7.2.6 (STAMATAKIS 2006). Morphology was not included. Data were divided into seven partitions, ribosomal (12S and 16S) [1], mitochondrial protein coding (COI and COII) codon positions (first [2], second [3] and third [4]), and nuclear protein coding (H3, wingless, and EF1 α) codon positions (first [5], second [6] and third [7]). One thousand non-parametric bootstrap replications were performed, estimating the parameters of a GTR-CAT model of nucleotide substitution independently from each partition. Every fifth bootstrap tree was then used as a starting tree for more thorough optimization of the real data using GTR-GAMMA. Results were examined and interpreted using FigTree (RAMBAUT 2006–2009).

2.3.3. Bayesian Methods (B)

A partitioned Bayesian analysis of combined molecular and morphological data was conducted using MrBayes 3.1.2 (HUELSENBECK & RONQUIST 2001). The molecular data were partitioned as described under the likelihood methods (section 2.3.2.) and parameters of a GTR+I+G substitution model were unlinked across partitions. Morphology was modeled with the MK1 default model. Two separate searches were conducted each with four Markov Chain Monte Carlo runs for 15,000,000 generations sampled every 2000th generation. The analyses began sampling from the stationary distribution almost immediately, so trees were combined from each run using a burnin of zero. Trees were pooled and summarized using the treeannotator module of BEAST (DRUMMOND et al. 2012) to find the topology with the maximum clade credibility. Results were examined and interpreted using FigTree (RAMBAUT 2006–2009).

3. Results

The parsimony analysis resulted in three equally optimal cladograms (consensus in Fig. 1, length = 11,414, CI = 25, RI = 32). The ML tree (Fig. 2) had a likelihood of -46758.223578. The Bayesian analysis resulted in a maximum clade credibility (MAX) tree shown in Fig. 3

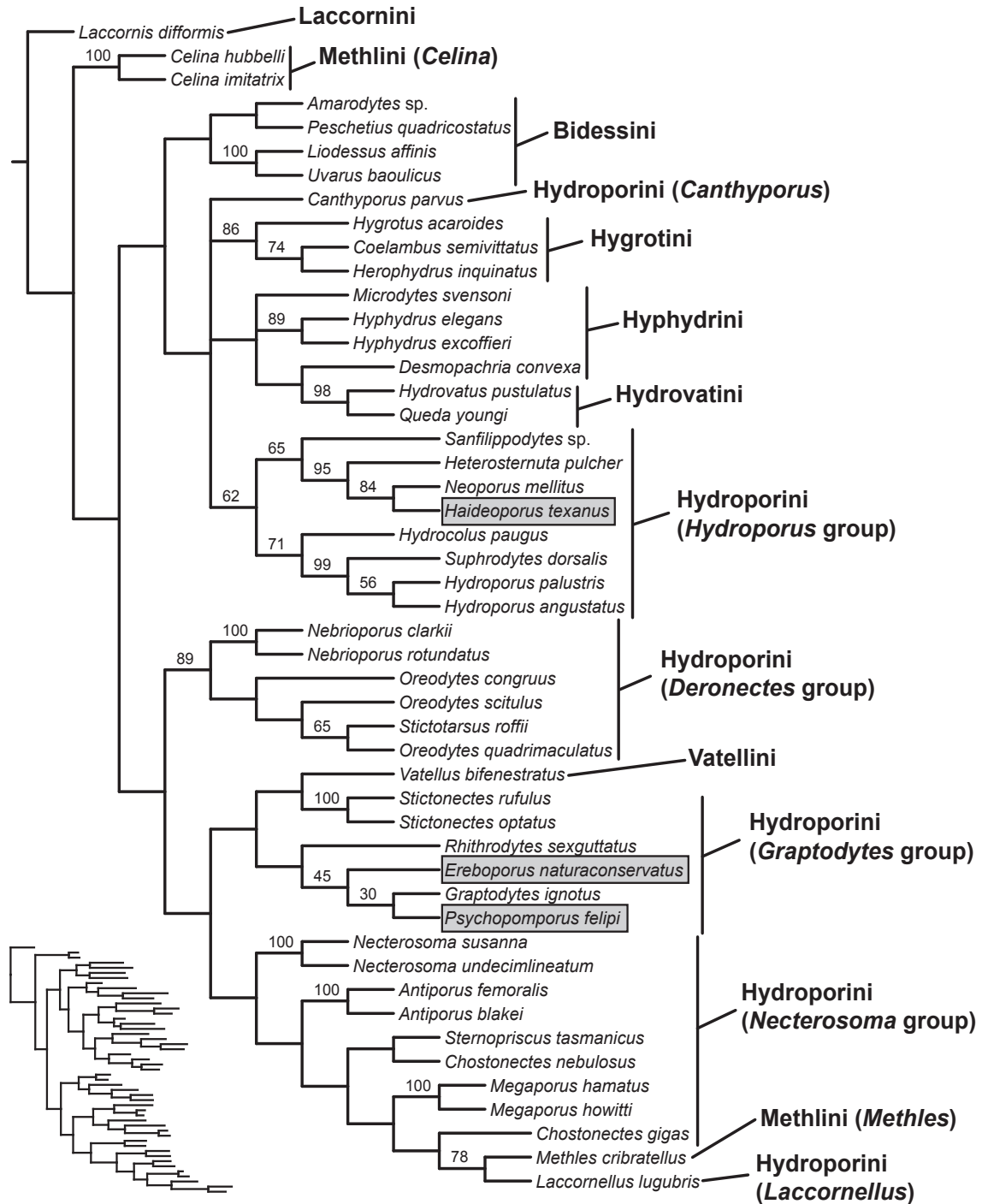


Fig. 1. Consensus cladogram of three most parsimonious trees from cladistic analysis of combined morphology and seven genes (length of trees = 11,414, CI = 25, RI = 32). Numbers above branches are bootstrap values. Small tree in lower corner is one of three parsimony trees (chosen arbitrarily) with relative branch lengths indicated with characters mapped using "fast" (ACCTRAN) optimization. Subterranean taxa marked with gray boxes.

(likelihood of -49446.8632). An SH test (SHIMODAIRA & HASEGAWA 1999) was conducted comparing the ML and MAX trees. The SH test results indicate that the MAX tree (which includes morphology whereas the ML tree does not) is not significantly worse than the ML tree ($ML_{BEST} = -46758.162949$; $ML_{MAX} = -46788.208029$; $D(LH) = -30.045080$; $SD = 29.314945$).

Support across the resulting trees from each optimality criterion is stronger for less inclusive groupings

(genera and tribes) than for more inclusive groupings (relationships among tribes). This is consistent with very long terminal branch lengths as compared with those in the tree 'backbone', which are very short in many cases. Conflict across optimality criteria also reflects this, with relationships congruent at the genus and tribe level, but not among tribes.

Congruent results across estimates at the tribe level include monophyly of Bidessini ($P_{bt} < 50$; ML

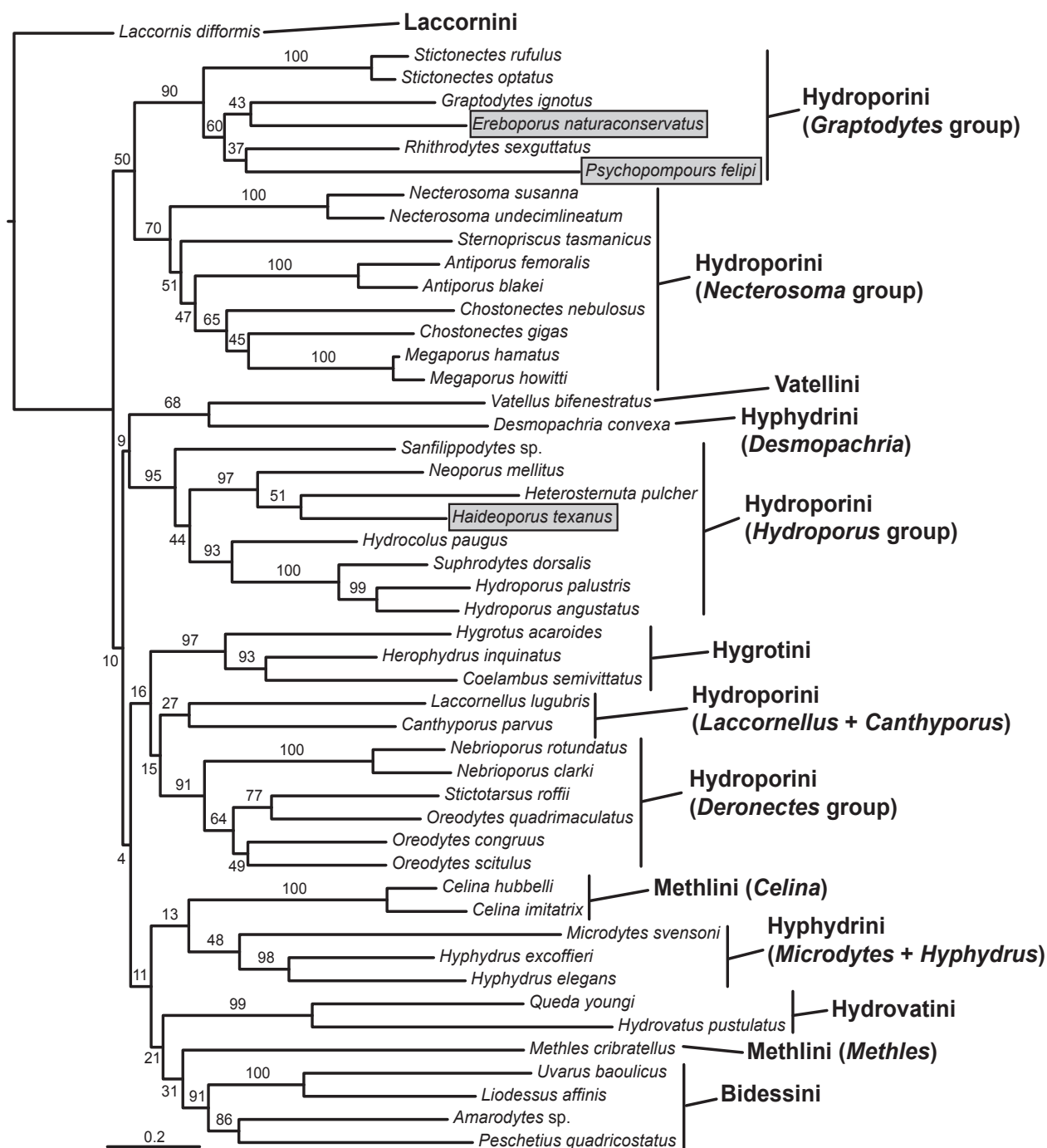


Fig. 2. ML tree estimated from molecular data. Numbers at branches are bootstrap values. Subterranean taxa marked with gray boxes.

bt = 91; B pp = 100), Hygrotini (P bt = 86; ML bt = 97; B pp = 100), and Hydrovatini (P bt = 98; ML bt = 99; B pp = 100). Hyphydrini is monophyletic in the Bayesian tree (pp = 99), but in the parsimony tree is paraphyletic with respect to Hydrovatini and in the ML tree is monophyletic except *Desmopachria* is resolved as sister to *Vatellus* (Vatellini). Methlini is monophyletic only in the Bayesian tree (pp = 100) with each of the other analyses resolving *Methles* separately from *Celina*. Hydroporini, as traditionally defined, is not monophyletic under any estimate. Monophyly of Laccornini and Vatellini was not examined in this analysis.

4. Discussion

4.1. Comparison between analyses

Parsimony, maximum likelihood, and Bayesian analyses recovered trees with very long terminal branches and much shorter internal branches. The latter appears to be related to incongruence among tree estimates since relationships subtended by short branches are the most

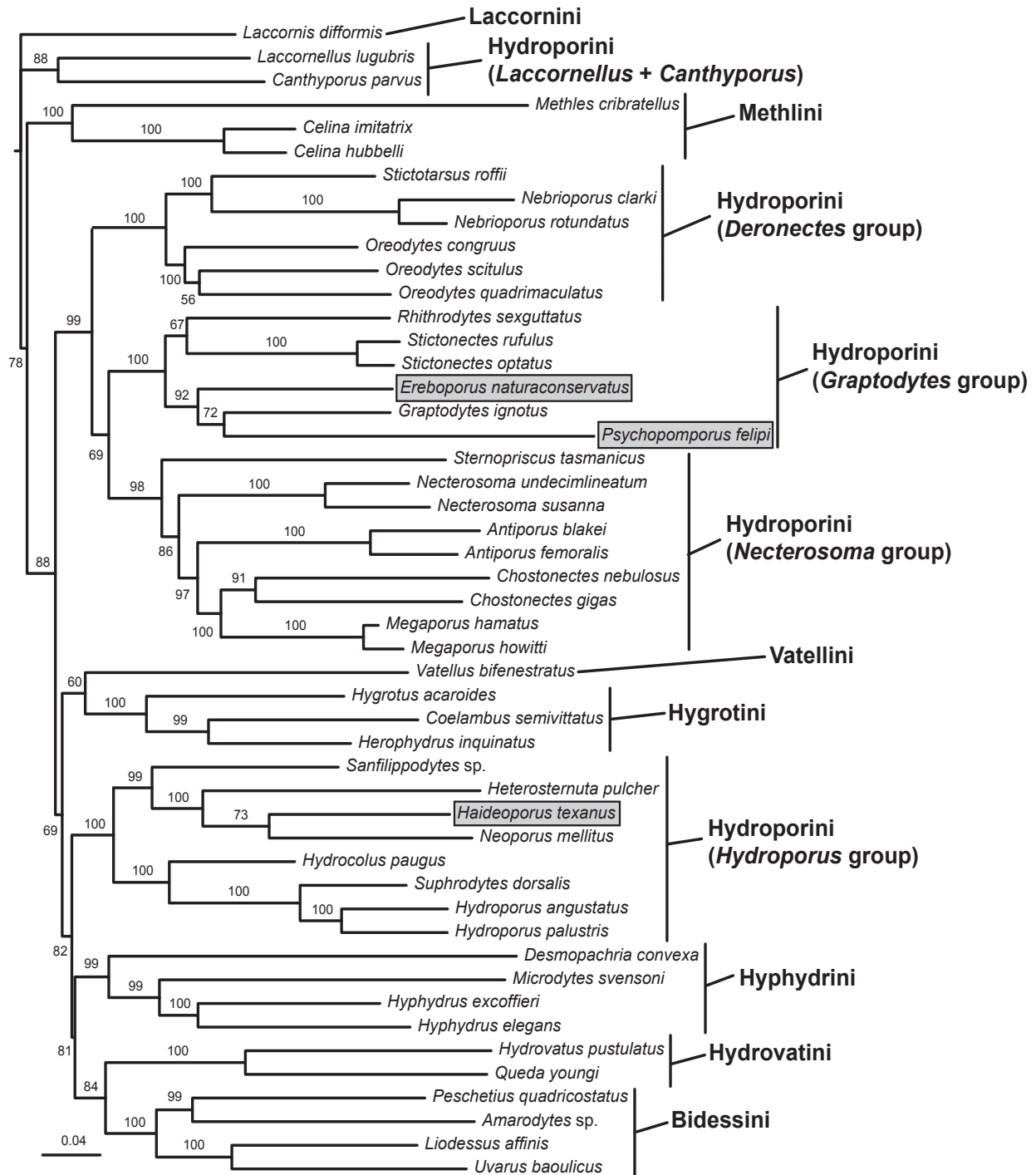
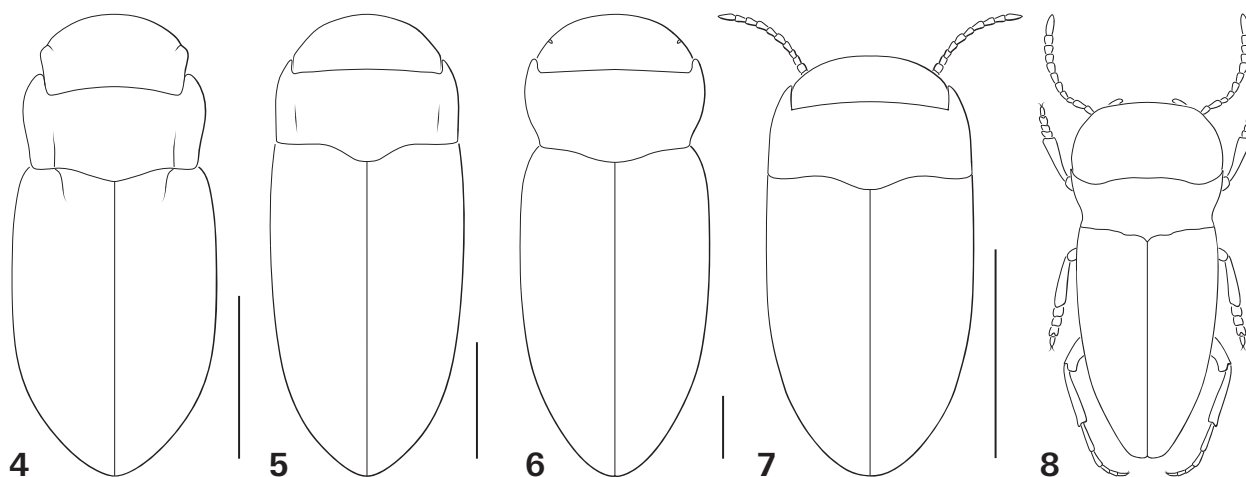


Fig. 3. Tree resulting from Bayesian analysis of combined DNA and morphological data. Numbers at branches are posterior probability values. Subterranean taxa marked with gray boxes.

rearranged among analyses and are the most poorly supported. Some of the results may be subject to long-branch effects causing discrepancy between the Bayesian/ML analyses and parsimony. The Bayesian analysis resulted in relationships that are most consistent with historical classification and morphology but are contradicted by the likelihood and parsimony results. These consistent relationships include 1) monophyly of Hyphydrini (pp = 99; BISTRÖM et al. 1997; MILLER 2001), 2) monophyly of Methlini (pp = 100; MILLER 2001; WOLFE 1985, 1988),

and 3) sister group relationship of Methlini, *Laccornellus* and *Canthyporus* with the rest of Hydroporinae except *Laccornis* (ROUGHLEY & WOLFE 1987; WOLFE 1985, 1988). The parsimony and likelihood analyses yielded some unexpected results that do not seem reasonable since they are at odds with well-founded historical ideas about classification and morphology and are relatively poorly supported such as, in likelihood, 1) sister relationship between *Desmopachria* and *Vatellus* (bt = 68), 2) sister relationship between *Methles* and Bidessini



Figs. 4–8. North American stygobitic diving beetles, habitus. 4: *Comaldessus stygius*. 5: *Stygoporus oregonensis*. 6: *Haedeoporus texanus*. 7: *Psychopompaporus felipi*. 8: *Ereboaporus naturaconservatus*. Scale bars = 0.5 mm.

(bt = 31), and 3) Methlini, *Laccornellus* and *Canthydrus* not resolved near *Laccornis*. Parsimony similarly resulted in some odd relationships including 1) Vatellini nested among the *Graptodytes* group of genera (bt < 50) and 2) *Methles* sister to *Laccornellus* and this clade nested within the *Necterosoma* group of genera (bt < 50). Because of anomalous and poorly-supported relationships of taxa resulting from both parsimony and likelihood (and absence of morphology in the likelihood analysis) our preferred conclusions about relationships are based on the Bayesian analysis (Fig. 3).

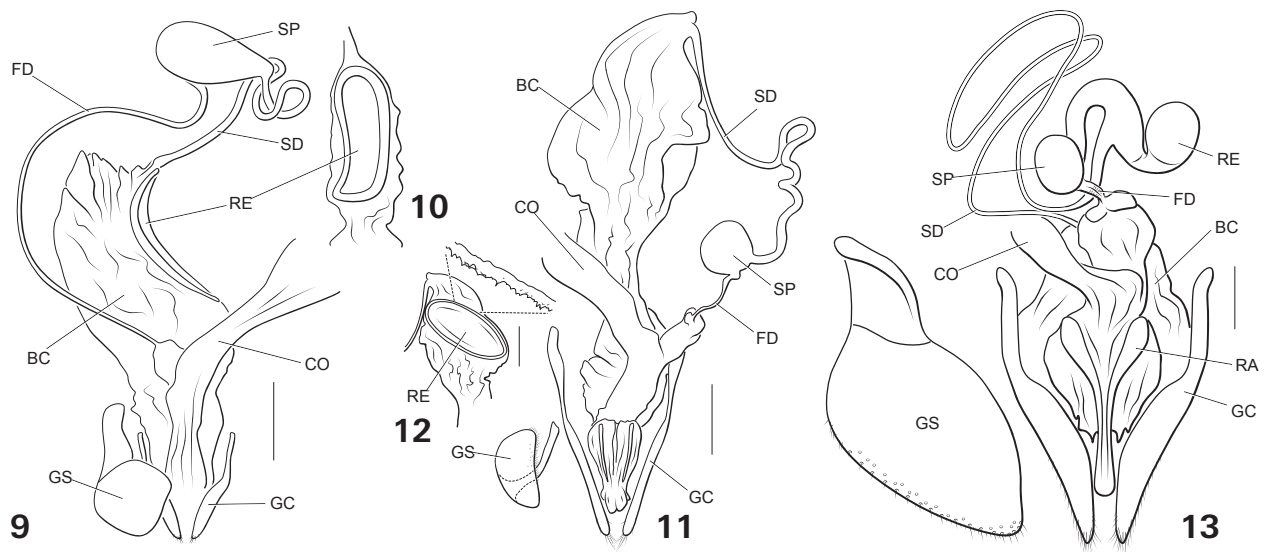
4.2. Relationships among Hydroporinae genera

This study focused primarily on resolving relationships of North American subterranean diving beetles and relationships among the Hydroporini (interpreted further below) and only secondarily on relationships among other Hydroporinae tribes and genera. Although taxon sampling among other hydroporines is not extensive, some interesting conclusions can be made based on these results. Bidessini is evidently monophyletic with strong support (Figs. 1–3), including the genera *Peschetius* and *Amarodytes*, confirming results presented by MILLER et al. (2006). Placement of *Peschetius* in Bidessini was questioned by RIBERA et al. (2008), but the presence of a spermathecal spine and a five-lobed transverse tooth of the proventriculus are convincing morphological synapomorphies of this genus along with other Bidessini taxa (MILLER et al. 2006), and the genus is retained in Bidessini here. Hydrovatini, including the genera *Queda* and *Hydrovatus*, is also monophyletic (pp = 100) supporting work by BISTRÖM (1990); however, Hydrovatini is not evidently near Methlini as sister to much of the remaining Hydroporinae as promoted by WOLFE

(1985, 1988). Hyphydrini is monophyletic (pp = 99) and closely associated with the monophyletic groups Hygro-tini (pp = 86) and Hydrovatini. *Pachydrus* and *Heterhydrus*, placed in Pachydrini by BISTRÖM et al. (1997), were not included here. The status of Pachydrini remains somewhat ambiguous based on recent analyses (MILLER 2001; RIBERA et al. 2008). Vatellini was not examined for monophyly, though there is little doubt that the group is monophyletic based on numerous synapomorphies as shown previously (MILLER 2005). Its relationships with other hydroporines remains ambiguous, however, with no clear resolution derived from these analyses or any other recent ones.

4.3. Relationships of North American subterranean diving beetles

Subterranean diving beetles typically are highly-modified for life in aquifers or caves with several related evidently convergent adult features (not all features occurring in the same combination in all taxa). These include 1) depigmentation, 2) flightlessness, often combined with reduced metathoracic wings, reduced flight musculature and fused elytra, 3) reduced or obliterated compound eyes, 4) reduction in swimming ability and related swimming adaptations such as natatory setae, 5) enhanced tactile setae, 6) cordate pronota with distinct constrictions between the pronotum and elytra, and 7) reduced prosternal processes often not reaching the metasternum (FRANCISCOLO 1983; MILLER et al. 2009b; SPANGLER 1986). North American taxa are consistent with other Dytiscidae around the world in having these modifications (Figs. 4–8). These features seem likely to be homoplasious and potentially confound actual relationships, and it has already been shown that, taken together, subterranean div-



Figs. 9–13. Female genitalia, diving beetles. **9–10:** *Ereboporus naturaconservatus*; **9:** ventral aspect (right gonocoxosternite omitted), **10:** receptacle on bursa, lateral aspect. **11–12:** *Stictonectes epipleuricus*; **11:** ventral aspect (right gonocoxosternite omitted), **12:** receptacle on bursa, dorsal aspect. **13:** *Nebrioporus subrotundus*, ventral aspect (right gonocoxosternite omitted). Scale bars = 0.25 mm. BC = Bursa copulatrix; CO = Common oviduct; FD = Fertilization duct; GC = Gonocoxa; GS = Gonocoxosternite; RA = Ramus; RE = Receptacle; SD = Spermathecal duct; SP = Spermatheca.

ing beetles of the world belong to several clades (BALKE et al. 2004; LEYS & WATTS 2008; MILLER et al. 2009b). SMRŽ (1981) placed the several known subterranean genera at that time into a tribe, Sietitiini Smrž, but this group, as he conceived it, is most likely polyphyletic as it was based on evidently convergent character states.

Since *Comaldessus stygius* (not included in this analysis) is unambiguously a member of Bidessini (SPANGLER & BARR 1995), Nearctic species of subterranean dytiscids belong to at least three only distantly-related groups. *Haedeoporus texanus* is most closely related to a clade of mainly Nearctic taxa including *Heterosternuta*, *Neoporus*, *Sanfilippodytes*, *Hydrocolus*, the Holarctic *Hydroporus* and the Palaearctic *Suphrodytes* (pp = 100, Fig. 3), called the “*Hydroporus* group” of genera (in part) by RIBERA et al. (2002, 2008). The other two included species, *Ereboporus naturaconservatus* and *Psychopompopus felipi* are each part of a clade that includes the Palaearctic *Stictonectes*, *Graptodytes*, and *Rithrodytes* (pp = 100, Fig. 3) and probably *Porhydrus* and *Metaporus* (not included), called the “*Graptodytes* group” of genera by RIBERA et al. (2002, 2008), RIBERA & FAILLE (2010), and SEIDLITZ (1887). The *Graptodytes* group includes several Palaearctic subterranean diving beetles including at least one species of *Graptodytes* and the genera *Iberoporus* and *Sietitia* which are also subterranean (ABEILLE DE PERRIN 1904; CASTRO & DELGADO 2001; RIBERA & FAILLE 2010). One characteristic of members of this group is an elongate ring-shaped structure on the bursa of the female which may be homologous with the receptacle of other Hydroporinae (Figs. 9–11, MILLER 2001). This feature is present in *Ereboporus* (Figs. 9, 10, MILLER et al. 2009), supporting this relationship. Female genitalic features are not known for *Psychopompopus*.

It is, perhaps, expected that *Haedeoporus texanus* would be nested within the *Hydroporus* group of genera (Figs. 1–3). Many of these taxa are lotic, often in relatively small seeps and streams. This is particularly true of *Hydrocolus* and *Sanfilippodytes* and also of certain *Neoporus* and *Heterosternuta* (LARSON et al. 2000; LARSON & LABONTE 1994). It may be a short evolutionary step from spring- and seep-inhabiting to a subterranean lifestyle, and *Haedeoporus* occurs well within the overall geographic range of the *Hydroporus* group of genera. Whether *Haedeoporus* is derived from within one of these genera, especially *Neoporus* or *Heterosternuta* (Figs. 1–3), will require a more comprehensive phylogenetic analysis of these closely related taxa to examine them for monophyly and placement of *H. texanus* among the genera and species.

Perhaps more unexpected is the evidence that *Ereboporus* and *Psychopompopus* are not closely related to the *Hydroporus* group, which is well represented in North America by epigeal species, or *Haedeoporus* (Figs. 1–3). Instead, they are nested within a different group of Hydroporini, the *Graptodytes* group, which is previously known exclusively from the Mediterranean region and Europe (RIBERA & FAILLE 2010), but includes no other known taxa in North America. Many *Graptodytes* group species are also found in lotic habitats, including small seeps and springs, and some of them are known to be subterranean including *Iberoporus* (CASTRO & DELGADO 2001), *Sietitia* (GUIGNOT 1925, 1931; RICHOUX 1980; RICHOUX et al. 1990) and one species of *Graptodytes* (RIBERA & FAILLE 2010). Surprisingly, however, the only known North American representatives of this primarily Palaearctic group (*E. naturaconservatus* and *P. felipi*) are from subterranean habitats suggesting a

potentially interesting evolutionary history between Palearctic and Nearctic representatives of this group. The considerable dissimilarity between the larvae of *Haedeoporus* and *Ereboporus* further supports their relatively distant relationships with each other (ALARIE et al. 2013).

It is not clear what the phylogenetic relationships are of *Stygoporus oregonensis*, the only known North American stygobitic species found outside of, but not in, the Edwards-Trinity aquifer. LARSON & LABONTE (1994) believed the species to be related to *Sanfilippodytes*, a member of the *Hydroporus* group of genera (see below). The evident longitudinal striae on the lateral surfaces of the pronotum (LARSON & LABONTE 1994) suggest this species could also be derived from within the *Graptodytes* group since this is a characteristic of many members of these genera, but its relationships remain to be carefully examined.

It is certainly possible (perhaps even likely) that the North American subterranean species are nested within other, more typically epigeal genera as has been shown to be the case with other subterranean diving beetle faunas of the world (e.g. BALKE et al. 2004; RIBERA & FAILLE 2010; LEYS & WATTS 2008). Until now, potential candidates for those phylogenetic affiliations were not known for the North American stygobionts. Relationships of *Haedeoporus* with *Neoporus* and *Heterosternuta*, and *Ereboporus* and *Psychopompus* with *Graptodytes*-group genera should be investigated in greater detail.

4.4. Relationships among genera of Hydroporini *sensu lato*

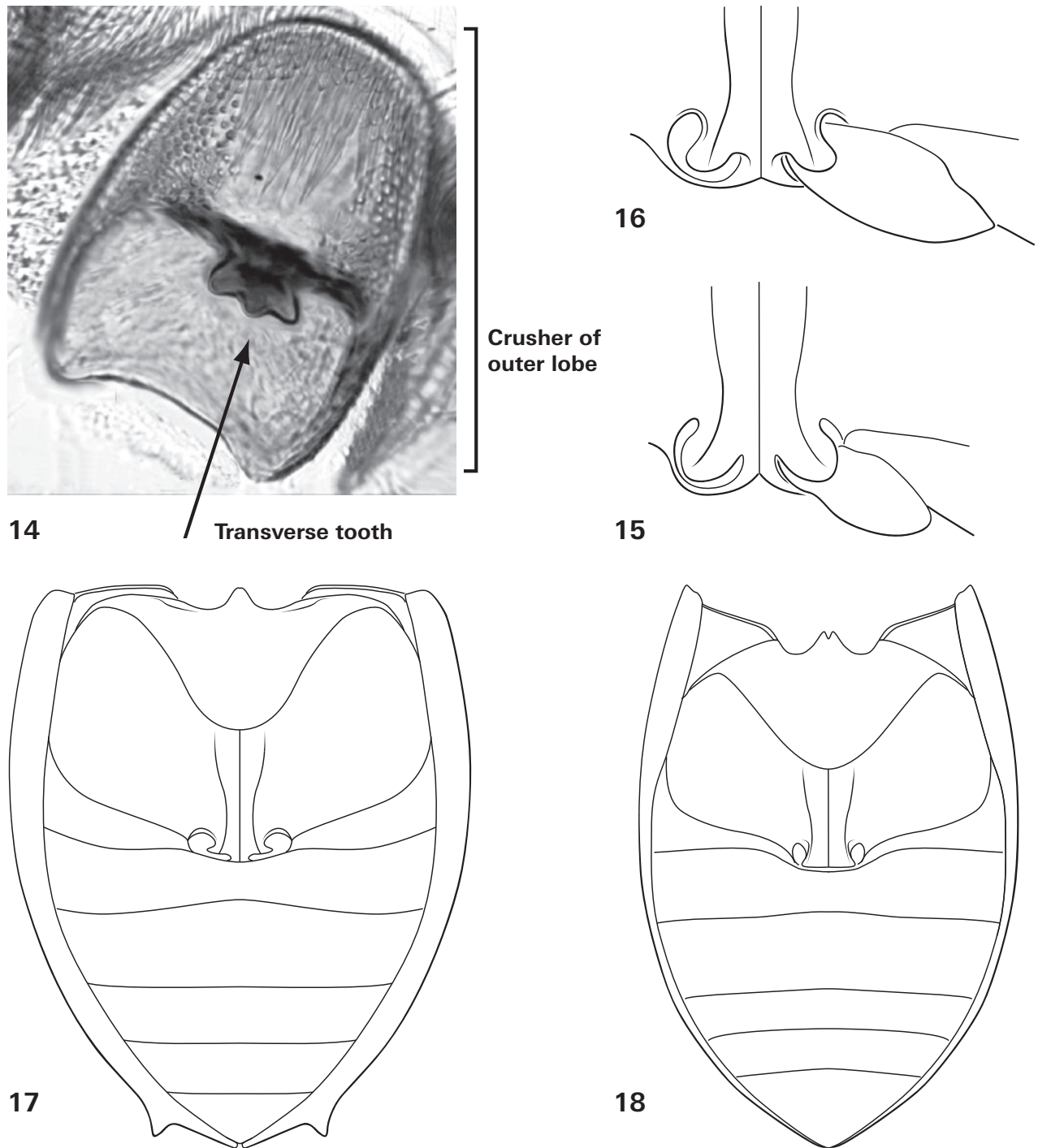
Based on these results, the tribe Hydroporini, as historically delimited, is not monophyletic. In addition to the *Hydroporus* group and the *Graptodytes* group (see above), there are at least three additional Hydroporini clades. The first is the “*Deronectes* group” of genera, which comprises mainly Holarctic taxa including *Oreodytes*, *Stictotarsus*, *Nebioporus* (pp = 100, Fig. 3) and probably *Deronectes*, *Scarodytes* and *Boreonectes* (ANGUS 2010; NILSSON & ANGUS 1992; RIBERA et al. 2002, 2008). The second group is the monophyletic “*Necterosoma* group” (RIBERA et al. 2002, 2008), which comprises taxa restricted to Australia including *Necterosoma*, *Chostonectes*, *Antiporus*, *Megaporus* and *Sternopriscus* (pp = 98, Fig. 3), and probably *Carabhydrus*, *Tiporus*, *Paroster*, *Barretthydrus*, and *Sekaliporus* as well (RIBERA et al. 2008). Finally, a third group includes the genera *Canthyporus* and *Laccornellus*, which are together monophyletic (see further discussion below). Each of these five genus groups is well-supported by this analysis, though relationships among them, as with other tribes in the Hydroporinae, are neither clear nor well-supported (Figs. 1–3).

Two Hydroporini genera here sampled, *Canthyporus* and *Laccornellus*, require some discussion, and their placement should be taken somewhat provisionally given

the considerable number of missing data (Table 1). In the consensus of the parsimony analysis (Fig. 1), *Canthyporus* is ambiguously resolved in a clade including the tribes Hygrotini, Hyphydrini, Hydrovatini and the *Hydroporus* group of Hydroporini genera, and *Laccornellus* is resolved sister to *Methles* and nested within the *Necterosoma* group of Hydroporini (Fig. 1). However, ML and Bayesian analyses (Figs. 2, 3) recovered these two groups as sister to each other, albeit with relatively low support (bt = 27, Fig. 2 and pp = 88, Fig. 3), possibly because of the fewer data available for analysis (Table 1). Likelihood places them well within the larger Hydroporinae and sister to the *Deronectes* group of Hydroporini whereas the Bayesian results have them as sister to all other Hydroporinae except *Laccornis*. This last result is noteworthy given the close relationships historically proposed between *Laccornis*, *Laccornellus* and *Canthyporus* (ROUGHLEY & WOLFE 1987 and at least some results presented by RIBERA et al. 2008). Given the unusual relationship between taxa resulting from the parsimony analysis (i.e., it seems unlikely that *Methles* and *Laccornellus* would be nested within the *Necterosoma* group of Hydroporini), it is not unreasonable to conclude that these two austral disjunct members of Hydroporinae form a monophyletic group (pp = 88), probably sister to the rest of Hydroporinae except *Laccornis* (Fig. 3).

4.5. Reclassification of Hydroporini *sensu lato*

Based on these results, and previous analyses by RIBERA et al. (2002, 2008), it seems clear that a reclassification of the genera heretofore placed in Hydroporini will be required to better reflect the phylogenetic relationships among them. Hydroporini historically has been a difficult tribe to diagnose since it includes Hydroporinae taxa remaining after other, more distinctive groups are placed in their own tribes. Historically, Hydroporini *sensu lato* has been diagnosed within the subfamily with the following character combination: 1) the metepisternum extending to the mexocoxal cavities, 2) the prosternal process extending to the metasternum between the mesocoxae (except in a few taxa including the North American *Stictotarsus minipi* Larson and several subterranean taxa which have been variously classified), 3) the metatarsal claws subequal in length, 4) the male lateral lobes of the aedeagus with a single segment, 5) the transverse tooth on the proventriculus without five elongate, finger-like lobes, 6) the medial portion of the metacoxa in a different plane from the base of the abdomen, 7) the metacoxal lobes prominent, 8) the female laterotergites absent, 9) the apex of the elytra and the last abdominal segment not acutely pointed, and 10) the metafemur along the dorsal margin broadly separated from the metacoxal lobes by the metatrochanter. Each of these diagnostic features is plesiomorphic within Hydroporinae (MILLER 2001). Al-



Figs. 14–18. Morphological features, Hydroporinae. **14:** Crusher of outer lobe of proventriculus, *Scaradytes halensis*. **15–16:** Medial portion of metacoxae, metacoxal lobes, left metatrochanter and base of left metafemur; **15:** *Celina immitatrix*; **16:** *Sanfilippodytes edwardsi*. **17–18:** Metasternum, abdomen and elytral epipleuron, ventral aspect; **17:** *Tiporus josepheni*; **18:** *Hydroporus notabilis*.

though the group is not well defined and we present some evidence that Hydroporini could use some reclassification (Figs. 1–3, MILLER 2001; RIBERA et al. 2002, 2008), because of lack of representative taxa and sequence fragments for some important taxa, we are hesitant to reclassify the group at this time. Acquisition of additional data from additional specimens is currently being planned that will hopefully clarify relationships such that appropriate reclassification can be developed for Hydroporini.

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6. References

- ABEILLE DE PERRIN E. 1904. Description d'un Coléoptère hypogé français. – Bulletin de la Société Entomologique de France **1904**: 226–228.
- ALARIE Y., GIBSON J.R., MILLER K.B. 2013. Descriptions of larvae of the North American endemic stygobiotic *Ereboporus naturaconservatus* Miller, Gibson & Alarie and *Haedeoporus texanus* Young & Longley (Coleoptera: Dytiscidae). – Tijdschrift voor Entomologie **156**: 1–10.
- ANGUS R.B. 2010. *Boreonectes* gen. n., a new genus for the *Stictotarsus griseostriatus* (De Geer) group of sibling species (Coleoptera: Dytiscidae), with additional karyosystematic data on the group. – Comparative Cytogenetics **4**: 123–131.
- BALKE M., WATTS C.H.S., COOPER S.J.B., HUMPHREYS W.F., VOGLER A.P. 2004. A highly modified stygobiont diving beetle of the genus *Copelatus* (Coleoptera, Dytiscidae): taxonomy and cladistic analysis based on mitochondrial DNA sequences. – Systematic Entomology **29**: 59–67.
- BISTRÖM O. 1990. Revision of the genus *Queda* Sharp (Coleoptera: Dytiscidae). – Quaestiones Entomologicae **26**: 211–220.
- BISTRÖM O., NILSSON A.N., WEWALKA G. 1997. A systematic review of the tribes Hyphyrini Sharp and Pachyrini n. trib. (Coleoptera, Dytiscidae). – Entomologica Fennica **8**: 57–82.
- BROWER A.V.Z., EGAN M.G. 1997. Cladistic analysis of *Heliconius* butterflies and relatives (Nymphalidae: Heliconiini): a revised phylogenetic position for *Eueides* based on sequences from mtDNA and a nuclear gene. – Proceedings of the Royal Society of London Series B, Biological Sciences **264**: 969–977.
- CASTRO A., DELGADO J.-A. 2001. *Iberoporus cermenius*, a new genus and species of subterranean water beetle (Coleoptera: Dytiscidae) from Spain. – Aquatic Insects **23**: 33–43.
- COLGAN D.J., McLAUCHLAN A., WILSON G.D.F., LIVINGSTON S.P., EDGEcombe G.D., MACARANAS J., CASSIS G., GRAY M.R. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. – Australian Journal of Zoology **46**: 419–437.
- DANFORTH B.N., SAUQUET H., PACKER L. 1999. Phylogeny of the bee genus *Halictus* (Hymenoptera: Halictidae) based on parsimony and likelihood analyses of nuclear EF-1 α sequence data. – Molecular Phylogenetics and Evolution **13**: 605–618.
- DRUMMOND A.J., SUCHARD M.A., XIE D., RAMBAUT A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. – Molecular Biology and Evolution **29**: 1969–1973.
- EDGAR R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. – Nucleic Acids Research **32**: 1792–1797.
- FRANCISCOLO M.E. 1983. Adaptation in hypogean Hydradephaga, with new notes on *Sanfilippodytes* (Dytiscidae and Phreatodytidae). P. 41 in: SATÔ M. (ed.), Special issue concerning the aquatic Coleoptera presented at the workshop of the XVI International Congress of Entomology in Kyoto, Japan, in 1980. Tokyo Tsûhan Service-sha Tokyo.
- GENECODES 1999. Sequencher, version 3.1.1. – Genecodes.
- GOLOBOFF P. 1995. NONA, version 2.0. – Published by the author.
- GUIGNOT F. 1925. Description d'un *Siettitia* nouveau du Midi de la France. – Bulletin de la Société Entomologique de France **1925**: 23–25.
- GUIGNOT F. 1931. Sur le *Siettitia avenionensis* Guig. et la valeur du genre *Siettitia* Ab. [Col. Dytiscidae]. – Bulletin de la Société Entomologique de France **36**: 243–246.
- HUELSENBECK J.P., RONQUIST F. 2001. MrBayes: Bayesian inference of phylogeny. – Bioinformatics **17**: 754–755.
- LARSON D.J., ALARIE Y., ROUGHLEY R.E. 2000. Predaceous diving beetles (Coleoptera: Dytiscidae) of the Nearctic Region, with emphasis on the fauna of Canada and Alaska. – National Research Council of Canada Research Press, Ottawa, Ontario, Canada. 982 pp.
- LARSON D.J., LABONTE J.R. 1994. *Stygoporus oregonensis*, a new genus and species of subterranean water beetle (Coleoptera: Dytiscidae: Hydroporini) from the United States. – The Coleopterists Bulletin **48**: 371–379.
- LEYS R., WATTS C.H. 2008. Systematics and evolution of the Australian subterranean hydroporine diving beetles (Dytiscidae), with notes on *Carabhydrus*. – Invertebrate Systematics **22**: 217–225.
- MADDISON W.P., MADDISON D.R. 2004. Mesquite: A modular system for evolutionary analysis, version 1.02.
- MILLER K.B. 2001. On the phylogeny of the Dytiscidae (Coleoptera) with emphasis on the morphology of the female reproductive tract. – Insect Systematics and Evolution **32**: 45–92.
- MILLER K.B. 2003. The phylogeny of diving beetles (Coleoptera: Dytiscidae) and the evolution of sexual conflict. – Biological Journal of the Linnean Society **79**: 359–388.
- MILLER K.B. 2005. Revision of the New World and southeast Asian Vatelini (Coleoptera: Dytiscidae: Hydroporinae) and phylogenetic analysis of the tribe. – Zoological Journal of the Linnean Society **144**: 415–510.
- 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.
- MILLER K.B., BERGSTEN J., WHITING M.F. 2009a. Phylogeny and classification of the tribe Hydaticini (Coleoptera: Dytiscidae): partition choice for Bayesian analysis with multiple nuclear and mitochondrial protein-coding genes. – Zoologica Scripta **38**: 591–615.
- MILLER K.B., GIBSON J.R., ALARIE Y. 2009b. North American stygobiotic diving beetles (Coleoptera: Dytiscidae: Hydroporinae) with description of *Ereboporus naturaconservatus* Miller, Gibson and Alarie, new genus and species, from Texas, U.S.A. – The Coleopterists Bulletin **63**: 191–202.
- MILLER K.B., EDGERLY J.S. 2008. Systematics and natural history of the Australian genus *Metoligotoma* Davis (Embioptera: Austrolembiidae). – Invertebrate Systematics **2**: 329–344.

- MILLER K.B., WOLFE G.W., BISTRÖM O. 2006. Phylogeny of the Hydroporinae and classification of the genus *Peschetius* Guignot, 1942 (Coleoptera: Dytiscidae). – *Insect Systematics and Evolution* **37**: 257–279.
- NILSSON A.N. 2010. Catalogue of Palearctic Dytiscidae (Coleoptera). Internet version 2010-01-01. – http://www2.emg.umu.se/projects/biginst/andersn/PALCATDYT_20100101.pdf.
- NILSSON A.N., ANGUS R.B. 1992. A reclassification of the *Deronectes* group of genera (Coleoptera: Dytiscidae) based on a phylogenetic study. – *Entomologica Scandinavica* **23**: 275–288.
- NIXON K.C. 2002. WinClada, version 1.00.08. – Published by the author.
- ORDISH R.G. 1976. Two new genera and species of subterranean water beetle from New Zealand (Coleoptera: Dytiscidae). – *New Zealand Journal of Zoology* **3**: 1–10.
- PESCHET R. 1932. Description d'un *Bidessus* nouveau hypogé de l'Afrique Occidentale Française (Coleoptera Dytiscidae). Pp. 571–574, Société Entomologique de France, Livre du Centenaire Paris.
- RAMBAUT A. 2006–2009. FigTree Tree Figure Drawing Tool, version 1.3.1. – Institute of Evolutionary Biology, University of Edinburgh.
- RIBERA I., FAILLE A. 2010. A new microphthalmic stygobitic *Graptodytes* Seidlitz from Morocco, with a molecular phylogeny of the genus (Coleoptera, Dytiscidae). – *Zootaxa* **2641**: 1–14.
- RIBERA I., HOGAN J.E., VÖGLER A.P. 2002. Phylogeny of hydrade-phagan water beetles inferred from 18S rRNA sequences. – *Molecular Phylogenetics and Evolution* **23**: 43–62.
- RIBERA I., VÖGLER A.P., BALKE M. 2008. Phylogeny and diversification of diving beetles (Coleoptera : Dytiscidae). – *Cladistics* **24**: 563–590.
- RICHOUX P. 1980. Les larves du coleoptere dytiscide phreatobie: *Siettitia avenionensis* Guignot. – *Memoires de Biospeleologie* **7**: 195–200.
- RICHOUX P., DALMON J., REYGROBELLET J.L. 1990. Données complémentaires sur la systématique et l'écologie de *Siettitia avenionensis* (Coleoptera: Dytiscidae). – *Annales de Limnologie* **26**: 39–44.
- ROUGHLEY R.E., WOLFE G.W. 1987. *Laccornellus* (Coleoptera: Dytiscidae), a new hydroporine genus from austral South America. – *Canadian Journal of Zoology* **65**: 1346–1353.
- SANFILIPPO N. 1958. Viaggio in Venezuela di Nino Sanfilippo, V: Descrizione de *Trogloguignotus concii*, n.gen. n.sp. di Dytiscidae freatobio. – *Annali del Museo Civico di Storia Naturale di Genova* **70**: 159–164.
- SEIDLITZ G. 1887. Bestimmungs-Tabelle der Dytiscidae und Gyrinidae des europäischen Faunengebietes. – *Verhandlungen des Naturforschenden Vereines in Brünn* **XXV**: 3–136.
- SHIMODAIRA H., HASEGAWA M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. – *Molecular Biology and Evolution* **16**: 1114–1116.
- SIMON C., FRATI F., BECKENBACH A., CRESPI B., LIU H., FLOOK P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. – *Annals of the Entomological Society of America* **87**: 651–701.
- SMRŽ J. 1981. Respiration – a new function of some hydroporine elytra (Coleoptera, Dytiscidae, Hydroporinae). – *Acta Entomologica Bohemoslovaca* **78**: 209–215.
- SPANGLER P.J. 1986. Insecta: Coleoptera. Pp. 62–72 in: BOTOSA-NEANU L. (ed.), *Stygofauna Mundi*. – E.J. Brill Leiden.
- SPANGLER P.J., BARR C.B. 1995. A new genus and species of stygobiotic dytiscid beetle, *Comaldessus stygius* (Coleoptera: Dytiscidae: Bidessini), from Comal Springs, Texas. – *Insecta Mundi* **9**: 301–308.
- STAMATAKIS A. 2006. RAXML – VI – HPC: Maximum likelihood-based phylogenetic analysis with thousands of taxa and mixed models. – *Bioinformatics* **22**: 2688–2690.
- SVENSON G.J., WHITING M.F. 2004. Phylogeny of Mantodea based on molecular data: Evolution of a charismatic predator. – *Systematic Entomology* **29**: 359–370.
- WATTS C.H.S., HANCOCK P.J., LEYS R. 2007. A stygobitic *Carabhydrus* Watts (Dytiscidae, Coleoptera) from the Hunter Valley in New South Wales, Australia. – *Australian Journal of Entomology* **46**: 56–59.
- WATTS C.H.S., HUMPHREYS W.F. 1999. Three new genera and five new species of Dytiscidae (Coleoptera) from underground waters in Australia. – *Records of the South Australian Museum* **32**: 121–142.
- WATTS C.H.S., HUMPHREYS W.F. 2000. Six new species of *Nirridessus* Watts and Humphreys and *Tjirtudessus* Watts and Humphreys (Coleoptera: Dytiscidae) from underground waters in Australia. – *Records of the South Australian Museum* **33**: 127–144.
- WATTS C.H.S., HUMPHREYS W.F. 2004. Thirteen new Dytiscidae (Coleoptera) of the genera *Boongurrus* Larson, *Tjirtudessus* Watts & Humphreys and *Nirriparti* Watts & Humphreys, from underground waters in Australia. – *Transactions of the Royal Society of South Australia* **128**: 99–129.
- WATTS C.H.S., HUMPHREYS W.F. 2006. Twenty-six new Dytiscidae (Coleoptera) of the genera *Limbodessus* Guignot and *Nirriparti* Watts & Humphreys, from underground waters in Australia. – *Transactions of the Royal Society of South Australia* **130**: 123–185.
- WHITING M.F. 2002. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. – *Zoologica Scripta* **31**: 93–104.
- WOLFE G.W. 1985. A phylogenetic analysis of plesiotypic hydroporine lineages with an emphasis on *Laccornis* des Gozis (Coleoptera: Dytiscidae). – *Proceedings of the Academy of Natural Sciences of Philadelphia* **137**: 132–155.
- WOLFE G.W. 1988. A phylogenetic investigation of *Hydrovatus*, *Methlini* and other plesiotypic hydroporines (Coleoptera: Dytiscidae). – *Psyche* **95**: 327–344.
- YOUNG F.N., LONGLEY G. 1976. A new subterranean aquatic beetle from Texas (Coleoptera: Dytiscidae-Hydroporinae). – *Annals of the Entomological Society of America* **69**: 787–792.

7. Appendix

Description of morphological characters used in cladistic analysis of Hydroporinae taxa (morphological character matrix: Table 4).

Head

1. Anterior clypeal margin. 0) Not produced or beaded; 1) Margin anteriorly produced or beaded.

Thorax

2. Scutellum. 0) Not externally visible; 1) Externally visible.
3. Pronotum lateral crease. 0) Absent; 1) Present.
4. Longitudinal sublateral basal striae on pronotum. 0) Absent; 1) Present.
5. Prosternal process. 0) Apically narrowed to pointed or rounded apex; 1) Apically broad, truncate.
6. Metacoxa and first visible abdominal sternite (= sternite II). 0) Not fused; 1) Fused.

Abdomen

7. Abdominal tergum VIII. 0) Apically rounded or obtusely angulate; 1) Apically sharply acute or acuminate.

Elytron

8. Anterior oblique epipleural carina. 0) Absent; 1) Present.
9. Elytral apices. 0) Not acuminate; 1) Distinctly acuminate.
10. Basal elytral stria. 0) Absent; 1) Present.

Legs

11. Posteromedial metacoxal lobes. 0) Absent; 1) Present, small; 2) Present, large. [additive]
12. Posteromedial metacoxal rim. 0) Discontinuous medially; 1) Continuous medially.
13. Metacoxa. 0) Medial portion not strongly concave laterally; 1) Medial portion strongly concave laterally with portion of metacoxa obscured in ventral aspect.
14. Metatarsal claws. 0) Unequal, posterior shorter than anterior; 1) Subequal in length.

Internal

15. Proventriculus. 0) Without five finger-like lobes on transverse tooth of outer lobe; 1) With five finger-like lobes on transverse tooth of outer lobe.

Female genitalia

16. Ringlike sclerite (receptacle) on bursa. 0) Absent; 1) Present.
17. Laterotergites. 0) Absent; 1) Present.
18. Receptacle. 0) Absent; 1) Present.
19. Spermathecal spine. 0) Absent; 1) Present.
20. Base of fertilization duct. 0) Without modifications; 1) With a heavily sclerotized, irregular ring.

Male genitalia

21. Lateral lobe of aedeagus. 0) Comprised of one segment; 1) Comprised of two segments.

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