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Defining the genus *Hydropsyche* (Trichoptera:Hydropsychidae) based on DNA and morphological evidence

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Abstract. In this paper, we review the history of Hydropsychinae genus-level classification and nomenclature and present new molecular evidence from mitochondrial cytochrome c oxidase subunit I (COI) and nuclear large subunit ribosomal ribonucleic acid (28S) markers supporting the monophyly of the genus *Hydropsyche*. Both molecular and morphological characters support a broad conservative definition of *Hydropsyche*. *Caledopsyche*, *Hydatomanicus*, and *Occutanspsyche* are synonymized with *Hydropsyche*. The following species groups are established: *Hydropsyche bronta* Group (generally corresponding with *Ceratopsyche* and *Hydropsyche morosa* and *newae* Groups), *Hydropsyche colonica* Group (generally corresponding with *Orthopsyche*), *Hydropsyche instabilis* Group (generally corresponding with *Hydropsyche* s.s.), and *Hydropsyche naumanni* Group (generally corresponding with *Occutanspsyche*). Molecular data recovered *Hydromanicus* as paraphyletic, and *Cheumatopsyche* and *Potamyia* as sister taxa. The genus names *Plectropsyche* and *Streptopsyche* are reinstated.

Key words: Hydropsychinae, systematics, *Hydropsyche*, nomenclatural instability, genitalic plasticity, sexual selection.

Hydropsychid caddisflies (Trichoptera:Hydropsychidae) are critical components of biomonitoring programs throughout their geographical range because of their high abundance and wide range of pollution tolerance values among species. However, the classification of genera within the subfamily Hydropsychinae has been subject to shifting generic nomenclature. Stable nomenclature systems are the foundation for biologists who use comparative biology to study the

evolutionary history of freshwater bioindicators like caddisflies. When nomenclatural changes obscure or do not reflect monophyly, the power of phylogeny as a comparative framework diminishes. As taxonomic hypotheses change, it is important to preserve “cognitive value” and monophyly (Scheffer 2005) with broad generic definitions across the global geographic range of a group, rather than relying on regional gaps in morphological and ecological characteristics that can lead to the elevation of regional species groups to nonmonophyletic genera.

Thus, our goal was to examine molecular and morphological characters to provide evidence supporting a stable genus-level nomenclature for the

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TABLE 1. Various classification schemes for the species originally described as *Hydropsyche bronta* Ross 1938.

Name	Taxonomic reference
<i>Hydropsyche bronta</i> (alternans group)	Ross 1938
<i>Symphitopsyche</i> (<i>Ceratopsyche</i>) <i>bronta</i>	Ross and Unzicker 1977
<i>Symphitopsyche bronta</i>	Schuster and Etnier 1978
<i>Ceratopsyche bronta</i>	Nielsen 1981
<i>Hydropsyche bronta</i> (morosa group)	Scheffer and Wiggins 1986
<i>Hydropsyche</i> (<i>Ceratopsyche</i>) <i>bronta</i>	Scheffer, Wiggins, and Unzicker 1986
<i>Hydropsyche</i> (<i>Ceratopsyche</i>) <i>bronta</i>	Tian et al. 1996
<i>Hydropsyche bronta</i> (newae group)	Mey 1998

subfamily Hydropsychinae and the *Hydropsyche* sensu lato (s.l.) lineage. Historically, *Hydropsyche* s.l. has included: *Abacaria*, *Aoteapsyche*, *Caledopsyche*, *Ceratopsyche*, *Herbertorossia*, *Hydatomanicus*, *Hydronema*, *Hydropsyche* (*Hydropsyche*), *Hydropsyche* (*Occutanspsyche*), *Mexipsyche*, *Orthopsyche*, and *Symphitopsyche*. If monophyletic, *Hydropsyche* s.l. is one of the most speciose lineages in all of Trichoptera with >500 described species (Morse 2009). Its members are found in Holarctic, Oriental, Afrotropical, and Australasian (minus Australian) streams and rivers, and their larvae exhibit a wide range of pollution tolerances (Resh and Unzicker 1975, Lenat 1993, Lenat and Resh 2001). Despite the ecological importance of the group, the evolutionary history of *Hydropsyche* s.l. has been obscured by the lack of: 1) a universally accepted definition of the genus *Hydropsyche*, 2) knowledge of the larvae, pupae, and females of most species, and 3) support for the phylogenetic position of *Hydropsyche* within the subfamily Hydropsychinae (Geraci et al. 2005, Scheffer 2005).

The history of Hydropsychinae generic classification has included much debate on the meaning of the names *Hydropsyche*, *Symphitopsyche*, and *Ceratopsyche*. The genus *Hydropsyche* Pictet, 1834, has been split into 10 genera and 3 subgenera (reviewed by Scheffer 2005). Ulmer (1907, 1951, 1957), Mosely (1941), McFarlane (1976), and Ross and Unzicker (1977) all described new genera based on adult males whose genitalia differed from *Hydropsyche* sensu stricto (s.s.) males. In North America, the above genus names also corresponded to larval characters (Schuster and Etnier 1978, Schuster 1984, Scheffer and Wiggins 1986), but larvae in most other parts of the world remain largely undescribed or unassociated, and hence their characters states unknown. Schmid (1979) broadly defined

the genus *Hydropsyche*, while noting that, at the time, it was the most morphologically homogeneous genus in all Trichoptera and that splitting the genus amounted, in his opinion, to taxonomic inflation. *Hydropsyche bronta* Ross, 1938 (the type species of *Ceratopsyche*), exemplifies the differing opinions on *Hydropsyche* nomenclature: this species has had 3 generic, 1 subgeneric, and 3 species-group names in its history (Table 1). These nomenclatural debates have resulted in some authors using *Ceratopsyche* as a genus (Merritt et al. 2008) or subgenus name (Tian et al. 1996), whereas others have rejected it altogether and have referred instead to *Hydropsyche* species groups (Scheffer and Wiggins 1986, Malicky and Chantaramongkol 2000, Mey 2003, Olah and Johanson 2008). Additional nomenclatural systems that have been used in major works on *Hydropsyche* s.l. across biogeographical regions are summarized in Table 2.

The first hypothesis of phylogenetic relationships among Hydropsychinae genera (Fig. 1A) was based on male genitalic characters (Ross and Unzicker 1977), but it did not consider the world fauna or outgroup relationships. Other studies of Hydropsychinae classification have focused on immature (Scheffer and Wiggins 1986, Wiggins 1996) or adult male stages (Schmid 1964, Mey 1998, 2003, 2005, Schmid 1998, Malicky and Chantaramongkol 2000, Olah and Johanson 2008). Based on a parsimony analysis of morphological characters from all life stages except eggs, Scheffer (2005) suggested synonymizing 5 genera with *Hydropsyche* (Fig. 1B, node C), but did not change genus-level nomenclature designations. Olah and Johanson (2008) examined morphological characters in a comparative (but non-matrix-based) study of Hydropsychinae, and formally synonymized: 1) *Ceratopsyche*, *Herbertorossia*, *Mexipsyche*, and *Symphitopsyche* with *Hydropsyche*, 2) *Aoteapsyche* with *Orthopsyche*, 3) *Plectropsyche* with *Cheumatopsyche*, 4) *Streptopsyche* with *Calosopsyche*, and 5) *Hydatomanicus* and *Hydatopsyche* with *Hydromanicus*. Thus, 2 different interpretations of similar morphological characters (Scheffer 2005, Olah and Johanson 2008) suggested 2 different genus-level nomenclature systems for Hydropsychinae.

We used molecular data from fragments of the mitochondrial (mt) cytochrome c oxidase subunit 1 (mtCOI) and nuclear large subunit ribosomal ribonucleic acid (rRNA) (28S) genes to infer phylogenetic relationships within Hydropsychinae. Our objectives were to test the monophyly of the *Hydropsyche* (sensu Scheffer 2005) lineage with multiple genes, and, in so doing, to examine outgroup relationships with reference to *Hydropsyche*. This approach allowed us to compare the deoxyribonucleic acid (DNA)-based

TABLE 2. Varying taxonomic treatments of species previously classified in the genera *Hydropsyche*, *Ceratopsyche*, *Mexipsyche*, and *Symphitopsyche*. These classifications do not fully overlap because they do not encompass all species or species groups, but they do represent a significant portion of the fauna and terminology used. Mey (2003) used the term “clade” to refer to a subdivision within a species group and Olah and Johanson (2008) used the term “cluster” to refer to a subdivision within a species group.

Ross and Unzicker 1977	Malicky and Chantaramongkol 2000
<i>Hydropsyche</i>	<i>angustipennis</i> group
<i>Symphitopsyche</i>	<i>annulata</i> group
S. (<i>Ceratopsyche</i>)	<i>asiatica</i> group
S. (<i>Symphitopsyche</i>)	<i>hamifera</i> group
<i>Mexipsyche</i>	<i>javanica</i> group
Scheffer, Wiggins, and Unzicker 1986	<i>formosana</i> group
<i>Hydropsyche</i>	<i>pluvialis</i> group
H. (<i>Ceratopsyche</i>)	<i>saranganica</i> group
H. (<i>Hydropsyche</i>)	<i>vasuomittra</i> group
Scheffer and Wiggins 1986	Olah and Johanson 2008
<i>depravata</i> group	<i>Hydropsyche</i> genus cluster:
<i>scalaris</i> group	<i>vasuomittra</i> group
<i>cuanis</i> group	<i>hamifera</i> group
<i>fulvipes-instabilis</i> group	<i>forcipata</i> cluster
<i>simulans</i> group	<i>hamifera</i> cluster
<i>bryanti-celebensis-annulata</i> group	<i>celebensis</i> cluster
<i>propinqua</i> group	<i>luzonica</i> cluster
<i>morosa</i> group	<i>pluvialis</i> group
<i>newae</i> subgroup	<i>pluvialis</i> cluster
Tian, Yang, and Li 1996	<i>rhomboana</i> cluster
<i>Hydropsyche</i>	<i>pallipenne</i> cluster
H. (<i>Ceratopsyche</i>)	<i>newae</i> group
H. (<i>Hydropsyche</i>)	<i>buergeri</i> group
H. (<i>Mexipsyche</i>)	<i>asiatica</i> group
H. (<i>Occutanspsyche</i>)	<i>ungulata</i> group
Mey 1998	<i>angustipennis</i> group
<i>newae</i> group	<i>guttata</i> cluster
<i>buergeri</i> group	<i>pellucidula</i> cluster
<i>hamifera</i> group	<i>instabilis</i> cluster
<i>formosana</i> group	<i>gifuana</i> group
Mey 2003	
<i>hamifera</i> group	
<i>hamifera</i> clade	
<i>calawiti</i> clade	
<i>faurai</i> clade	
<i>javanica</i> clade	
<i>polyacantha</i> clade	
<i>vasuomittra</i> group	

topology with that inferred from parsimony analysis of morphological characters (Fig. 1B; Scheffer 2005) and to test whether previously designated nomenclature systems communicated monophyly.

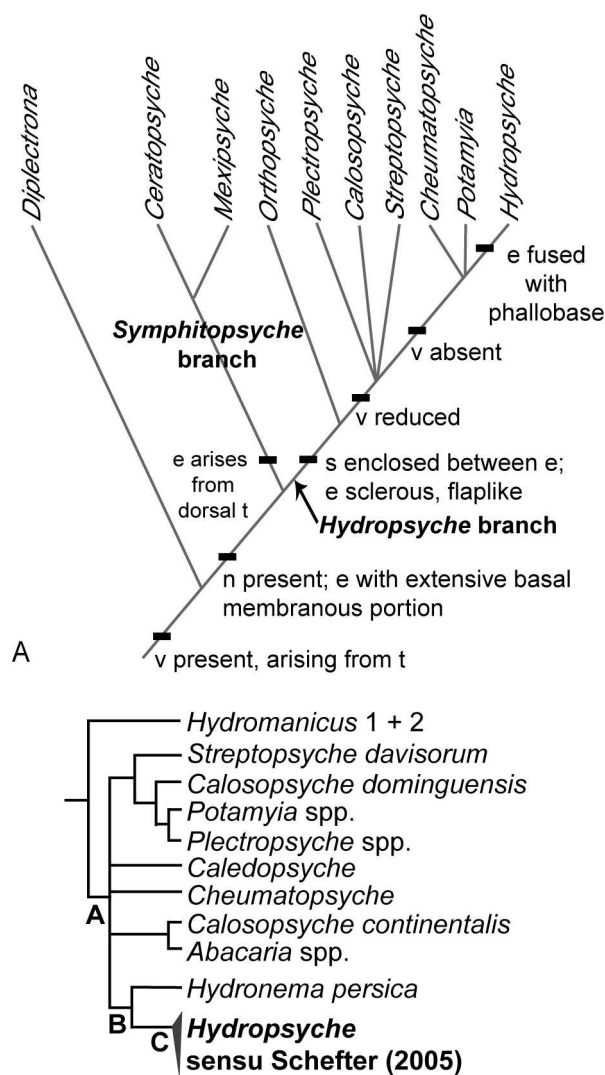


FIG. 1. Phylogenetic hypotheses of Hydropsychinae generic relationships based on morphology. A.—Relationships among Hydropsychinae species based on male genital morphology, adapted from the text of Ross and Unzicker (1977). B.—Maximum parsimony strict consensus tree of Hydropsychinae inferred from adult male morphology data, redrawn and condensed from Scheffer (2005). e = endotheal process, n = endophallus, s = phallotremal sclerites, t = endotheca (or endotheal membrane), v = ventral endotheal lobe.

Methods

DNA sequences were obtained for as many representative species of *Hydropsyche* sensu Scheffer (2005) as possible. Additional exemplars for DNA sequencing were chosen to maximize species group representation (Scheffer and Wiggins 1986, Malicky and Chantaramongkol 2000, Mey 2003, Olah and

Johanson 2008). Specimens were obtained from the Clemson University Arthropod Collection, Nanjing Agricultural University (China), Smithsonian's National Museum of Natural History, the University of Minnesota Insect Collection, and Dr. Hans Malicky. Additional DNA sequences for Hydropsychinae taxa were downloaded from GenBank (Kjer et al. 2001, Zhou et al. 2007). Our analysis consisted of 2 tiers. For the 1st tier, we sequenced the D2 variable region of the nuclear 28S rRNA gene and 657 base pairs (bp) of the COI gene region for 60 Hydropsychinae exemplars (D2COI data set; Appendix 1). These gene fragments were chosen because they trace species and genus boundaries reliably for hydropsychid caddisflies (Zhou et al. 2007) and provide phylogenetic signal at both shallow (COI) and deeper (28S) phylogenetic levels (Kjer et al. 2001, 2002). COI data were generated in collaboration with the Trichoptera Barcode of Life Campaign (<http://www.trichopterabol.org>).

A 2nd-tier data set was assembled consisting of the COI gene sequence and the sequences for the D1, D2, and D3 regions of the 28S rRNA gene (28SCOI data set; Appendix 2) for 12 species representing all available Hydropsychinae genera (both currently and previously recognized genera). The D1 and D3 regions were included with D2 because they varied little in length, had fewer alignment-ambiguous nucleotides, and thus, potentially provided more characters at deeper levels of phylogeny. Three species of *Hydromanicus* were included that represented types 1 (*Hydromanicus* nr. *truncatus* Betten) and 2 (*Hydromanicus* nr. *canaliculatus* Li, Tian, and Dudgeon) from Scheffer's (2005) analysis. Fresh specimens of *Abacaria*, *Hydromanicus seychellensis* Ulmer (*Hydromanicus* type 3, Scheffer 2005), *Hydronema*, and *Schmidopsycha* were not available for DNA analysis. The D2 fragment from *Calosopsycha continentalis* Flint & Bueno-Soria or *Cheumatopsycha hoogstraali* (Ross) (referred to hereafter and in figures as *Plectropsycha hoogstraali* Ross to reflect the updated nomenclature described below) and the D1 and D3 fragments from *Hydromanicus* nr. *canaliculatus* Li, Tian, and Dudgeon or *Hydrosycha instabilis* (Curtis) could not be sequenced.

DNA extraction, polymerase chain reaction, sequencing

Genomic DNA was extracted from either 1 leg or from the entire animal using Qiagen DNeasy Kits (Qiagen, Hilden, Germany) and standard protocols. For some specimens the following modifications were made. An initial volume of 20 µL of Proteinase K was added to the Qiagen ATL buffer, and the legs or entire animals were incubated at 55°C for 24 to 48 h. An

additional 20 µL of Proteinase K was added to the buffer every 24 h. Polymerase chain reaction (PCR) amplification of the 28S rRNA fragment was performed on 1 µL of genomic DNA from each species in 25-µL reactions according to the following recipe: 12.5 µL of Qiagen Taq PCR Master Mix, 5.0 µL Qiagen Q solution, 1.0 µL of each 10-µmol oligonucleotide primer, and 4.5 µL of double distilled (dd) H₂O. The primers used were D1-UP ([5'-GGAGGAAAAGAACTAACAAGGATT-3'] paired with D1-DN [5'-CAACTTTCCCTTACGGTACT-3']; D2UP-4 [5'-GAGTTCAAGAGTACGTGAAACCG-3'] paired with D2DN-B [5'-CCTTGGTCCGTGTTTCAAGAC-3']; and D3-UP [5'-ACCCGTCTTGAAACACGGAC-3'] paired with D3-DN [5'-CTATCCTGAGGGAACTTCGGA-3']).

Purified PCR products were sequenced on an ABI 3730XL or 3130XL sequencer (Applied Biosystems, Foster City, California) using BigDye® Terminator v3.1 (Applied Biosystems) and standard reaction parameters. Each gene fragment was sequenced bidirectionally and then assembled as contig files using either Sequencher (v.4.0.5; Gene Codes Corporation, Ann Arbor, Michigan) or LaserGene (v.6; DNASTAR, Inc., Madison, Wisconsin). Acquisition of the COI barcode region was done at the Canadian Centre for DNA Barcoding, University of Guelph, Canada. Standard barcoding protocols were followed (Ivanova et al. 2006, deWaard et al. 2008). Genomic DNA was extracted using an AcroPrep™ 96 1-mL filter plate (PALL) with 3.0-µm glass fiber (Ivanova et al. 2006). DNA was eluted in 40 µL of distilled (d) H₂O. Full-length COI barcodes were amplified using 2 primer sets: LepF1 ([5'-ATTCAACCAATCATAAA GATATTGG-3']/LepR1 [5'-TAAACTTCTGGATGT CCAAAAAATCA-3']) (Hebert et al. 2004) and LCO1490 ([5'-GGTCAACAAATCATAAAGATATTGG-3']/HCO2198 [5'-TAAACTTCAGGGTGACCAAA AAATCA-3']) (Folmer et al. 1994). Standard DNA barcoding protocols were followed for COI sequencing as described by deWaard et al. (2008) and Hajibabaei et al. (2005). COI barcodes and detailed specimen information were deposited in the Barcode of Life Data (BOLD) Systems (Ratnasingham and Hebert 2007) as part of the Trichoptera Barcode of Life Campaign (<http://www.trichopterabol.org>).

Alignment

Edited 28S rRNA D1 and D3 sequences were aligned following the Trichoptera secondary structural model provided by Kjer et al. (2001). Alignment of hydrogen-bonded stems and stem-and-loop numbering for the D2 fragment followed models available at: <http://hymenoptera.tamu.edu/rna/index.php> (Gil-

lespie 2004, Gillespie et al. 2005). Regions of expansion and contraction (REC) and regions of ambiguous alignment (RAA) were excluded from the analysis (aligned DNA data set available from <http://rci.rutgers.edu/~insects/pdata.htm>).

Phylogenetic analyses

Bayesian analysis was performed with MrBayes (v.3.1.2; Ronquist and Huelsenbeck 2003). Gaps were coded as “–”, and missing data were coded as “?” for all analyses. The consensus tree produced in each analysis was rooted a posteriori with *Calosopsycha parander* (Botosaneanu) (referred to hereafter as *Streptopsycha parander* (Botosaneanu) and in figures to reflect the updated nomenclature described below) because this species was found to be the sister taxon to the rest of Hydropsychinae in previous analyses (Geraci 2007).

D2COI data set

The data were partitioned into 28S rRNA (424 nucleotides [nts] including gaps) and COI (657 nts), and 2 different model schemas were used. In the first Bayesian analysis, the general time reversible + time invariant + Γ (GTR+I+ Γ) model was applied to both partitions, as recommended by MrModeltest (v.2.2; J. A. A. Nylander, Uppsala University, Uppsala, Sweden). A Mixed GTR–Codon model was used in a 2nd analysis, with the GTR model applied to the 28S rRNA partition and the Codon model to the COI partition. The GTR model had 6 Γ rate categories, whereas the Mixed model had 4 categories. Both analyses were run with default values for other model prior parameters; revmat, statefreq, shape, and Pinvar were unlinked. Each analysis had 4 Metropolis-coupled Markov Chain Monte Carlo (MCMC) chains (3 heated and 1 cold) that were run for 5 million generations (with 10% of the trees discarded as burn-in). GARLI (v.0.951; Zwickl 2006) was used to analyze the D2COI 60-taxon data set using the maximum likelihood criterion under the GTR model with default parameters. The most likely tree topology was rooted a posteriori with *Streptopsycha parander* (Botosaneanu) and right-ladderized using TreeView (v.1.6.6; Page 1996). HyPhy (v.0.99; Kosakovsky Pond et al. 2004) then was used to calculate nonsynonymous substitution rates for each branch. Likelihood parameters were optimized on the GARLI maximum likelihood topology based on the D2COI data set for 60 taxa. Parsimony analyses were done with PAUP (v.4.10b; Sinauer, Sunderland, Massachusetts; Swofford 1999). Heuristic searches with tree bisection and reconnection (TBR) branch swapping were done for each data

set, and strict consensus trees were constructed for each analysis. Bootstrap analyses were run for each data set (10,000 replicates, stepwise addition).

28SCOI data set

The data were partitioned into 28S rRNA (979 nts including gaps) and COI (657 nts). Two analyses were run using MrBayes. The GTR model analysis applied the GTR+I+ Γ model to both partitions. The Mixed model analysis applied the GTR+I+ Γ model to the 28S rRNA partition, and the Codon model to the COI partition. The GTR model had 6 Γ rate categories in each analysis, whereas the Codon model had 4 Γ rate categories. Default values were used for all other parameters, and revmat, statefreq, shape, and Pinvar all were unlinked. Four Metropolis-coupled MCMC chains (3 heated and 1 cold) were run for 3 million generations (with 10% burn-in) for the GTR model analysis, and for 10 million generations (with 20% burn-in) for the Mixed model analysis. PHASE (v.2.0; Hudelot et al. 2003) also was used to analyze the 28SCOI data set in a Bayesian framework to enable partitioning of 28S rRNA stem-and-loop regions. The 28S rRNA data were partitioned into loops and hydrogen-bonded stems according to secondary structure, and the COI data were partitioned into codon positions. The RNA7+I+ Γ model with 6 Γ categories was applied to hydrogen-bonded stems, the reverse + time invariant + Γ (REV+I+ Γ) model with 6 Γ categories was applied to loops, and the YNH98 codon model was applied to the COI partition. The MCMC chains were run using a random start chain and model parameters for 1 million burn-in iterations and 10 million sampling iterations (sampling period = every 100 iterations).

Results

All analyses recovered a monophyletic *Hydropsyche* clade that subsumes these previously established genus-group names: *Aoteapsyche*, *Caledopsyche*, *Ceratopsyche*, *Herbertorossia*, *Hydatomanius*, *Hydropsyche* (*Hydropsyche*), *Hydropsyche* (*Occutanspsyche*), *Mexipsyche*, and *Orthopsyche*. The consensus trees produced by both GTR and Mixed GTR/Codon model Bayesian analyses of the D2COI data set for 60 taxa recovered 100% posterior probability (p.p.) support for *Hydropsyche* (Fig. 2A, B). The topology of *Hydropsyche* derived from molecular data is congruent with the parsimony-derived topology inferred from morphology (Scheffer 2005) except for the placement of the *Caledopsyche* exemplary species (*Hydropsyche atalanta* (Scheffer & Ward) and *H. C.J.G. sp. NC2*). *Caledopsyche* was not erected based on genitalic characters, but

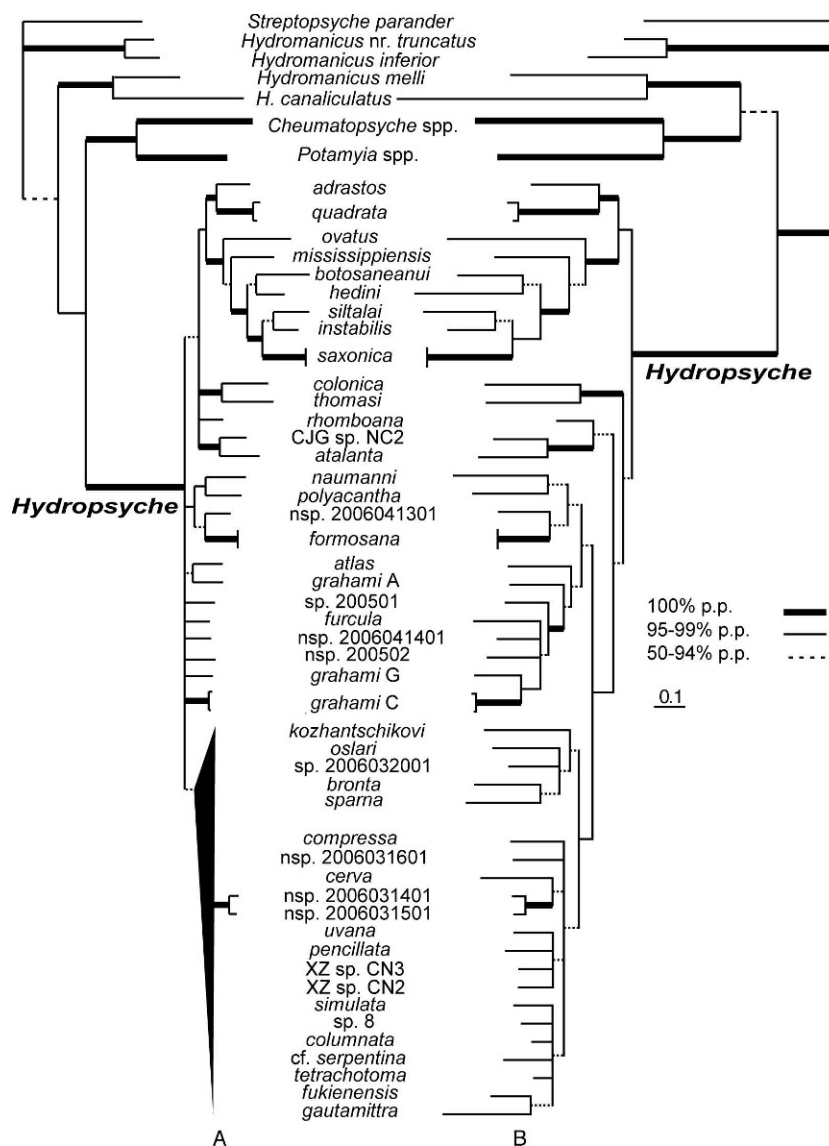


FIG. 2. Bayesian consensus phylogenies show the strongly supported monophyly of *Hydropsyche*. Trees were inferred from 1081 nucleotides of the mitochondrial cytochrome c oxidase subunit I [COI] and D2 region of nuclear large subunit ribosomal ribonucleic acid (28S D2) for 60 *Hydropsychinae* species under 2 model schema. A.—Consensus phylogeny from the Mixed (general time reversible [GTR]/Codon) model (weakly supported nodes beyond *Hydropsyche* were collapsed a posteriori for aesthetic reasons). B.—Consensus phylogeny from the GTR model. Thick solid lines, thin solid lines, and dashed lines signify nodes with 100%, 95 to 99%, and <95% posterior probability support, respectively. Nomenclature changes outlined in our paper are reflected. Numbers and codes in species names refer to BOLD sample identification numbers (see Appendix 1).

rather on a wing vein autapomorphy (Kimmins 1953). However, DNA characters support the placement of *Caledopsyche* species within *Hydropsyche*. Chinese species previously classified as *Mexipsyche* (*Hydropsyche grahami* A, C, and G Banks; *H. furcula* Tian & Li) did not form a monophyletic lineage, and *Hydropsyche grahami* Banks might contain a series of cryptic lineages, an observation supported by a larger data set of COI barcodes (XZ, unpublished data). Olah and

Johanson (2008) synonymized *Hydatomanicus* with *Hydromanicus*, but DNA data supported with 100% p.p. *Hydromanicus ovatus* (previously *Hydatomanicus ovatus*) (Li, Tian, & Dudgeon) as belonging to *Hydropsyche* (Fig. 2A, B). This conclusion also is supported by larval morphology (Zhou 2007). The placement of *Hydropsyche ovatus* (Li, Tian, & Dudgeon) as the basal species within the *H. instabilis* Group in both Bayesian analyses (Fig. 2A, B) suggests

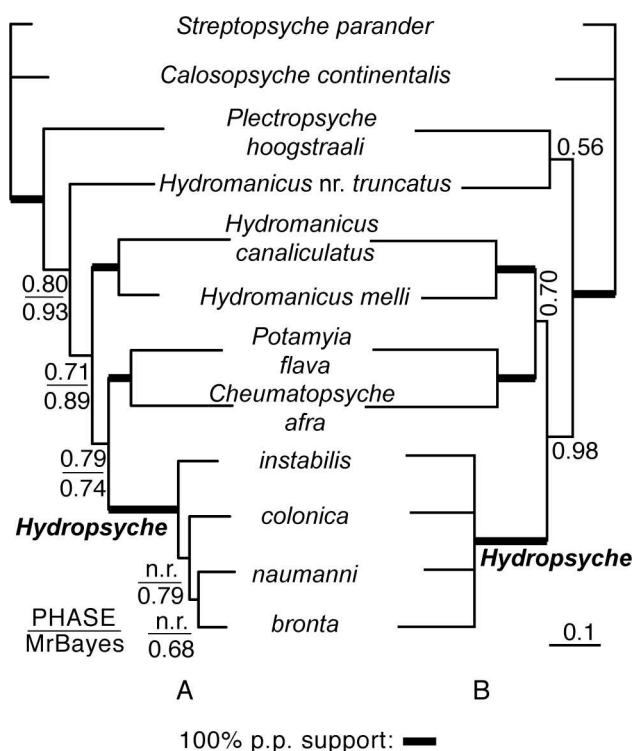


FIG. 3. Bayesian consensus phylogenies based on 1636 nucleotides of the nuclear large subunit ribosomal ribonucleic acid (28S) regions D1, D2, and D3 and mitochondrial cytochrome c oxidase subunit I (COI) gene fragments for 12 Hydropsychinae taxa mirrored strong support for *Cheumatopsyche*, *Potamyia*, and *Hydropsyche* monophyly, but recovered a paraphyletic *Hydromanicus*. A.—Topology recovered by both PHASE and MrBayes under a Mixed (general time reversible [GTR]/Codon) model. The top number in each pair is the value in PHASE, and the bottom number is the value in MrBayes. B.—Topology recovered by MrBayes using a GTR model for both partitions. Numbers at nodes represent posterior probability values; thick solid lines represent 100% posterior probability (p.p.) support. Nomenclature changes outlined in our paper are reflected. n.r. = not recovered.

that it belongs to that species group. Exemplars of *H. (Hydrocheumatopsyche)* Marlier were not available to us, so that subgenus distinction is retained here. DNA from *Hydromanicus seychellensis* Ulmer, African *Symphitopsyche*, or any *Abacaria* or *Hydronema* exemplars were unavailable to us, so the current nomenclature for those groups is maintained here.

Bayesian analyses of the 28SCOI data set also recovered 4 *Hydropsyche* sensu Scheffer (2005) exemplars as a monophyletic clade with 100% p.p. support (Fig. 3A, B). Parsimony analyses supported *Hydropsyche* as monophyletic except when only COI nucleotides were used (data not shown). HyPhy analysis recovered a nonsynonymous substitution

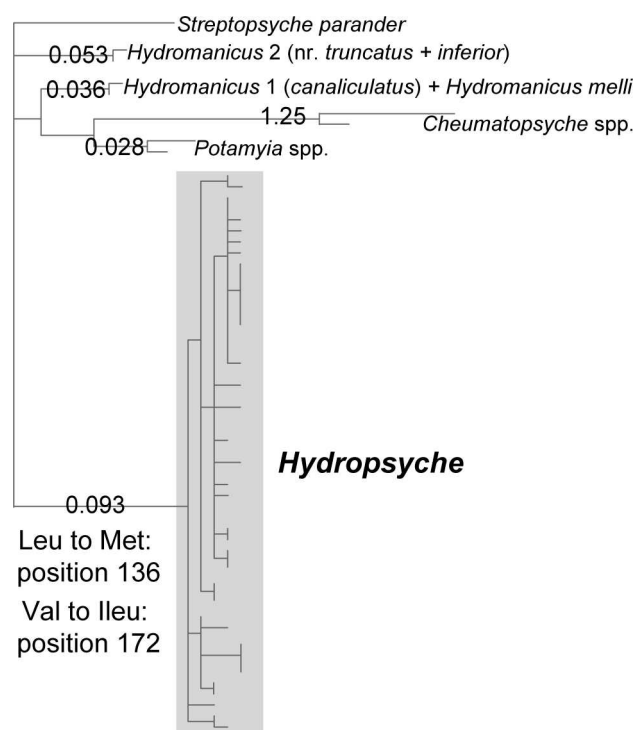


FIG. 4. Maximum likelihood topology for 60 species of Hydropsychinae scaled according to nonsynonymous mitochondrial cytochrome c oxidase subunit I (COI) substitution rates calculated in the program HyPhy. Two amino acid changes occurred in the ancestor to *Hydropsyche* and are synapomorphies for that genus. Rates are presented on nodes for each lineage. Likelihood parameters were optimized on the maximum likelihood topology recovered from GARLI ML software using a general time reversible + invariant time + Γ (GTR+I+ Γ) model on 1081 nucleotides of the D2 region of nuclear large subunit ribosomal ribonucleic acid [28S D2] and COI gene fragments. Nomenclature changes outlined in this paper are reflected. Leu = leucine, met = methionine, val = valine, Ileu = isoleucine.

rate for the *Hydropsyche* branch (0.093) that was similar to that of the branches for other genera within Hydropsychinae (Fig. 4). The only branch that had a higher rate (0.125) was for *Cheumatopsyche*. Further examination of the translated amino acids revealed 2 unreversed substitutions at positions 136 and 172 (out of 219 total amino acids translated from the 657-nucleotide COI fragment; Fig. 4). These COI amino acid substitutions are synapomorphies for *Hydropsyche*, as defined here (see *Genus diagnosis* below).

Bayesian analyses for the 28SCOI data set recovered conflicting topologies with regard to relationships among *Cheumatopsyche*, *Potamyia*, and *Hydromanicus* s.s. (Scheffer's type 2; Fig. 3A, B). The Mixed model analyses that accounted for COI codon position recovered the clade (*Cheumatopsyche* spp. + *Potamyia*

spp.) as the sister taxon to *Hydropsyche* but with only 79% p.p. support (Fig. 3A). However, the GTR model analysis recovered (*Hydromanicus canaliculatus* + *Hydromanicus melli*) as sister to (*Cheumatopsyche* spp. + *Potamyia* spp.) with 70% p.p. support, but strongly (defined here as >95% p.p.) supported that clade as sister to *Hydropsyche* (Fig. 3B). The conflicting topologies recovered by the GTR vs Mixed models for the larger taxon sample (Figs 2A vs 3B) mirrored this uncertainty in outgroup relationships. Scheffer (2005) recovered *Hydronema* as sister to *Hydropsyche*, as it was defined at the time. The sister taxon to *Hydropsyche* remains equivocal because specimens of *Hydronema* were not available for DNA extraction. However, *Cheumatopsyche* and *Potamyia* were recovered as sister taxa with 100% p.p. support (Figs 2A, B, 3A, B). This relationship is not congruent with the parsimony topology inferred from morphology (Fig. 1B) of Scheffer (2005), but it produces the same genus-level nomenclature system.

The 28S rRNA and COI data also suggest that *Hydromanicus* (sensu Olah and Johanson 2008) is not monophyletic and needs formal taxonomic revision (Figs 2A, B, 4). The paraphyly of *Hydromanicus* also was supported by morphology data (Fig. 1B; Scheffer 2005) and by examination of larval characters of some Chinese *Hydromanicus*. The larvae of *Hydromanicus canaliculatus* Li, Tian, & Dudgeon and *H. melli* (Ulmer) share synapomorphies (e.g., head glabrous, anterior margin of frontoclypeal apotome asymmetric, deeply excised, etc.), and both are distinctly different from those of *Hydromanicus* nr. *truncatus* Betten (Zhou 2007). Last, we revert to the previous nomenclature for *Streptopsyche parander* (Botosaneanu) and *Plectropsyche hoogstraali* Ross because our topology and resulting classification was congruent with that inferred from morphological characters in a parsimony framework (Scheffer 2005). Neither study supported the synonymy of *Streptopsyche* with *Calosopsyche* or the synonymy of *Plectropsyche* with *Cheumatopsyche* (sensu Olah and Johanson 2008). Therefore, revised nomenclature is used in all figures and in Appendices 1 and 2 for clarity and ease of comparison to Scheffer's (2005) topology.

Proposed classification

Hydropsychinae genera.—Our analyses lead us to propose the following classification of the genera of *Hydropsychinae*:

Family Hydropsychidae Curtis, 1835 Subfamily Hydropsychinae Curtis, 1835

GENUS *Abacaria* Mosely, 1941

GENUS *Calosopsyche* Ross & Unzicker, 1977

GENUS *Hydropsyche* Pictet, 1834

SUBGENUS *Hydropsyche* Pictet, 1834

Hydropsyche bronta Group (generally corresponding with *Ceratopsyche* and *H. morosa* and *newae* Groups)

Hydropsyche colonica Group (generally corresponding with *Orthopsyche*)

Hydropsyche instabilis Group (generally corresponding with *Hydropsyche* s.s.)

Hydropsyche naumanni Group (generally corresponding with *Occutanspsyche*)

SUBGENUS *Hydrocheumatopsyche* Marlier, 1962

GENUS *Cheumatopsyche* Wallengren, 1891

SUBGENUS *Abacarioides* Marlier, 1961

SUBGENUS *Achirocentra* Marlier, 1961

SUBGENUS *Cheumatopsyche* Wallengren, 1891

SUBGENUS *Cheumatopsychodes* Marlier, 1961

SUBGENUS *Ethiopsyche* Marlier, 1962

GENUS +*Electrodipletrona* Ulmer, 1912

GENUS *Hydromanicus* Brauer, 1865

Synonym GENUS *Hydatopsyche* Ulmer, 1926
(Olah and Johanson 2008:14)

GENUS *Hydronema* Martynov, 1914

GENUS +*Palaehydropsyche* Wichard, 1983

GENUS *Plectropsyche* Ross, 1947

GENUS *Potamyia* Banks, 1900

GENUS *Schmidopsyche* Olah & Scheffer 2008

GENUS *Streptopsyche* Ross & Unzicker, 1977

Hydropsyche.—Furthermore, our analyses lead us to recognize the following synonyms for *Hydropsyche*:

Genus *Hydropsyche* Pictet, 1834

Type species: *Hydropsyche cinerea* Pictet [subsequent designation Ross 1944:86, = *Hydropsyche instabilis* (Curtis, 1834)].

Synonym *Aoteapsyche* McFarlane, 1976, type species: *Hydropsyche raruraru* McFarlane (original designation); considered a synonym of *Hydropsyche* by Scheffer 2005:148 (synonymized with *Orthopsyche* by Olah and Johanson 2008:164).

Synonym *Caldra* Navás, 1924, type species: *Caldra nigra* Navás (original designation); synonymized with *Hydropsyche* by Botosaneanu and Malicky 1978:344, synonymy not confirmed in this study.

Synonym *Ceratopsyche* Ross & Unzicker, 1977, type species: *Hydropsyche bronta* Ross (original designation); synonymized as a subgenus of *Hydropsyche* by Scheffer et al. 1986:68, reduced to synonym of *Hydropsyche* by Olah and Johanson 2008:56.

Synonym *Caledopsyche* Kimmins, 1953, type species: *Caledopsyche cheesmanae* Kimmins (original designation); NEW SYNONYM.

Synonym *Herbertorossia* Ulmer, 1957, type species: *Hydromanicus unguatus* Ulmer (original designation); synonymized with *Hydropsyche* by Scheffer 2005:148 and Olah and Johanson 2008:56.

Synonym *Hydatomanicus* Ulmer 1951, type species: *Hydromanicus verrucosus* Ulmer (original designation); synonymized as a subgenus of *Hydropsyche* by Malicky and Chantaramongkol 2000:791–860 (considered a synonym of *Hydromanicus* by Olah and Johanson, 2008:14).

Synonym *Mexipsyche* Ross and Unzicker, 1977, type species: *Mexipsyche dampfi* Ross and Unzicker (original designation); synonymized with *Hydropsyche* by Scheffer 2005:148 and Olah and Johanson 2008:56.

Synonym *Occutanspsyche* Li and Tian, 1989, type species: *Hydropsyche polyacantha* Li and Tian (original designation); described originally as a subgenus of *Hydropsyche*; reduced to NEW SYNONYM of *Hydropsyche* in this study.

Synonym *Orthopsyche* McFarlane, 1976, type species: *Hydropsyche fimbriata* McLachlan (original designation); synonymized with *Hydropsyche* by Scheffer 2005:148.

Synonym *Plesiopsyche* Navás, 1931, type species: *Plesiopsyche alluaudina* Navás (original designation); synonym of *Symphitopsyche* according to Ross and Unzicker 1977:304–305, synonymy not confirmed in this study.

Synonym *Symphitopsyche* Ulmer, 1907, type species: *Hydropsyche mauritiana* McLachlan (monobasic); synonymized with *Hydropsyche* by Scott 1983:319, Scheffer 2005:148, and Olah and Johanson 2008:56.

Genus diagnosis

The following synthetic diagnosis combines morphological characters described by Scheffer (2005) and molecular characters from this study of the 28S rRNA D2 fragment and COI gene.

Adults (character numbers refer to those by Scheffer 2005).—The pro-episternal setal wart (Character 4) is a synapomorphy for *Hydropsyche* (Scheffer 2005). Other characters have been shown to vary in some *Hydropsyche* taxa (see Scheffer 2005, for further discussion of each of these characters). Maxillae each has its 2nd maxillary segment subequal in length to its 3rd, and its 5th segment is subequal in length to its segments 1–4 combined (Characters 1 and 2) (Banks 1914, Ross 1944, Ulmer 1951). A tarsal setal bundle is present on each foretarsus of the male (Character 5) (Ulmer

1951). Each forewing has its crossvein *cu* at or near the thyridial nygma, not close to crossvein *m-cu* (Character 6) (Ross 1944, Ulmer 1951). Each hind wing has its crossvein *m-cu* present and conspicuous (Character 11). The dorsum of the head has 7 warts (Character 3). Posterior lobes are present on segments X–XI of the female (Character 40).

Larvae.—The submentum is cleft (Character 46). The foretrochantin is biramous (Character 47), and a pair of large sclerites occurs in the intersegmental fold posterior to the prosternal plate in *Hydropsyche* species (Schuster and Etnier 1978, Morse and Holzenthal 2008), however, these character states also can be found in some Chinese *Hydromanicus* species (Zhou 2007).

DNA characters.—All *Hydropsyche* species examined had an “A–C” bulge in stem 2–2' of the 28S rRNA D2 fragment. The secondary structure of the D2 fragment for *Hydropsyche instabilis* (Curtis) is illustrated in Fig. 5. *Hydropsyche* also is characterized by 2 mtCOI amino acid changes: leucine to methionine at position 136 (out of 219 amino acids), and valine to isoleucine at position 172 (Fig. 4).

Distribution.—Afrotropical (AT), Australasian (AU), East Palearctic (EP), Nearctic (NA), Neotropical (NT), Oriental (OL), West Palearctic (WP). The distribution of *Hydropsyche* within the Neotropical Region is limited and not yet fully known. *Hydropsyche* species have not been found in Australia, but are known from New Zealand, New Caledonia, Indonesia, and other islands in the Australasian Biogeographic Region.

Discussion

Our objectives were to test the monophyly of *Hydropsyche* (Scheffer 2005) and its relationships to other hydropsychine genera based on DNA data. Both model-based and parsimony trees support a broad definition of *Hydropsyche* that is largely, but not entirely, congruent with that inferred from morphology data (Scheffer 2005). *Hydropsyche*, as defined in our paper, has diagnostic morphological characters for both adults and larvae and from both the COI amino acid and 28S rRNA D2 data sets. However, the resolution and support beyond the *Hydropsyche* node is inconsistent (Fig. 2A, B). We support the use of species group names for groups whose members possess apomorphic, diagnostic morphological, behavioral, or ecological characters. Expanded taxon sampling from existing species groups and genera is needed to analyze the phylogenetic relationships within *Hydropsyche*. In particular, more sampling of the Nearctic and Oriental *Hydropsyche bronta* Group, *Mexipsyche* (generally corresponding to the *Hydropsyche propinqua* Group), and African *Symphitopsyche*

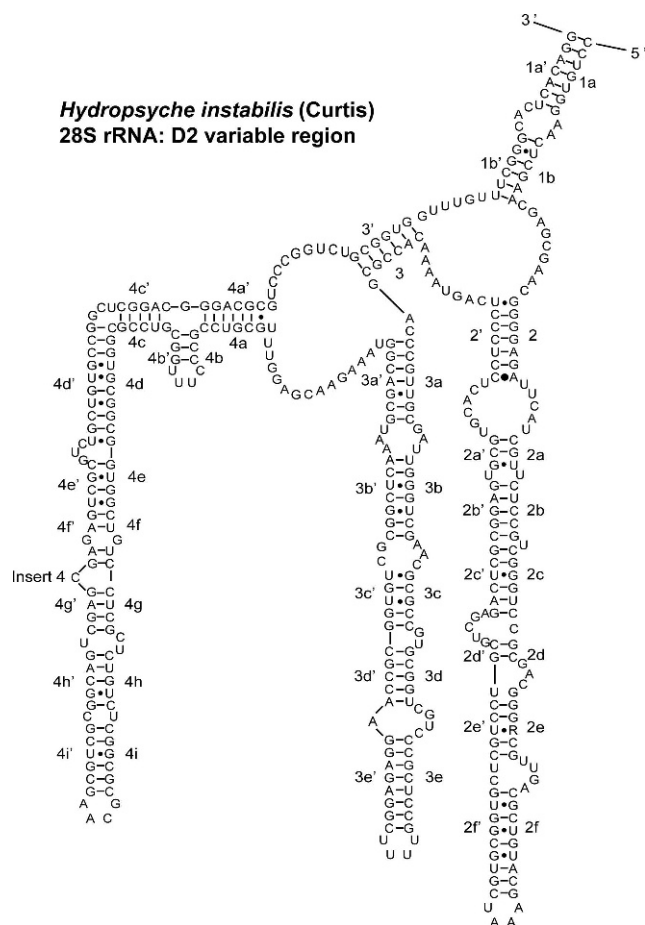


FIG. 5. Two-dimensional visualization of the secondary structure of the D2 region of nuclear large subunit ribosomal ribonucleic acid (28S) D2 variable region of the exemplar specimen of *Hydropsyche instabilis* (Curtis). *Hydropsyche instabilis* is the senior synonym of *Hydropsyche cinerea* (Pictet), the type species of genus *Hydropsyche* (Fischer, 1963:51). Canonical pairings are represented by dashes, guanine-uracil (G-U) pairing by small black circles, and noncanonical pairings by large black circles in between nucleotide letters. The noncanonical adenine-cytosine (A-C) pair at the base of the loops between stems 2 and 2a is a synapomorphy for *Hydropsyche*.

species are needed to test the monophyly of those taxa and standardize species group terminology.

We suggest that the geographically restricted use of non-matrix-based interpretations of male genitalic structures to establish genera (e.g., Fig. 1A), in the absence of corroborating female, larval, or DNA characters, can contribute to nomenclatural instability. The link between genitalic diversity and stability of generic definitions in Hydropsychidae can be seen by comparing the taxonomic histories of *Cheumatopsyche* and *Hydropsyche*. *Cheumatopsyche* has not been split into multiple genera, and the description of *Cheuma-*

topsycha subgenera (Marlier 1961, 1962a, b) did not obscure the definition of the genus. If the *Hydropsyche* and *Cheumatopsyche* lineages have comparable distributions and species numbers (Table 3), why was *Hydropsyche* split in so many contested ways, whereas *Cheumatopsyche* was not? One possible reason is differing taxonomic philosophies of describers (i.e., lumpers vs splitters), but this reason is unlikely because many of the same authors described species from both groups. We suggest, in agreement with Schmid (1979), that perhaps too much emphasis was placed on phallic characters for defining higher-level taxa in Hydropsychidae systematics without considering that some of these structures might have evolved convergently or in parallel. *Cheumatopsyche* lacks the diversity in phallic structures that *Hydropsyche* species display (Scheffer 2005, Korecki 2006). Furthermore, if only the North American fauna is considered, the distinction between the *Hydropsyche* and *Ceratopsyche* male genitalic forms is more pronounced because the species that display intermediate morphological forms (Fig. 2A, B) are not found in the Nearctic Region.

Phylogenetic relationships among *Hydropsyche* species groups might be illuminated by further examination of homology relationships among phallic morphology characters. However, insect male genitalia have been shown to be complex and subject to sexual selection (Eberhard 1985, 2004, Hosken et al. 2001, Hosken and Stockley 2004, House and Simmons 2005), and their evolution is driven by mating systems (Arnqvist 1998, Arnqvist et al. 2000) or coevolution via reproductive conflict (Cordoba-Aguilar 2002, Ronn et al. 2007). Relying on such potentially plastic characters to define or synonymize genera in the absence of corroborating evidence, or to infer phylogeny without firmly establishing homology among phallic characters, could lead to classification via functional analogy or convergent evolution instead of via shared ancestry. As DNA sequencing campaigns continue to assist in the association of life stages (Zhou et al. 2007, Zhou 2009), the immatures and females of more species will be described, and we will be able to use a combined evidence approach to revise species group relationships for *Hydropsyche*.

Our study demonstrates that revisionary taxonomy at the generic level is important to both basic phylogenetics and applied research. As we continue to gain appreciation for the value of combined data sets that include structural attributes from all life stages plus molecular characters from multiple genes, consistent generic definitions become increasingly important. Consistency is needed to avoid creating chimera taxa from unrelated species that happen to

TABLE 3. Comparison of *Hydropsyche* and *Cheumatopsyche* species numbers, taxonomy, distribution, and phallic characters.

Variable	<i>Hydropsyche</i>	<i>Cheumatopsyche</i>
Approximate number of species*	580	340
Distribution	Cosmopolitan except for Australia and South America	Cosmopolitan except for Australia and South America
Taxonomic divisions	10 genera	5 subgenera
Endothelial processes	Either membranous or sclerotized	Always sclerotized
Phallotremal sclerites	Dorsal or apical	Always apical, between sclerotized flaps

* based on the current Trichoptera World Checklist values

have the same genus name (e.g., *H. canaliculatus* + *H. nr. truncatus*) and using those chimera in combined evidence phylogenetic analyses. Both morphological and molecular data support *Hydropsyche* as a species-rich and widespread monophyletic lineage that is characterized by at least 2 synapomorphic amino acid changes in the mitochondrial COI genome and 1 secondary structural change in the nuclear 28S rRNA genome. The evolutionary history of *Hydropsyche* subgenera and species groups is long and complex (as evidenced by its wide geographic range; Appendix 1), probably with multiple colonization and extinction events at both local and global scales. Mey (2003) inferred that Southeast Asia was the center of taxonomic diversity for *Hydropsyche*, and that *Hydropsyche* species are relatively recent immigrants to the Afrotropics (Mey 2005). A revision of the World *Hydropsyche* species that examines morphological and molecular diversity across the entire geographic scope of the genus is needed to test such biogeographical and ecological hypotheses regarding the evolutionary history of species groups.

Last, our analysis provides a basic framework for future applied research on hydropsychid larvae. We know from decades of bioassessment data that larvae of North American *Hydropsyche* species display a particularly wide range of pollution tolerance values (Resh and Unzicker 1975, Lenat 1993, Lenat and Resh 2001), but we do not yet know why. Is phylogenetic signal inherent in this pattern: i.e., are sister species more likely to have similar tolerance values than are more distantly related species within the Hydropsychinae? Molecular approaches like DNA barcoding are being used to expedite larval–adult associations (Zhou et al. 2007), and biomonitoring programs continue to expand worldwide (Morse et al. 2007). Differences in larval physiology and behavior might explain why different Hydropsychidae species have different pollution tolerance values (Petersen and Petersen 1984, Vuori 1994, Vuori and Kukkonen 1996, Tessier et al. 2000a, b, c, d, Illes et al. 2001, Buchwalter and Luoma 2005, Buchwalter et al. 2008). Our ability to study the mechanisms driving those

physiological and behavioral differences in a phylogenetic context will depend on taxonomists and ecologists in different parts of the world basing hydropsychine generic classification on monophyly and using the name *Hydropsyche* consistently.

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APPENDIX 1. Exemplar specimens used for the analyses with the D2COI data set (mitochondrial cytochrome c oxidase subunit I [COI] and D2 region of nuclear large subunit ribosomal ribonucleic acid ([28S D2]), their collection locality, GenBank accession numbers, and BOLD (Barcode of Life Data System; Ratnasingham and Hebert 2007) sample identification (ID) numbers.

Species	Country	GenBank accession number		BOLD sample ID number
		28S D2	COI	
<i>Cheumatopsyche lateralis</i> (Barnard)	South Africa	EU254438	HM102227	07CJCAD-0022
<i>Cheumatopsyche triangularis</i> (Ulmer)	South Africa	EU254435	HM102228	07CJCAD-0019
<i>Hydromanicus melli</i> (Ulmer)	China	HM167431	HM102230	07CJCAD-0012
<i>Hydromanicus inferior</i> Chantaramongkol & Malicky	Thailand	HM167432	HM102229	07CJCAD-0038
<i>Hydromanicus</i> nr. <i>canaliculatus</i> Li, Tian, & Dudgeon	China	EF513893	HM102231	CNCAD_0211
<i>Hydromanicus</i> nr. <i>truncatus</i> Betten	China	EU254432	HM102232	07CJCAD-0014
<i>Hydrosyche adraetos</i> Malicky & Chantaramongkol	Thailand	HM167433	HM102233	07CJCAD-0042
<i>Hydrosyche atalanta</i> (Scheffer & Ward)	New Caledonia	HM167434	HM102234	07CJCAD-0032
<i>Hydrosyche atlas</i> Malicky & Chantaramongkol	Bhutan	HM167435	HM102235	07CJCAD-0044
<i>Hydrosyche botosaneanui</i> Marinkovic	Italy	HM167436	HM102236	07CJCAD-0045
<i>Hydrosyche bronta</i> Ross	USA	HM167437	HM102237	KKCAD-0265
<i>Hydrosyche colonica</i> McLachlan	New Zealand	HM167438	HM102241	07CJCAD-0060
<i>Hydrosyche columnata</i> Martynov	China	EF513980	HM102242	CNCAD_0238
<i>Hydrosyche compressa</i> Li & Tian	China	EF513906	HM102243	CNCAD_0056
<i>Hydrosyche cerva</i> Li & Tian	China	EF513944	HM102238	CNCAD_0166
<i>Hydrosyche formosana</i> A Ulmer	China	EF13958	HM102244	CNCAD_0216
<i>Hydrosyche formosana</i> B Ulmer	China	EF513959	HM102245	CNCAD_0217
<i>Hydrosyche fukienensis</i> Schmid	China	EF513900	HM102246	CNCAD_0032
<i>Hydrosyche furcula</i> Tian & Li	China	EF513896	HM102247	CNCAD_0019
<i>Hydrosyche gautamittra</i> Schmid	China	EF513922	HM102248	CNCAD_0124
<i>Hydrosyche grahami</i> A Banks	China	EF513931	HM102249	CNCAD_0153
<i>Hydrosyche grahami</i> C Banks	China	EF513899	HM102251	CNCAD_0031
<i>Hydrosyche grahami</i> C Banks	China	EF513921	HM102250	CNCAD_0122
<i>Hydrosyche grahami</i> G Banks	China	EF514001	HM102252	CNCAD_0262
<i>Hydrosyche</i> sp. 200501	China	HM167439	HM102274	07CJCAD-0013
<i>Hydrosyche</i> nsp. 2006041401	China	EF514002	HM102263	CNCAD_0263
<i>Hydrosyche hedini</i> Forsslund	China	EF513985	HM102253	CNCAD_0243
<i>Hydrosyche instabilis</i> (Curtis) ^a	Austria	HM167440	HM102254	07HMCAD-0091
<i>Hydrosyche kozhantschikovi</i> Martynov	China	EF13975	HM102255	CNCAD_0233
<i>Hydrosyche mississippiensis</i> Flint	USA	HM167441	HM102256	07CJCAD-0029
<i>Hydrosyche naumanni</i> Malicky	Indonesia	EU254434	HM102257	07CJCAD-0016
<i>Hydrosyche osleri</i> Banks	USA	HM167442	HM102264	07CJCAD-0063
<i>Hydrosyche ovatus</i> (Li, Tian, & Dudgeon)	China	EF513902	HM102265	CNCAD_0036
<i>Hydrosyche penicillata</i> Martynov	China	HM167446	HM102266	CNCAD_0253
<i>Hydrosyche polyacantha</i> Li & Tian	China	EF513950	HM102267	CNCAD_0184
<i>Hydrosyche quadrata</i> A Li & Dudgeon	China	EF513943	HM102268	CNCAD_0165
<i>Hydrosyche quadrata</i> B Li & Dudgeon	China	EF513956	HM102269	CNCAD_0202
<i>Hydrosyche rhomboana</i> Martynov	China	EF513991	HM102270	CNCAD_0251
<i>Hydrosyche saxonica</i> McLachlan	Austria	HM167443	HM102271	07CJCAD-0040
<i>Hydrosyche</i> cf. <i>serpentina</i> Schmid	China	EF513972	HM102239	CNCAD_0230
<i>Hydrosyche siltalai</i> Doehler	Austria	HM167444	HM102272	07HMCAD-0003
<i>Hydrosyche simulata</i> Mosely	China	EF513924	HM102273	CNCAD_0130
<i>Hydrosyche sparna</i> Ross	USA	HM167445	HM102277	07CJCAD-0027
<i>Hydrosyche tetrachotoma</i> Li & Tian	China	EF513963	HM102278	CNCAD_0221
<i>Hydrosyche thomasi</i> Wise	New Zealand	EU254446	HM102279	07CJCAD-0052
<i>Hydrosyche uvana</i> Mey	Thailand	HM167447	HM102280	07CJCAD-0043
<i>Hydrosyche</i> C/JG sp. NC2	New Caledonia	EU254421	HM102240	07CJCAD-0003
<i>Hydrosyche</i> nsp. 2006031401	China	EF513976	HM102259	CNCAD_0234
<i>Hydrosyche</i> nsp. 2006031501	China	EF513977	HM102260	CNCAD_0235
<i>Hydrosyche</i> nsp. 2006031601	China	EF513971	HM102261	CNCAD_0229
<i>Hydrosyche</i> nsp. 200502	China	EF513913	HM102258	CNCAD_0099
<i>Hydrosyche</i> nsp. 2006041301	China	EF513895	HM102262	CNCAD_0011

APPENDIX 1. Continued.

Species	Country	GenBank accession number		BOLD sample ID number
		28S D2	COI	
<i>Hydropsyche</i> sp. 8	China	EF514009	HM102276	CNCAD_CR09
<i>Hydropsyche</i> sp. 2006032001	China	EF513974	HM102275	CNCAD_0232
<i>Hydropsyche</i> XZ sp. CN2	China	EF513904	HM102281	CNCAD_0045
<i>Hydropsyche</i> XZ sp. CN3	China	EF513920	HM102282	CNCAD_0118
<i>Potamyia chekiangensis</i> (Schmid)	China	EF513892	HM102283	CNCAD_0102
<i>Potamyia flava</i> (Hagen)	USA	HM167448	HM102284	KKCAD-0284
<i>Streptopsyche parander</i> (Botosaneanu)	Dominican Republic	EU254455	HM167458	KKCAD-0007

^a Different specimens, both identified as *H. instabilis*, were used to sequence the D2 (07CJCAD-0039) and the COI (07HMCAD-0091), but the COI sequences of the 2 specimens were identical.

APPENDIX 2. Exemplar specimens used for the analyses with the 28SCOI data set (nuclear large subunit ribosomal ribonucleic acid (rRNA) [28S] regions D1, D2, and D3 and mitochondrial cytochrome c oxidase subunit I [COI]), their collection locality, GenBank accession numbers, and BOLD (Barcode of Life Data System; Ratnasingham and Hebert 2007) sample identification (ID) numbers. n.s. = not sequenced.

Species	Country	BOLD sample ID number	Genbank accession numbers			
			COI	28S D1	28S D2	28S D3
<i>Calosopsyche continentalis</i> Flint & Bueno—Soria	Costa Rica	KKCAD-0261	HM167459	HM167450	n.s.	HM167454
<i>Cheumatopsyche lateralis</i> (Barnard)	South Africa	07CJCAD-0022	HM102227	EU312016	EU254438	EU254465
<i>Hydromanicus melli</i> (Ulmer)	China	07CJCAD-0012	HM102254	EU312008	EU254430	EU254461
<i>Hydromanicus</i> nr. <i>truncatus</i> Betten	China	07CJCAD-0014	HM102232	EU312010	EU254432	EU254463
<i>Hydromanicus</i> nr. <i>canaliculatus</i> Li, Tian, & Dudgeon	China	CNCAD_0211	HM102231	n.s.	EF513893	n.s.
<i>Hydromanicus bronta</i> Ross	USA	KKCAD-0265	HM102237	AF436214	HM167437	AF436344
<i>Hydropsyche colonica</i> McLachlan	New Zealand	07CJCAD-0060	HM102241	AF436215	HM167438	AF436335
<i>Hydropsyche instabilis</i> (Curtis) ^a	Austria	07HMCAD-0091	HM102254	n.s.	HM167440	n.s.
<i>Hydropsyche naumanni</i> Malicky	Indonesia	07CJCAD-016	HM102257	EU312012	EU254434	HM167457
<i>Plectropsyche hoogstraali</i> Ross	Costa Rica	07CJCAD-0034	HM167460	HM167451	n.s.	HM167455
<i>Potamyia flava</i> (Hagen)	USA	KKCAD-0284	HM102284	HM167452	HM167448	HM167456
<i>Streptopsyche parander</i> (Botosaneanu)	Dominican Republic	KKCAD-0007	HM167458	HM167449	EU254455	HM167453

^a Different specimens, both identified as *H. instabilis*, were used to sequence the D2 (07CJCAD-0039) and the COI (07HMCAD-0091), but the COI sequences of the 2 specimens were identical