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Phylogeny, classification and evolution of the water scavenger beetle tribe Hydrobiusini inferred from morphology and molecules (Coleoptera: Hydrophilidae: Hydrophilinae)

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Abstract. The water scavenger beetle tribe Hydrobiusini contains 47 species in eight genera distributed worldwide. Most species of the tribe are aquatic, although several species are known to occur in waterfalls or tree mosses. Some members of the tribe are known to communicate via underwater stridulation. While recent morphological and molecular-based phylogenies have affirmed the monophyly of the tribe as currently circumscribed, doubts remain about the monophyly of included genera. Here we use morphological and molecular data to infer a species-level phylogeny of the Hydrobiusini. The monophyly of the tribe is decisively supported, as is the monophyly of most genera. The genus *Hydrobius* was found to be polyphyletic, and as a result the genus *Limnohydrobius* **stat. rev.** is removed from synonymy with *Hydrobius*, yielding three new combinations: *L. melaenus* **comb.n.**, *L. orientalis* **comb.n.**, and *L. tumbius* **comb.n.** Recent changes to the species-level taxonomy of *Hydrobius* are reviewed. The morphology of the stridulatory apparatus has undergone a single remarkable transformation within the lineage, from a simple, unmodified pars stridens to one that is highly organized and complex. We present an updated key to genera, revised generic diagnoses and a list of the known distributions for all species within the tribe.

Introduction

The water scavenger beetle tribe Hydrobiusini is a relatively small lineage of 47 described species in eight genera within the large, cosmopolitan beetle family Hydrophilidae (Short & Fikáček, 2011; Table 2). While at least one representative of the tribe is found in all biogeographic regions except the Antarctic, the genera of Hydrobiusini exhibit an enigmatic array of geographic distributions (Fig. 1). For example, there are monotypic endemic genera found in Western Australia (*Hybogralius* d'Orchymont), New Caledonia (*Limnocyclus* Balfour-Browne), northern Argentina (*Hydramara* Knisch) and eastern North America (*Sperchopsis* LeConte). Conversely, the genus *Hydrobius* Leach has a completely Holarctic distribution and the genus *Limnoxenus* Motchulsky has representatives in Europe, South Africa and Australia, and even a significant radiation in the Hawaiian Islands (Short & Liebherr, 2007). Additionally, one species (*Hydrobius fuscipes* Linnaeus) occurs throughout North America, Europe and northern Asia – possibly the largest indigenous range for any water scavenger beetle (but see Fossen *et al.*, 2016).

Many members of the Hydrobiusini are also known to exhibit stridulatory communication. Stridulation is well documented in several aquatic insect groups, including multiple independent groups of aquatic Coleoptera (Aiken, 1985). Among these groups, perhaps none is better studied than the Hydrophilidae, in which taxa with acoustic behaviour have been known for more than 100 years. In all known cases within the family (including the Hydrobiusini) the sound is produced via an abdominal–elytral mechanism: the abdomen (laterosternite 3: pars stridens) moves up and down with respect to the elytral interior (plectrum), with roughened patches on each scraping together. Interestingly, the morphology of

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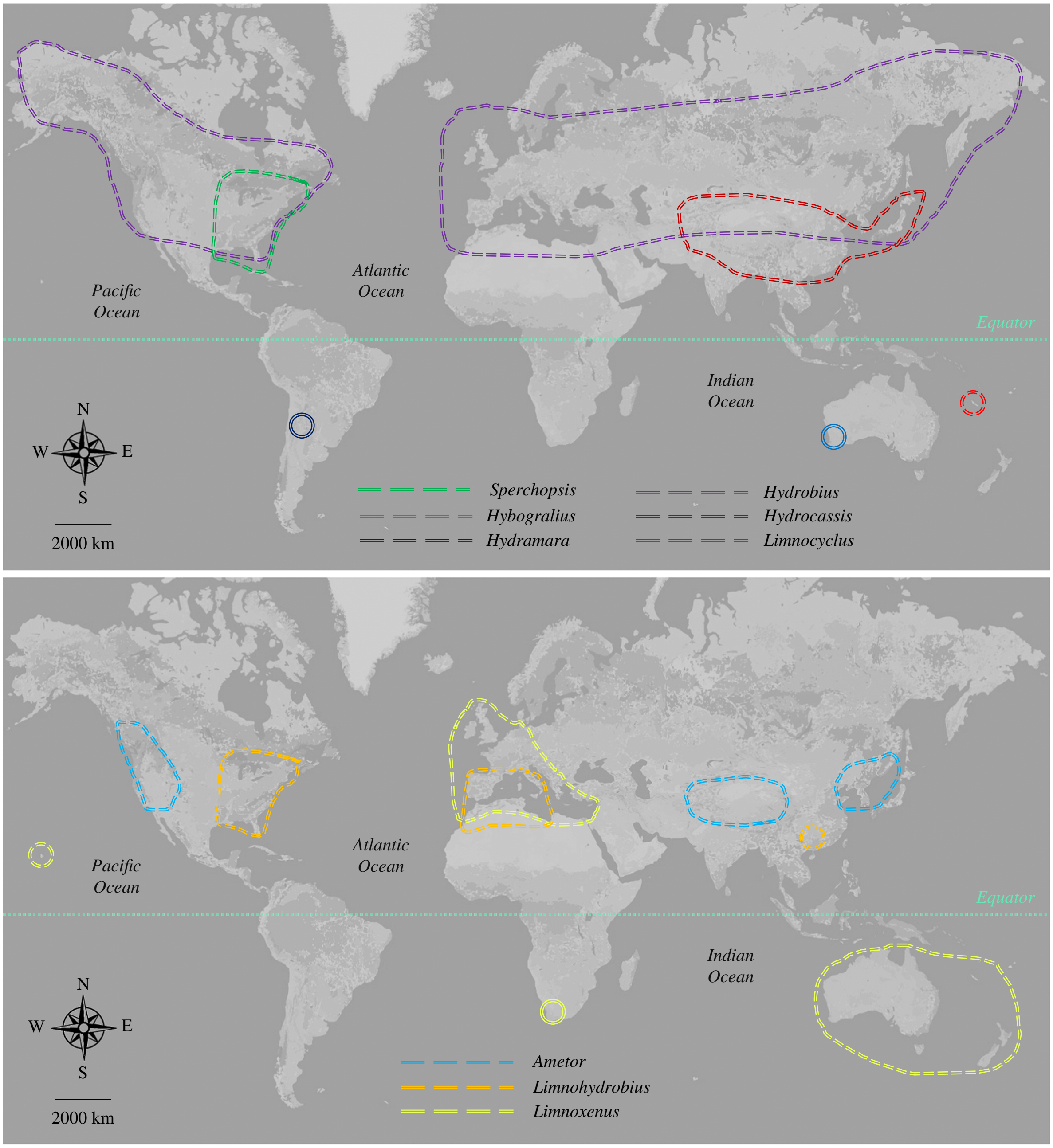

Fig. 1. Generalized distribution of the genera of Hydrobiusini. [Colour figure can be viewed at wileyonlinelibrary.com].

these sound-producing organs varies widely within the Hydrobiusini, ranging from a simple field of unorganized cuticular microtrichia to a complex pars stridens across the taxa. Stridulatory behaviour in water scavenger beetles was first documented in the Hydrobiusini and discussed by Balfour-Browne (1910) in his review of the biology of *H. fuscipes*. The mechanism of that species and that of *Limnoxenus niger* (Gmelin) were reviewed and illustrated by Marcu (1932a,b). The most extreme and notable cases of bioacoustics in the Hydrophilidae involve the genera *Berosus* Leach and *Tropisternus* Solier, in which the sound can occasionally be heard from several feet away (Spangler, 1960). In several case studies on these two genera, stridulatory signals have been documented to be both species-specific and serve multiple functions, including both disturbance (or ‘stress’) calls when disturbed and sexual signalling between males and females – both sexes stridulate via the same mechanism (Van Tassel, 1965; Ryker, 1972).

A number of taxa within the Hydrobiusini are known to produce audible stridulatory sounds, including *H. fuscipes* (Balfour-Browne, 1910), *Hydrobius tumidus* (A. Short, personal

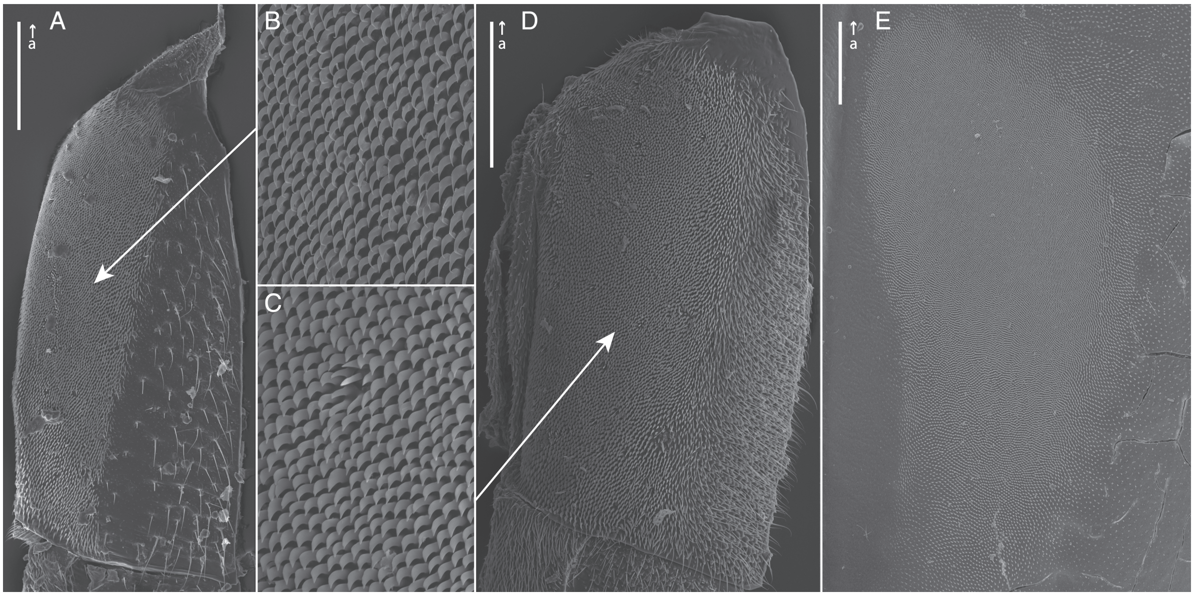

Fig. 2. Scanning electron micrographs of the stridulatory apparatus of hydrobiusine taxa. Third laterosternite of hydrobiusine taxa with an 'unmodified' pars stridens: (A, B) *Hydrobius fuscipes*; (C, D) *Ametor scabrosus*. Interior of the elytra, showing the plectrum: (E) *Limnocyclus puncticeps*. The anterior orientation is indicated by an arrow adjacent to a small letter 'a'. Scale bars = 0.2 mm.

observation), *Hydramara argentina* (Archangelsky, 2000) and several *Limnoxenus* species (e.g. Marcu, 1932b; Williams, 1936). Although they all stridulate via the same general abdominal–elytral mechanism, the morphology of these organs varies between taxa and they have never been examined in a phylogenetic context. Examination of the bioacoustic morphology of the tribe in the context of a robust phylogeny will help to elucidate if a general pattern of stridulatory evolution exists, and if it is applicable to other occurrences within the family and within other lineages of beetles.

The composition of the tribe has fluctuated moderately over time, and at one point it contained as many as 25 genera (d'Orchymont, 1916, 1919a,b). In the first comprehensive cladistic analysis of the family, Hansen (1991) narrowed the concept of the Hydrobiusini to just five relatively similar-appearing genera (*Hydrobius*, *Hybogralius*, *Limnoxenus*, *Hydramara*, and *Limnocyclus*), and lowered its rank to a subtribe of Hydrophilini. In that study, Hansen (1991) found weak morphological support for a sister-group relationship between the Hydrobiusini and the Hydrophilini. A subsequent cladistic analysis that incorporated newly available larval data suggested a sister-group relationship to the Sperchopsini (Archangelsky, 2004).

Recent cladistic analyses based on adult morphology (Short & Liebherr, 2007) and molecular data (Short & Fikáček, 2013) have supported the monophyly of the Hydrobiusini as currently circumscribed (e.g. to include the former Sperchopsini, which was found by those studies to be nested within the Hydrobiusini). However, the relationships among taxa within the tribe have been less clear. For example, the nominal genus *Hydrobius* has itself been suggested to be nonmonophyletic, and other internal generic relationships have not been decisively resolved.

In this study, we use morphological and molecular data to infer a species-level phylogeny of the Hydrobiusini to: (i) clarify relationships within the tribe and ensure its classification accurately reflects its evolutionary history; and (ii) understand the evolution of complex stridulatory morphology within water scavenger beetles.

Material and methods

Taxon sampling and morphology

The molecular dataset included seven genera out of the eight described and 19 species out of the 47 described in the tribe Hydrobiusini (File S1). We also included representatives of multiple other genera of the family Hydrophilidae as outgroups (Table 1). For the morphological dataset, we used the matrix from Short & Liebherr (2007), which we modified to include a few additional in-group taxa, including *Ametor latus* (Horn), *Hydrocassis* sp. and the recently described *Hydrobius orientalis* Jia & Short. This morphological matrix, comprising 42 characters (including several characters related to the stridulatory structure) and 24 in-group species, allowed the inclusion of some of the most important missing taxa from our molecular dataset (Tables 1, File S2, File S3 and File S4). In particular, the monotypic genus *Hydramara* for which we did not have molecular data was included along with one species of *Hydrobius* and three species of *Limnoxenus*. Overall we were able to reconstruct phylogenetic relationships among all genera and ~50% of the species richness in this tribe. The remainder of the species for which we did not have molecular data were not coded, because they did not present morphological differences with respect to

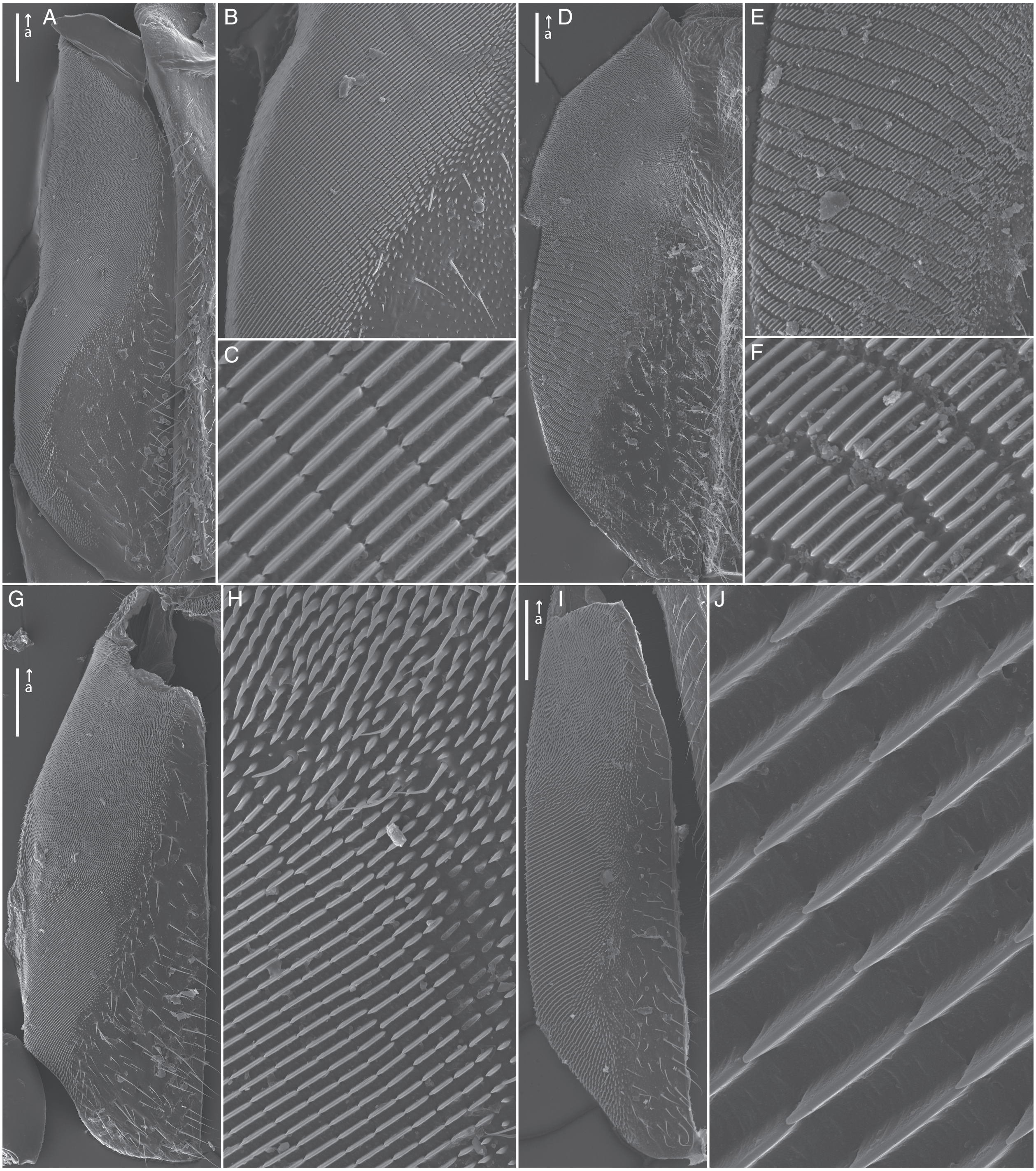

Fig. 3. Scanning electron micrographs of laterosternite 3 of hydrobiusine taxa with a modified, or 'complex', pars stridens. (A–C) *Limnocyclus puncticeps*; (D–F) *Hydramara argentina*; (G–H) *Limnoxenus zealandicus*; (I–J) *Limnoxenus kauaiensis*. The anterior orientation is indicated by an arrow adjacent to a small letter 'a'. Scale bars = 0.2 mm.

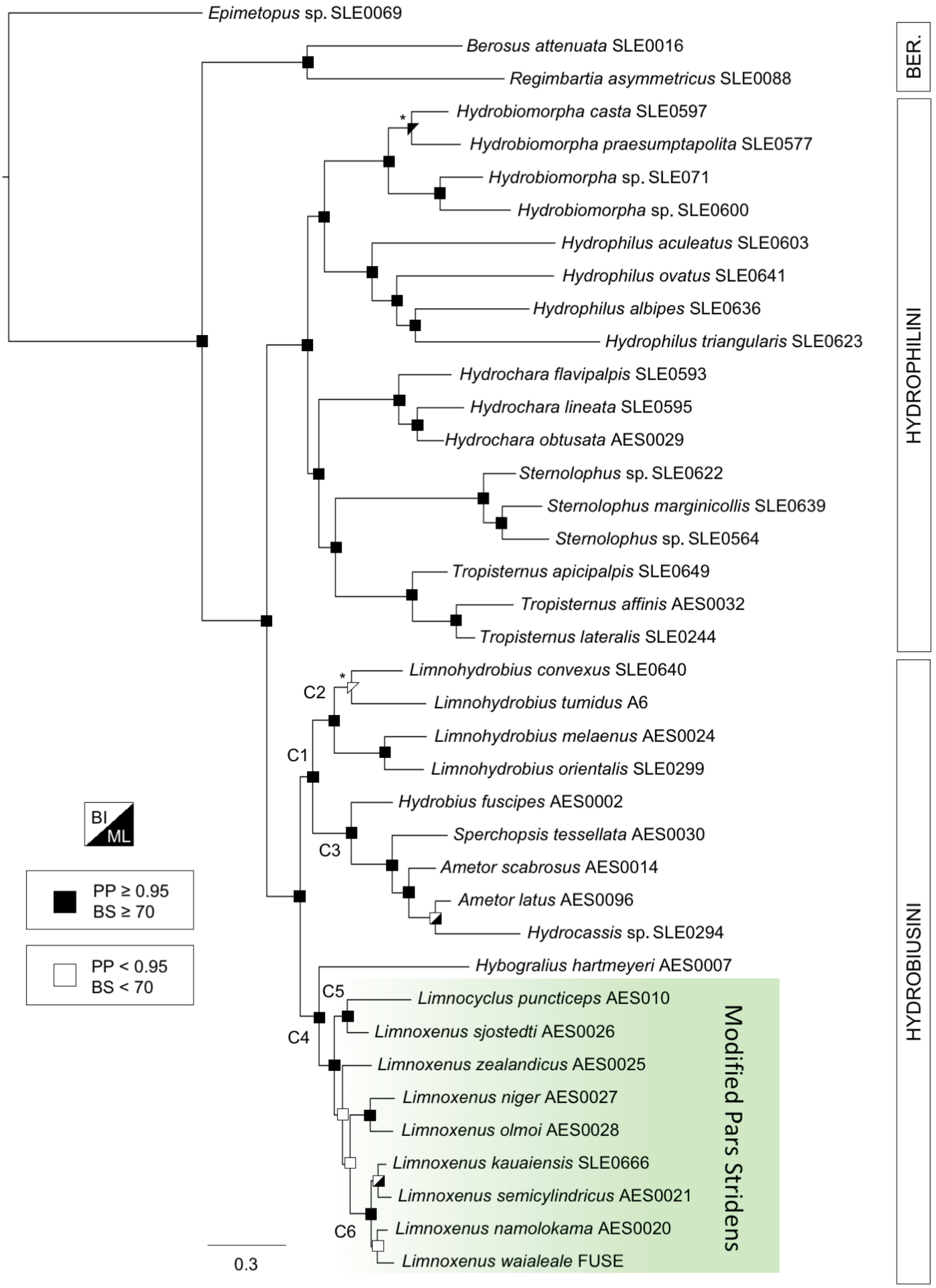

Fig. 4. Molecular phylogeny of the Hydrobiusini. BI, Bayesian inference; ML, maximum likelihood; PP, posterior probability; BS, bootstrap support. Asterisks indicate nodes that were not recovered in ML. [Colour figure can be viewed at wileyonlinelibrary.com].

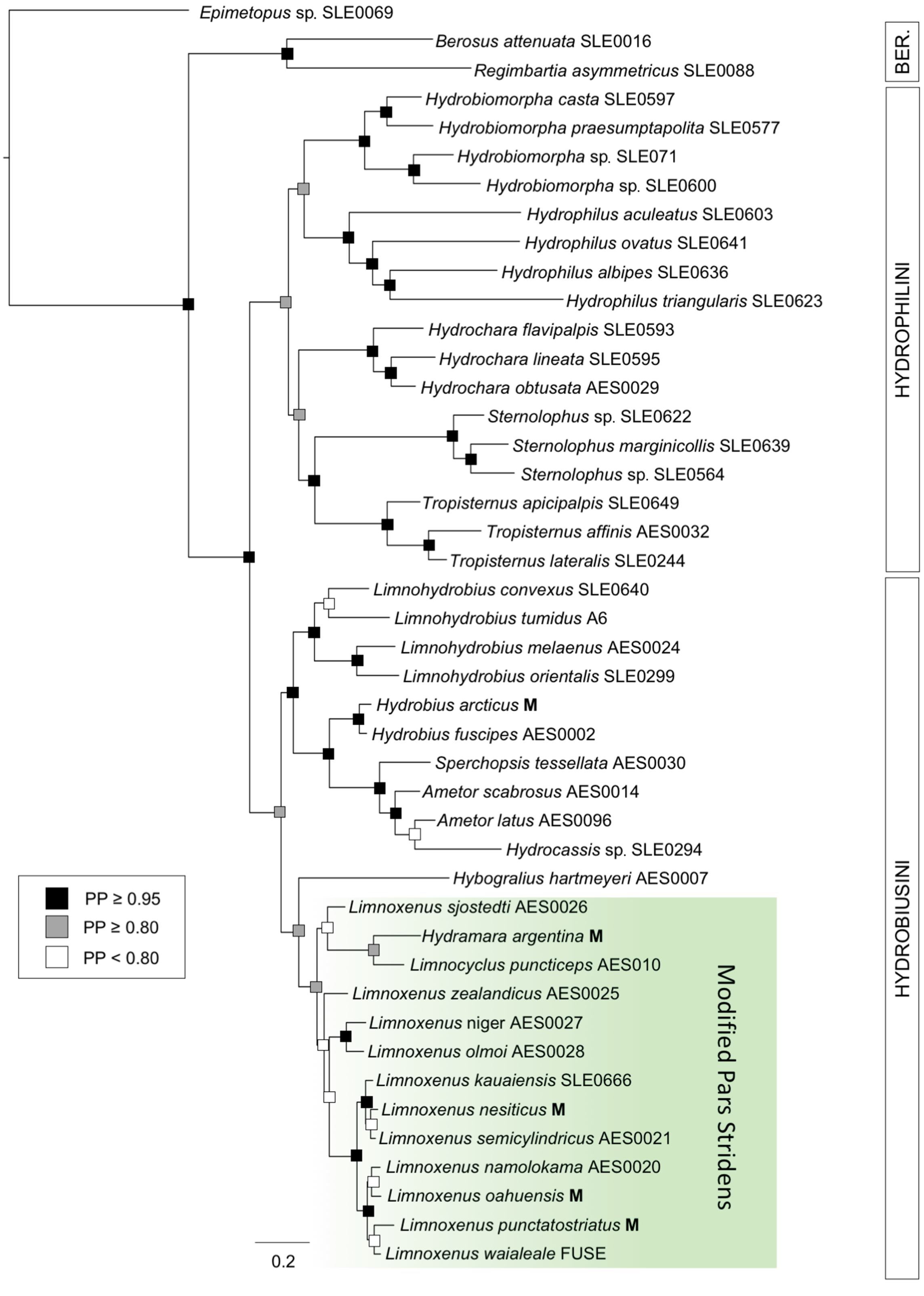

Fig. 5. Total evidence phylogeny of the Hydrobiusini (morphology + DNA). PP, posterior probability. Bolded 'M' indicates taxa that were represented in the analysis by morphology only. [Colour figure can be viewed at wileyonlinelibrary.com].

Fig. 6. Dorsal habitus images of selected genera of Hydrobiusini. (A) *Sperchopsis tessellata*; (B) *Ametor rugosus*; (C) *Hydrocassis baoshanensis*; (D) *Hydrobius fuscipes* (ex. Canada); (E) *Limnoxenus niger*; (F) *Limnohydrobius melaenus*. Scale bar = 5.0 mm. [Colour figure can be viewed at wileyonlinelibrary.com].

our coding, therefore creating an artefactual polytomy in preliminary topologies. Moreover, most of these species (16) are concentrated in the relatively homogenous genus *Hydrocassis* Fairmaire and unlikely to affect our broader phylogeny.

DNA extraction and amplification

Specimens for molecular analysis were preserved in 95% ethanol and kept frozen at −20°C, and were prepared for extraction by separating the thorax and abdomen between the pronotum and elytra using sterilized forceps. Total genomic DNA was extracted from entire beetles with blood and tissue kits (Qiagen, Valencia, CA, U.S.A.) according to the manufacturer instructions and subsequently stored at −20°C. We used the same polymerase chain reaction protocols as in Bloom *et al.* (2014) to amplify and sequence the following gene fragments: *cytochrome oxidase subunit 1* (COI, 822 bp), *arginine kinase* (Ark, 696 bp), 18S (~1791 bp) and 28S (~972 bp). The DNA sequences were edited in GENEIOUS R8.0.5 (Biomatters, http://www.geneious.com/), aligned using MUSCLE (Edgar, 2004) and the reading frames checked in MESQUITE 3.02 (http://mesquiteproject.org). New sequences were deposited in GenBank (accession numbers KY554443–KY554473).

Molecular phylogenetics and ancestral state reconstruction

We used Bayesian inference (BI) to reconstruct phylogenetic relationships using two different datasets. We first generated

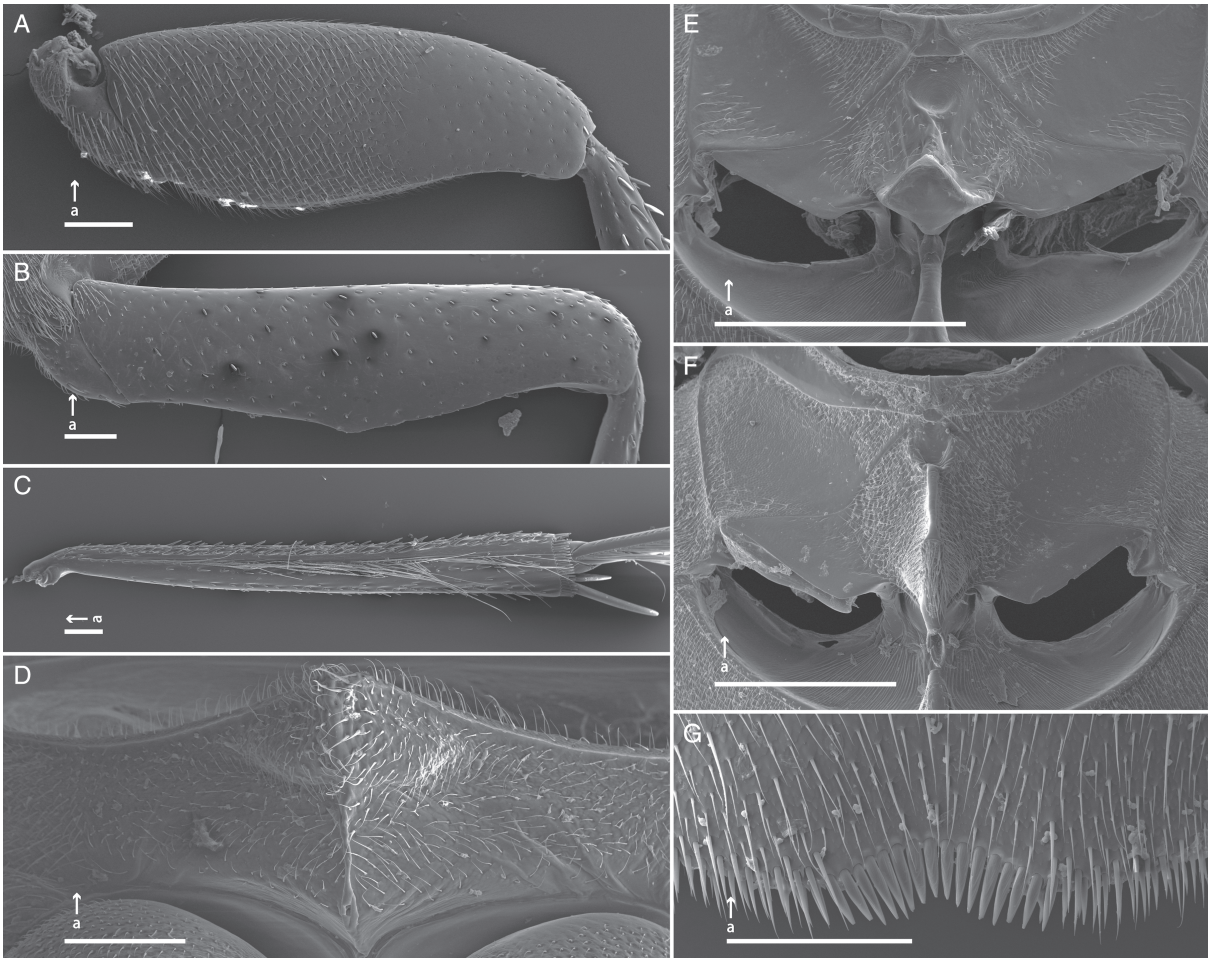

Fig. 7. Scanning electron micrographs of selected diagnostic characters of the Hydrobiusini. (A) *Hydrobius fuscipes*, metafemora; (B) *Limnocyclus puncticeps*, metafemora; (C) *Limnocyclus puncticeps*, metatibiae; (D) *Limnoxenus zealandicus*, prosternum; (E) *Limnohydrobius melaenus*, mesosternum; (F) *Limnoxenus zealandicus*, mesosternum; (G) *Limnoxenus zealandicus*, apex of fifth abdominal ventrite showing emargination. The anterior orientation is indicated by an arrow adjacent to a small letter 'a'. Scale bars: A–D, G = 0.2 mm; E, F = 1.0 mm.

a dataset of the four concatenated gene fragments, hereafter referred to as 'molecular dataset'. A second dataset referred to as 'combined dataset' was generated by combining the concatenated gene fragments and the morphological matrix in order to reconstruct the phylogenetic relationships of the taxa for which we did not have molecular data. For the 'molecular dataset', the partitions and corresponding optimal models of substitution were searched under PARTITIONFINDER 1.1.1 (Lanfear *et al.*, 2012) using the '*greedy*' algorithm and the '*mrbayes*' set of models. The Akaike information criterion corrected (AICc) was used to compare the fit of the different models of substitution. The morphological matrix was assigned the Mk model for morphology (Lewis, 2001) as implemented in MRBAYES 3.2 (Ronquist *et al.*, 2012), assuming gamma-distributed rate variation across characters. The BI analyses of the 'molecular' and 'combined' datasets were performed using MRBAYES 3.2.6 (Ronquist *et al.*, 2012) as implemented in CIPRES (Miller *et al.*, 2010). Two simultaneous and independent runs consisting of eight MCMC runs (one cold and seven incrementally heated) were run for 30 million generations, with a tree sampling every 1000 generations to calculate posterior probabilities (PPs). We assessed convergence of the runs by investigating the average standard deviation of split frequencies and effective sample size (ESS) of all parameters in TRACER 1.5 (http://BEAST.bio.ed.ac.uk/Tracer). A value of ESS >200 was acknowledged as a good indicator of convergence. All posterior trees that predated the time needed to reach a log-likelihood plateau were discarded as burn-in, and the remaining samples were summarized to generate a 50% majority rule consensus tree.

We also used IQ-TREE (Nguyen *et al.*, 2015) as implemented on the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at) to cross-check the results of the MRBAYES analysis in a maximum likelihood (ML) framework. The concatenated dataset was partitioned with the same scheme as in the MRBAYES analyses and the best-fit models of substitution were searched using the '*Auto*' function on the IQ-TREE web server based on the AICc. We performed 1000 ultrafast bootstrap replicates

Table 1. Taxon sampling.

Tribe	Genus	Species	Code	Region	CO1	ARK	18S	28S	Morphology
–	*Epimetopus*	sp.	SLE0069	–	x	x	x	x	–
Berosini	*Berosus*	*asymmetricus*	SLE0088	–	x	–	x	x	–
	Regimbartia	*attenuata*	SLE0016	–	x	x	x	x	–
Hydrobiusini	*Ametor* (2/6)	*latus*	SLE0096	West Nearctic	x	x	x	–	x
		scabrosus	AES0014	West Nearctic	x	x	x	x	x
	Hybogralius (1/1)	*hartmeyeri*	SLE0007	Australian	x	–	x	x	x
	Hydramara (1*/1)	*argentina**	–	Neotropics	–	–	–	–	x
	Hydrobius (6*/9)	*arcticus**	–	West Palearctic	–	–	–	–	x
		convexus	SLE0640	West Palearctic	x	x	x	x	x
		fuscipes	SLE0002	Holarctic	x	x	x	x	x
		melaenus	AES0024	East Nearctic	x	x	x	x	x
		orientalis	SLE0299	East Palearctic	x	x	x	–	x
		tumidus	A6	East Nearctic	x	x	x	x	x
	Hydrocassis (1/17)	sp.	SLE0294	East Palearctic	x	x	x	x	x
	Limnocyclus (1/1)	*puncticeps*	AES0010	New Caledonia	x	x	x	x	x
	Limnoxenus (11*/11)	*kauaensis*	SLE0666	Hawaii	x	x	x	x	x
		namolokama	AES0020	Hawaii	x	–	x	x	x
		*nesiticus**	–	Hawaii	–	–	–	–	x
		niger	AES0027	West Palearctic	x	–	x	x	x
		*oahuensis**	–	Hawaii	–	–	–	–	x
		olmoi	AES0028	West Palearctic	x	–	x	–	x
		*punctatostriatus**	–	Hawaii	–	–	–	–	x
		semicylindricus	AES0021	Hawaii	x	x	x	x	x
		sjostedti	AES0026	South Africa	x	x	x	x	x
		waialeale	FUSE	Hawaii	x	x	x	x	x
		zealandicus	AES0025	South Pacific	x	x	x	x	x
	Sperchopsis (1/1)	*tessellata*	AES0030	East Nearctic	x	x	x	x	x
Hydrophilini	*Hydrobiomorpha*	*casta*	SLE0597	–	x	x	x	x	–
	Hydrobiomorpha	*praesumptapolita*	SLE0577	–	x	x	x	x	–
	Hydrobiomorpha	sp.	SLE0071	–	–	x	x	x	–
	Hydrobiomorpha	sp.	SLE0600	–	x	x	x	x	–
	Hydrochara	*flavipalpis*	SLE0593	–	x	x	x	x	–
	Hydrochara	*lineata*	SLE0595	–	x	x	x	x	–
	Hydrochara	*obtusata*	AES0029	–	x	x	x	x	–
	Hydrophilus	*aculeatus*	SLE0603	–	x	x	x	x	–
	Hydrophilus	*albipes*	SLE0636	–	x	x	x	x	–
	Hydrophilus	*ovatus*	SLE0641	–	x	x	x	x	–
	Hydrophilus	*triangularis*	SLE0623	–	x	x	x	x	–
	Sternolophus	*marginicollis*	SLE0639	–	x	x	x	x	–
	Sternolophus	sp.	SLE0564	–	x	x	x	x	–
	Sternolophus	sp.	SLE0622	–	x	x	x	x	–
	Tropisternus	*affinis*	AES0032	–	x	x	x	x	–
	Tropisternus	*apicipalpis*	SLE0649	–	x	x	x	x	–
	Tropisternus	*lateralis*	SLE0244	–	x	x	x	x	–

(Minh *et al.*, 2013) to investigate nodal support across the topology.

We performed an ancestral state reconstruction in MESQUITE 3.2 (Maddison & Maddison, 2017) using parsimony optimization on the total evidence phylogeny to examine the origin of the complex stridulatory file.

Results

Phylogenetic analysis

Analyses of both molecular (BI and ML) and combined (BI) datasets resulted in topologically congruent trees (Figs 4, 5). The Hydrobiusini was strongly supported as monophyletic [PP = 1.0/bootstrap support (BS) = 93, molecular dataset/PP = 0.85, combined dataset]. Within the Hydrobiusini, there are two strongly supported primary clades, the first comprising *Hydrobius* and the former sperchopsine genera *Sperchopsis*, *Ametor* and *Hydrocassis* (1.0/98/0.99), while the second contains the remaining genera, *Hybogralius*, *Limnoxenus*, *Limnocyclus* and *Hydramara* (1.0/97/0.85). Within the first clade, the genus *Hydrobius* was recovered as polyphyletic, with representatives of the *H. fuscipes* complex (*H. fuscipes* and *Hydrobius arcticus*) resolved as sister to the sperchopsine genera (1.0/100/0.99), while the remaining *Hydrobius* taxa also form a strongly supported clade (0.99/98/0.99). Within the second clade, the enigmatic *Hybogralius hartmeyeri* is decisively

Table 2. List of described species of Hydrobiusini and their known distribution.

Species	Distribution	References
Ametor elongatus Ji & Schödl, 1988	China (Sichuan)	Hansen (1999)
A. latus (Horn, 1873)	Canada (BC), USA (CA, ID, OR, WA)	Hansen (1999)
A. rudesculptus Semenow, 1900	China (Yunnan, Tibet, Sichuan), Bhutan, Nepal, India (Himachal Pradesh, Sikkim) Tajikistan	Hansen (1999), Schödl (2000) and Jia & Zhao (2013)
A. rugosus (Knisch, 1924)	China (Yunnan, Tibet), Nepal, Bhutan, India (Himachal Predesh, Sikkim, Uttar Pradesh, West Bengal)	Hansen (1999) and Schödl (2000)
A. scabrosus (Horn, 1873)	Canada (AB, BC), China (Jilin), Russia (Far East), South Korea, USA (AK, CA, ID, OR, UT, WA, WY)	Hansen, 1999 and Lee & Ahn (2008)
A. xizangensis, Jia & Zhao, 2013	China (Tibet)	Jia & Zhao (2013)
Hybogralius hartmayeri (Régimbart, 1908)	Australia (Western Australia)	Hansen (1999)
Hydramara argentina (Knisch, 1925)	Argentina, ?Paraguay (doubtful)	Hansen (1999)
Hydrocassis anhuiensis Ji & Schödl, 1998	China (Anhui, Jiangxi)	Hansen (1999)
H. baoshanensis Schödl & Ji (1995)	China (Yunnan)	Hansen (1999)
H. gansu Jia & Zhao (2013)	China (Gansu)	Jia & Zhao (2013)
H. hebaueri Schödl, 2000	China (Guandong), Laos	Jia & Zhao (2013)
H. imperialis (Knisch, 1921)	China (Anhui, Jiangxi, Hunan, Fujian)	Jia & Zhao (2013)
H. jengi Sato, 1998	Japan	Hansen (1999)
H. lacustris (Sharp, 1884)	Japan	Hansen (1999)
H. metasternalis Schödl & Ji (1995)	China (Yunnan), Thailand	Schödl (2000)
H. mongolica Liu, Ji, & Jeng, 2008	China (Inner Mongolia)	Liu *et al.*(2008)
H. pseudoscapha Ji & Schödl, 1998	China (Anhui)	Hansen (1999)
H. scapha d'Orchymont (1942)	China (Anhui, Hunan, Guangxi, Jianxi, Zhejian, Guizhou, Fujian, Guangdong, Sichuan), Vietnam	Hansen (1999) and Jia & Zhao (2013)
H. scaphoides d'Orchymont (1942)	Burma, China (Yunnan)	Hansen (1999)
H. scapulata Deyrolle & Fairmaire, 1878	China (Gansu, Hebei, Shanxi, Shaanxi, Sichuan)	Hansen (1999), Schödl (2000) and Jia & Zhao (2013)
H. schillhammeri Schödl & Ji (1995)	China (Yunnan)	Hansen (1999)
H. sichuana (Ji & Schödl, 1998)	China (Sichuan)	Hansen (1999)
H. taiwana Sato, 1971	China (Taiwan)	Hansen (1999)
H. uncinata Ji & Schödl, 1998	China (Yunnan), Laos, Thailand	Schödl (2000)
Hydrobius arcticus Kuwert, 1980	Russia, Finland, Norway, Sweden, ?Turkey	Hansen (1999) and Mart *et al.*(2006)
H. fuscipes Linnaeus (1758)	Widespread in the Palearctic. CA (All provinces and territories), USA (['widespread'] AK, CA, CT, DE, IN, MI, NV, NY, VT, WI).	Hansen (1999) and Short (2005)
H. pauper Sharp, 1884	Japan	Hansen (1999)
H. pui Jia, 1995	China (Qinghai)	Hansen (1999)
H. punctistriatus Jia, 1995	China (Heilongjiang)	Hansen (1999)
Limnocyclus puncticeps Balfour-Browne (1939)	New Caledonia	Hansen (1999)
Limnohydrobius convexus (Brullé, 1835) **stat. rev.**	France, Italy, Portugal, Spain, Algeria, Tunisia, Morocco	Hansen (1999), Incekara & Bouzid (2007) and Touaylia (2011).
L. melaenus (Germar, 1824) **comb.n.**	Canada (NB, NS, ON, QB), USA (DE, IL, IN, WI, NC) ['northeastern, westward to IL, ID and WI; southward to NC']	Hansen (1999) and Short (2005)

Table 2. Continued

Species	Distribution	References
L. orientalis Jia & Short (2009) **comb.n.**	China (Guangdong)	Jia & Short (2009)
L. tumidus (LeConte, 1855) **comb.n**.	USA (ME, NY, PA, FL, MS, IL, DE) ['From ME, NY, & PA to FL and MS, west to IL'].	Hansen (1999) and Short (2005)
Limnoxenus kauaiensis Short & Liebherr (2007)	USA (Hawaii)	Short & Liebherr (2007)
L. namolokama Short & Liebherr (2007)	USA (Hawaii)	Short & Liebherr (2007)
L. nesiticus (Sharp, 1908)	USA (Hawaii)	Short & Liebherr (2007)
L. niger (Gmelin, 1790)	Austria, Britain, Bulgaria, Croatia, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Israel, Italy, Lebanon, Netherlands, Poland, Romania, Syria, ?Turkey, Yugoslavia, Algeria (records from Iberian peninsula need confirmation as may be confused with *L. olmoi*)	Hansen (1999) and Incekara & Bouzid (2007)
L. oahuensis Short & Liebherr 2007	USA (Hawaii)	Short & Liebherr (2007)
L. olmoi Hernando & Fresneda, 1994	Portugal, Spain	Hansen (1999)
L. punctatostriatus Short & Liebherr, 2007	USA (Hawaii)	Short & Liebherr (2007)
L. semicylindricus (Eschscholtz, 1822)	USA (Hawaii)	Short & Liebherr (2007)
L. sjostedti Knisch, 1924	South Africa	Hansen (1999)
L. waialeale Short & Liebherr, 2007	USA (Hawaii)	Short & Liebherr (2007)
L. zealandicus (Broun, 1880)	Australia (Victoria), New Zealand, New Caledonia	Hansen (1999) and Short (2010)
Sperchopsis tessellata Ziegler, 1844	Canada (NB, NS, ON, QB), USA (AL, AR, CT, DE, FL, GA, IL, IN, MD, MA, MI, MS, MO, NJ, NY, NC, OH, PA, SC, TX, VT, VA, WI)	Hansen (1999) and Short (2005)

resolved as sister to (*Limnoxenus* + *Hydramara* + *Limnocyclus*) (1.0/97/0.85). The genus *Limnoxenus* is not recovered as monophyletic (0.99/95/0.84), as the taxon pair *Hydramara* + *Limnocyclus* (–/–/0.84) are resolved as sister to *Limnoxenus sjostedti*, which in turn is found to be sister to the remaining *Limnoxenus*. The Hawaiian *Limnoxenus* form a well-supported monophylum (PP = 0.99/0.99).

Hydrobiusine stridulatory morphology

Two primary forms of the pars stridens on laterosternite 3 were observed among taxa of Hydrobiusini. The third laterosternite was relatively unmodified (e.g. Fig. 2) in the genera *Hydrobius*, *Limnohydrobius*, *Ametor*, *Sperchopsis*, *Hydrocassis* and *Hybogralius*. In these taxa, the mesal half of the sclerites is densely covered with small, hook-shaped unarticulated microtrichia that do not display any organized patterns of arrangement.

In the genera *Hydramara*, *Limnocyclus* and *Limnoxenus*, the postero-mesal region of the laterosternite has been transformed into a field of highly organized cuticular plates that are arranged into dense transverse rows, which together form a large 'file' (e.g. Fig. 3). In the genus *Limnoxenus*, the file structure is very uniform, with the plates tightly packed and uniform in size (Fig. 3G–J). In *Hydramara*, the cuticular plates are arranged in rows, but there is little secondary organization, leaving broad gaps in the file structure (Fig. 3D–F), and the rows of cuticular plates are not uniform in width. The genus *Limnocyclus* possesses a file similar to *Limnoxenus*, but the teeth are extremely close (Fig. 3A–C) such that the region appears nearly smooth when viewed under light microscopy.

The ancestral state reconstruction recovered a single origin of the highly organized stridulatory file at the base of the *Hydramara* + *Limnocyclus* + *Limnoxenus* clade. The character represents an unreversed synapomorphy for the lineage.

Discussion

Classification of the Hydrobiusini

While our analyses affirm the current circumscription of the tribe, we find strong support for several incompatibilities between the inferred evolutionary history and internal classification of the Hydrobiusini. Most problematic is that the genus *Hydrobius* itself is not monophyletic, which had been suggested in prior analyses based on morphology (Short & Liebherr, 2007) and molecular data (Short & Fikáček, 2013). Specifically, the *H. fuscipes* complex is shown to be sister to the former sperchopsine genera, *Sperchopsis*, *Ametor* and *Hydrocassis*, while the remaining species of *Hydrobius* form a separate clade (Fig. 5). Consequently, we believe it is time to separate *Hydrobius* into two genera, one for *H. fuscipes* and its relatives (= *Hydrobius* s.s.) and one for the remaining four species hitherto classified in the genus. A new genus-group name is not required as the name *Limnohydrobius* can be removed from synonymy with *Hydrobius* to serve this purpose (see 'Taxonomy' for further details).

Our single representative of *Hydrocassis* renders the two included species of *Ametor* paraphyletic, suggesting that the two genera may not be reciprocally monophyletic. This situation was actually precisely foreseen by Schödl & Ji (1995) in their taxonomic revision of both genera; they recognized that *A. latus* exhibits intermediate characters between *Ametor* and *Hydrocassis* and may render the former paraphyletic. Indeed, *A. latus* groups with *Hydrocassis* in our analysis rather than *Ametor*. We did not have extensive taxon sampling of these genera (e.g. three of 23), and did not include any Old World representatives of *Ametor*. While morphological and now molecular data suggest that the current classification of these two genera is unstable, we refrain from making any formal changes now pending further study.

The paraphyly of *Limnoxenus*, particularly given the morphological similarity among *L. sjostedti*, *L. niger*, *L. olmoi* and *L. zealandicus*, was surprising, although perhaps it should not have been. Our analyses suggest that *Limnocyclus* and *Hydramara* may represent very derived lineages within *Limnoxenus* rather than evolutionarily distinct genera. Short & Liebherr (2007) found that while there was strong morphological support for *Limnoxenus* + *Limnocyclus* + *Hydramara*, support for a monophyletic *Limnoxenus* was equivocal. However, given that DNA-grade specimens of *Hydramara* were not avaible for this study and the relatively poor internal node support within *Limnoxenus*, we prefer to maintain these two lineages as separate genera until their status can be further clarified.

Species boundaries within Hydrobius ***sensu n.***

The species that remain with the genus *Hydrobius* (s.s.) form a closely related species complex that has proved difficult to parse. For the last 70 years, *H. fuscipes* has been considered a widespread species with a Holarctic range, commonly occurring throughout large swaths of North America and the Palearctic region. Several other more localized species have been generally recognized as separate taxa: *H. arcticus* from Fennoscandinavia and Russia, *H. pauper* from Japan, and *H. pui* and *H. punctistriatus* from China. Numerous additional morphological variants of *H. fuscipes* have also been described from North America and Europe, not to mention many junior synonyms. In total, there are more than 20 names folded under *H. fuscipes* that were at one time considered varieties, subspecies or even full species (Hansen, 1999).

Recently, Fossen *et al.* (2016) took a multifaceted approach to examining species boundaries within the *H. fuscipes* complex within northern Europe. Using three gene fragments and morphology, they tested species boundaries within the complex in northern Europe. This careful study provided much-needed insight into the complex and demonstrated that there is notable genetic structure and measurable morphological variation within the clade, and that these two are probably correlated. To provide formal names to the clades identified as species from Scandinavia, they reinstated two synonyms of *H. fuscipes* (*H. f.* var. *subrotundus* and *H. f.* var. *rottenbergii*) to species status, and essentially redefined *H. fuscipes* proper to a very narrow area in Europe.

While the findings of Fossen *et al.* (2016) are compelling in that there are probably multiple cryptic or incipient lineages that may warrant species status, we do not believe there is yet sufficient data or understanding of the group to translate this knowledge into nomenclatural acts. Rather, we believe that doing as they propose will introduce significant nomenclatural instability. As they focused on a small part of the range of *H. fuscipes* (northern Europe), they do not provide a framework for how the lineages outside northern Europe which have previously been treated as *H. fuscipes* may be delimited or treated. Additionally, they acknowledge that some of the names they reinstated to full species may not be properly applied to the lineages they identified. For these reasons, we do not recognize the elevation of these two names and move them back to synonymy with *H. fuscipes*.

Stridulatory evolution

When the two morphological types of laterosternite 3 (organized and unorganized microstructure) are reconstructed on the total evidence phylogeny (DNA + morphology), there is a single change within the Hydrobiusina from the more commonly observed 'unorganized' type to the highly organized 'file' form (Fig. 5). Additionally, the change to the organized file is unreversed, all taxa in the clade formed by the genera *Limnocyclus*, *Hydramara* and *Limnoxenus* possess this morphology. The morphological structure differs from all other known 'organized' files in the family in which each 'tooth' of the file is formed from single cuticular projection (e.g. *Berosus*), further supporting the idea that they are not homologous in origin. The Hydrophilini, sister group to the Hydrobiusini, exhibit a laternosternite morphology similar to the 'unorganized type' and lack any file organization.

Previous hypotheses on the origin of morphologically complex stridulation within the Hydrophilidae suggested that the teeth in the file are derived from the hooked microtrichia which are found in taxa with an otherwise unmodified pars stridens (e.g. Maillard & Sellier, 1970). These microtrichia are probably homologous with those involved in the wing-binding mechanism in other Staphyliniformia (Hansen, 1997). These assertions were based on examining the structure in individual taxa rather than a phylogenetic context. This hypothesis appears to be supported in the case of the Hydrobiusina: simple stridulation precedes complex file stridulation and the microstructure of the file teeth indicates they are homologous with the unmodified microtrichia found in around the margins of laterosternite 3.

Key to the genera of Hydrobiusini

Modified from Hansen (1991):

1. Elytral margins serrate in posterior half (e.g. Fig. 6A, B). Northern hemisphere 2

– Elytral margins smooth along entire margin (e.g., Fig. 6E, F). Worldwide 4

2. Posterior margin of fifth abdominal ventrite truncate, without medial emargination. Anterior margin of pronotum deeply emarginate. Eastern North America . . . . *Sperchopsis*

– Posterior margin of fifth abdominal ventrite with medial emargination (e.g. Fig. 7G). Posterior margin of pronotum not deeply emarginate. Oriental, eastern Palearctic and western Nearctic regions 3

3. Elytral interstices with simple or double punctation, smooth between punctures (Fig. 6C). Epipleura moderately oblique . . . . *Hydrocassis*

-Elytral interstices not smooth between punctures, but with distinct granulate or rugose sculpturing or microreticulation. Epipleura horizontal anteriorly . . . . *Ametor*

4. Posterior margin of fifth abdominal ventrite with median emargination (Fig. 7G). Size variable. Variously distributed *5*

– Posterior margin of fifth abdominal ventrite entire, without median emargination. Size <5.5 mm … Western Australia . . . . *Hybogralius*

5. Prosternum strongly tectiform, usually forming a median keel (Fig. 7D). Mesosternum usually with a strongly elevated median keel (Fig. 7F) . . . . *Limnoxenus*

– Prosternum flat to only weekly raised medially, never with a median keel. Mesosternum without a strongly elevated median keel, but often with a longitudinal process or tubercle (e.g. Fig. 7E) 6

6. Metafemora entirely glabrous (Fig. 7B). Southern hemisphere 7

– Metafemora pubescent on at least basal half (Fig. 7A). Northern hemisphere 8

7. Meso- and metatibiae with row of dense natatory setae on dorsal face (Fig. 7C). New Caledonia . . . . *Limnocyclus*

– Meso- and metatibiae without row of dense natatory setae on dorsal face. Argentina . . . . *Hydramara*

8. Process of the mesoventrite forming a weak to strongly elevated transverse ridge. Body moderately dorsoventrally compressed (Fig. 6D) . . . . *Hydrobius*

– Process of the mesoventrite forming either a high longitudinal keel or a strongly elevated, broad tubercle (Fig. 7E). Body strongly convex . . . . *Limnohydrobius*

Taxonomy

Family Hydrophilidae
  Subfamily Hydrophilinae

Tribe Hydrobiusini Mulsant, 1844

Hydrobiaires Mulsant, 1844: 116. Type genus: *Hydrobius* Leach, 1815.

Hydrobiates Mulsant, 1844: 117. Type genus: *Hydrobius* Leach, 1815.

Sperchopsini Hansen, 1991: 108. Type genus: *Sperchopsis* LeConte. Synonymized by Short & Fikáček, 2013: 15.

Diagnosis. Size 4.0–13.5 mm. Body hemispherical to elongate; dorsum light brown to black. Maxillary palps short, less than the width of the head; all segments inwardly curved. Antennae with nine antennomeres. Prosternum with or without longitudinal carina. Elytra with systematic punctures and ten rows of serial punctures. Elytral margin serrate or not. Mesoventrite variable, with or without elevation. Third laterosternite with or without an organized stridulatory file. Apex of abdomen truncate or with apical emargination (except *Hybogralius*).

Genus *Ametor* Semenow, 1900

Ametor Semenow, 1900: 614. Type species: *Ametor rudesculptus* Semenow by monotypy.

Genus *Hydrobius* Leach, 1815 sensu n.

Hydrobius Leach, 1815: 96. Type species: *Dytiscus fuscipes* Linnaeus, by subsequent designation of Hope (1838: 125).

Genus *Limnohydrobius* Reitter, 1909 stat.n.

Limnohydrobius Reitter, 1909: 357. Type species: *Hydrobius convexus* Brullé by monotypy.

Genus *Limnoxenus* Motschulsky, 1853

Limnoxenus Motschulsky, 1853: 10. Type species: *Hydrophilus oblongus* Herbst (= *Hydrobius niger* Gmelin) by monotypy.

Genus *Limnocyclus* Balfour-Browne, 1939

Limnocyclus Balfour-Browne, 1939: 375. Type species: *Limnocyclus puncticeps* Balfour-Browne by original designation.

Genus *Hydramara* Knisch, 1925

Hydramara Knisch, 1925: 2. Type species: *Hydrobius argentina* Knisch by monotypy. First described as a subgenus of *Hydrobius* Leach, then elevated to genus by d'Orchymont, 1942: 25.

Genus *Hybogralius* d'Orchymont, 1942

Hybogralius d'Orchymont, 1942: 20. Type species: *Hydrobius Hartmeyeri* Régimbart, 1908 by original designation.

Genus *Hydrocassis* Fairmaire, 1878

Hydrocassis Fairmaire, 1878: 88. Type species: *Hydrocassis scapulata* Fairmaire by monotypy.

Hydrocyclus Sharp, 1884: 451. Type species: *Hydrocyclus lacustris* Sharp by monotypy.

Synonymized by d'Orchymont, 1928: 93.

Genus *Sperchopsis* LeConte, 1861

Sperchopsis LeConte, 1861: 47. Type species: *Spercheus tessallata* Ziegler by original designation.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12239

File S1. Nexus file of aligned DNA matrix.

File S2. Nexus file of total evidence (combined DNA and morphological) matrix.

File S3. Morphological characters and character states.

File S4. Differential diagnoses, distribution and biology of the genera of Hydrobiusini.

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