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Larvae and adults of Vietnamese species of *Drepanocentron* and *Hydromanicus* (Trichoptera: Xiphocentronidae, Hydropsychidae)

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ABSTRACT

Genetic sequencing (with mtCOI) was used to associate larvae with two new Vietnamese caddisfly (Trichoptera) species from Bach Mã National Park, *Hydromanicus calyx* n. sp. (Hydropsychidae) and *Drepanocentron dentatum* n. sp. (Xiphocentronidae). Adult *Drepanocentron dentatum* n. sp. is distinguishable by the toothy dorsal margin of the male inferior appendages; the most similar species, *D. vercaius*, has teeth on the ventral, but not the dorsal margins of the inferior appendages. The larva of *D. dentatum* is fully described herein, the first larva to be described in the genus *Drepanocentron*; it can be separated from the other two known xiphocentronid larvae (*Xiphocentron messapus* and *Cnodocentron yavapai*) by the smooth, thin mesal margin of each mandible. Adult *Hydromanicus calyx* n. sp. is distinguishable by the cupped apices of the male harpagones; this character is absent in morphologically similar species (*H. nieuwenhuisi*, *H. abiud*, and *H. serubabel*). The larva of *H. calyx* is morphologically most similar to that of *H. inferior*, but can be distinguished by the presence of faint muscle scars interrupting the lateral margins of the stridulatory areas on the venter of the head.

Key words: DNA barcoding, Parsimony, Maximum Likelihood, Bayesian analysis, morphology, genitalia

INTRODUCTION

There are more than 16,000 species of caddisflies (Morse 2018 and unpublished data), making Trichoptera the 7th largest insect order with respect to species richness (Morse 2016). The greatest percentages of species known from larvae are those of the Holarctic Region. However, the Oriental Region is the most speciose, as "it contains more than double the recorded species for each of the other regions, except the Neotropics" (de Moor & Ivanov 2008). According to the Trichoptera World Checklist there are now 5,788 trichopteran species known from the Oriental Region, yet larvae of only 91 (0.016%) of the species in that region of the world are known (Morse 2018).

Resh & Unzicker (1975) and Morse *et al.* (2017) urged their readers to associate larvae and adult species of aquatic insects and create diagnostic keys rather than continue to evaluate environmental impact using genus-level identifications. They argued that species-level identifications would greatly improve water quality assessments. Our goal is to associate yet-unidentified larvae of Trichoptera with their identifiable adults and to prepare illustrated morphological descriptions so that the newly described larvae will be identifiable to species. As density of larval species descriptions increases, future studies to assign tolerance values to the newly recognizable larval forms will be possible (e.g., Lenat 1993).

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There are three ways to associate adult and larval caddisflies: First by rearing the larvae, secondly by capture of mature pupae with their shed larval sclerites (metamorphotype method, Milne 1938), and thirdly by applying molecular techniques (Morse *et al*. 2017). Rearing can be time-consuming work and is often unsuccessful. Metamorphotypes of a given species are present for only a few days each year. Thus, we used genetic sequencing to match undescribed caddisfly larvae with known and identifiable adults using principles and methods of Zhou *et al*. (2007), except that we used the phylogenetic methods of Caterino and Tishechkin (2006) in place of the neighbor-joining method used by the other authors. According to Huelsenbeck (1995), maximum likelihood analysis is more statistically robust than neighbor-joining and is more likely to "estimate the correct phylogeny even when the assumptions of the phylogenetic method are violated." This is especially important because "the idealized assumptions underlying phylogenetic methods are most likely violated with real data" (Huelsenbeck 1995).

In this study we associated and described larvae of two new Vietnamese species, one in the genus *Drepanocentron* (Xiphocentronidae) and one in *Hydromanicus* (Hydropsychidae). There are 41 species of *Drepanocentron* globally, all from the Oriental Region; this study represents the first larval description for any species of the genus. There are 71 *Hydromanicus* species globally, 70 from the Oriental Region, of which the larvae of only 7 species were previously known, including larvae of *H. adonis* (Malicky & Chantaramongkol 1996) and *H. klanklini* Malicky & Chantaramongkol 1993 by Prommi *et al*. 2006; and *H. abiud* Malicky & Chantaramongkol 1993, *H. inferior* Chantaramongkol & Malicky 1995, and *H. malayanus* Banks 1931 by Prommi & Permkam (2015); and *H. canaliculatus* Li *et al*. 1990 and *H. umbonatus* Li 1993 (in Tian *et al*. 1993) by Zhou (2007).

MATERIAL AND METHODS

Sampling

Specimens were collected in June and July 2017 in Bach Mã National Park (*Výờn quốc gia Bạch Mã*), a protected area in central Vietnam, near the city of Huế. Field data were recorded, such as date of sampling, names of all collectors, GPS coordinates of sampling site, detailed description of the site and sampled habitats, weather conditions, and other general ecological observations. Upstream and downstream photographs were taken at each site.

Larvae were collected using D-frame dip nets and kick screens and by examining rocks and debris. Adults were collected in battery-operated light traps each consisting of a BioQuip (Rancho Dominguez, CA, USA) black light tube laid over a shallow white pan filled with 95–100% ethanol. Light traps were operated starting approximately 20 minutes after sunset and ran for at least 1 hour or until the number of incoming insect specimens declined. Larvae and adults were preserved in the field in 95–100% ethanol.

Adult males were identified to species when possible. Male genitalia were cleared for identification using methods modified from those of Blahnik & Holzenthal (2004) and Blahnik *et al.* (2007). Lactic acid was chosen because it often causes the phallus to evert, aiding description and, unlike potassium hydroxide (KOH), is unlikely to over-clear the cuticle (Blahnik *et al.* 2007). Adult specimens were identified using the atlas by Malicky (2010) and primary literature published since 2010. Larvae were identified to genus using the key by Morse *et al.* (1994) and primary literature.

All specimens were sorted into glass, screwcap vials. Specimens for which we attempted to extract DNA were vouchered; each voucher label is marked with the DNA extraction number. Representatives of each adult and larval form are deposited in the Vietnam National Museum of Nature, Hanoi, Vietnam (VMN) and the Clemson University Arthropod Collection, Clemson, South Carolina, USA (CUAC).

Molecular work

We attempted to sequence the mtCOI gene for representative adults of each species collected. Larvae from each site collected were sequenced due to the possibility that they could be the same species as adults collected at the same location. Each specimen for which sequencing was attempted received a unique extraction number beginning with the initials VN, with the same extraction number used to refer to specific specimens throughout the study. Unique PCR numbers also were assigned because some extractions were run multiple times using different PCR methods in order to amplify DNA.

For adult male specimens, a leg was used for DNA extraction, and broken in two places to expose muscle tissue. For large larval specimens the insect was cut in half between the thorax and abdomen, the anterior half was used for extraction. For very small adult and larval specimens the entire body was used for extraction. This process dissolved soft tissue and muscle, but left behind sclerotized structures and cuticle, similar to the effects of clearing with lactic acid or KOH.

Extraction protocols from the Thermo Scientific® GeneJet extraction kit were used. A 25 µL polymerase chain reaction (PCR) was conducted following extraction to amplify the DNA. The universal mtCOI barcode primers (LCO1490 and HCO2198) were used for all PCRs. The master mix formula varied slightly with different runs as we made minor adjustments to find what amounts provided the most successful results. Generally, we found the master mix formula shown in Table 1 to be most successful, (although we did not optimize this exhaustively). One µL of template DNA was added to each tube.

Tubes were put into an Eppendorf Master® cycler nexus gradient PCR machine, and run using the parameters shown in Table 2.

TABLE 1. Master mix reagent quantities used for most PCR runs.

Master Mix Reagent	volume per reaction
ddH_20	16.75 µL
Taq buffer	2.5 µL
dNTP mix	2.5 µL
Primer 1 (LCO1490)	1 µL
Primer 2 (HCO2198)	1 µL
Dream Taq (ThermoFisher Scientific)	0.25 µL

TABLE 2. PCR program used throughout study.

	Temp.	Seconds
Initial Denaturation.	95	180
Denaturation	94	30
Annealing	50	30
Extension	72	45
Final extension.	72	180
Cycles:	35*	

* "35 cycles" pertains only to denaturation, annealing, and extension (not the initial and final steps).

Gel electrophoresis was conducted in order to determine if PCR was successful. PCR products were sent to Macrogen USA (Rockville, MD) for Sanger sequencing of mitochondrial COI. All sequences obtained have been uploaded to the Barcode of Life Database (BOLD) under the project name: "Genco". Sequences for this project are also available in the appendix to M. Genco's Master's Thesis (2018).

Tree Building

Forward and reverse sequences for each specimen were aligned using Clustal Omega® (Sievers *et al*. 2011) to check that they were in agreement. Sequences from each primer were combined and checked/edited by hand in Geneious® (Biomatters Ltd., Auckland, NZ). Each aligned sequence was put into a Nexus file using Microsoft Notepad (a simple text editor) along with its species name and the extraction number used to identify the exact specimen.

Additional sequences from BOLD were added to the Nexus file and aligned with the rest of the sequences by hand. Process identifications were included (with the hyphen removed from before the last two digits) so that the exact sequences can be traced back to BOLD.

Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) j-model comparison tests (version 2.1.9) were run to choose the model that best fits the data and include only necessary parameters. For both tests, the most complex model (GTR+I+G) was selected as the best fit.

Fig. 1

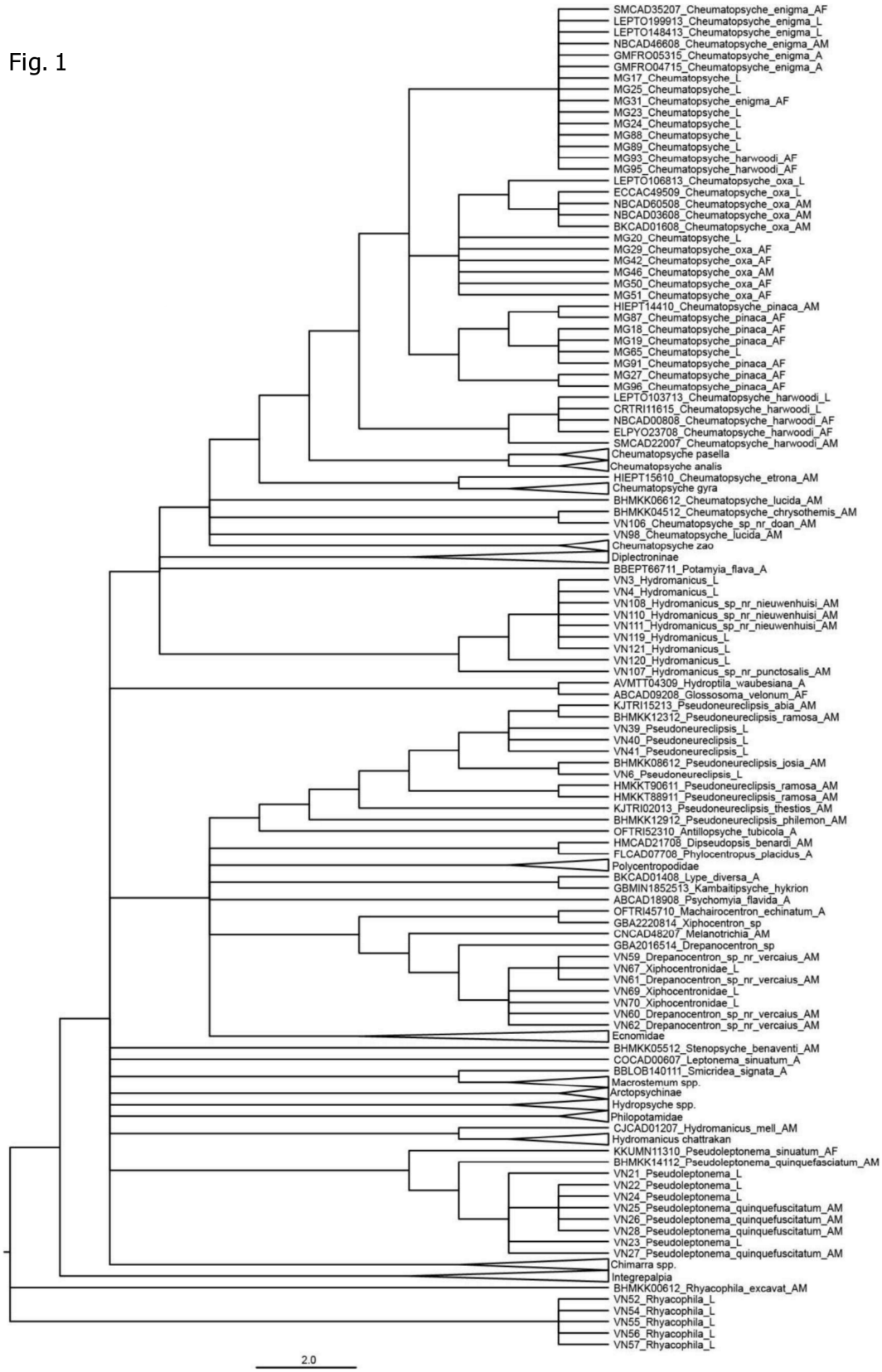


FIGURE 1. Majority rule consensus tree assembled from most parsimonious trees. Extraction numbers beginning with VN indicate the specimen was collected in Vietnam, MG indicates it was collected in North or South Carolina (USA). BOLD process ID's are included for outgroups. A = adult specimen, L = larva, M = male, and F = female.

Fig. 2

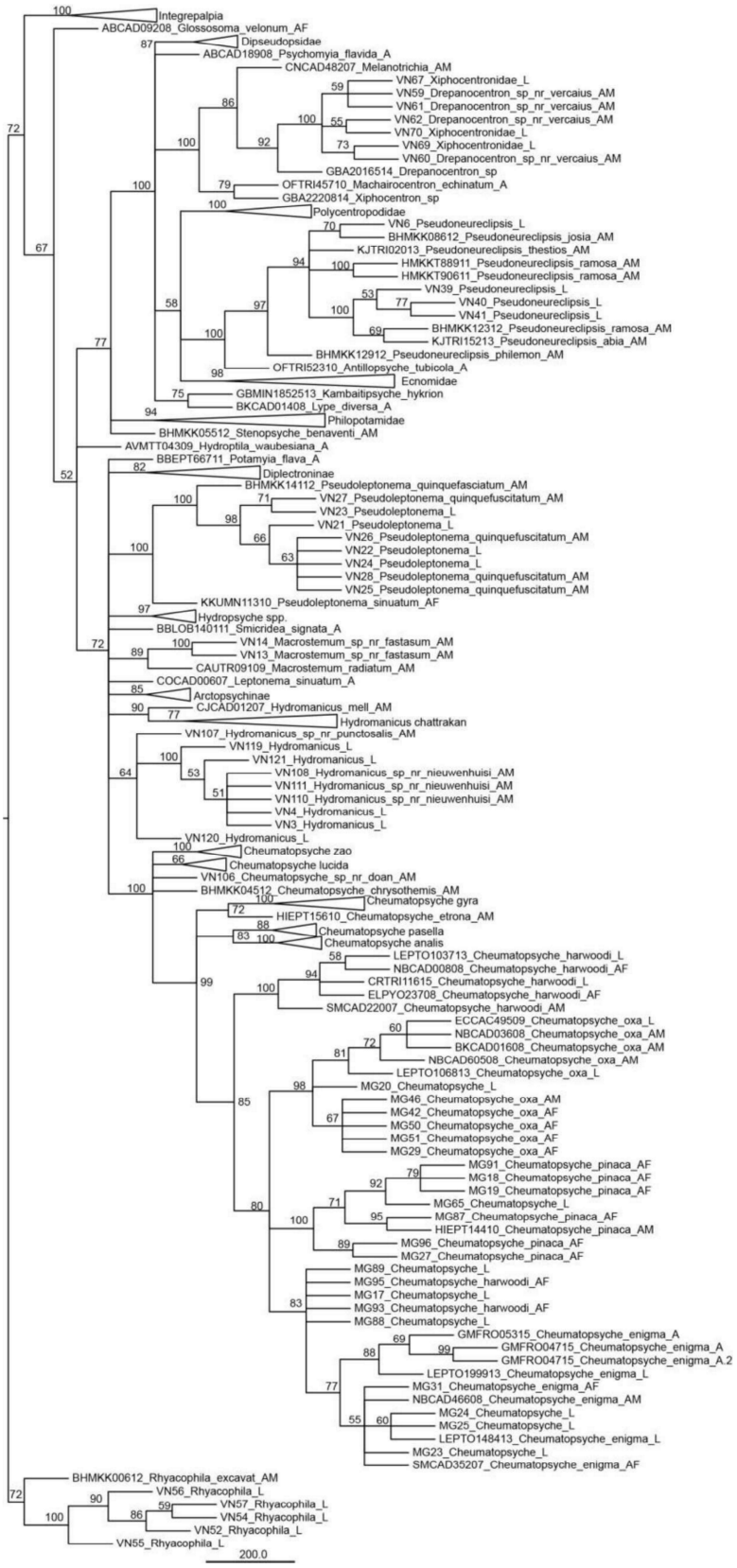


FIGURE 2. Majority rule consensus tree assembled from maximum likelihood trees. Bootstrap values are indicated on branches. Extraction numbers beginning with VN indicate the specimen was collected in Vietnam, MG indicates it was collected in the North or South Carolina (USA). BOLD process ID's are included for out-groups. A = adult specimen, L = larva, M = male, and F = female.

Fig. 3

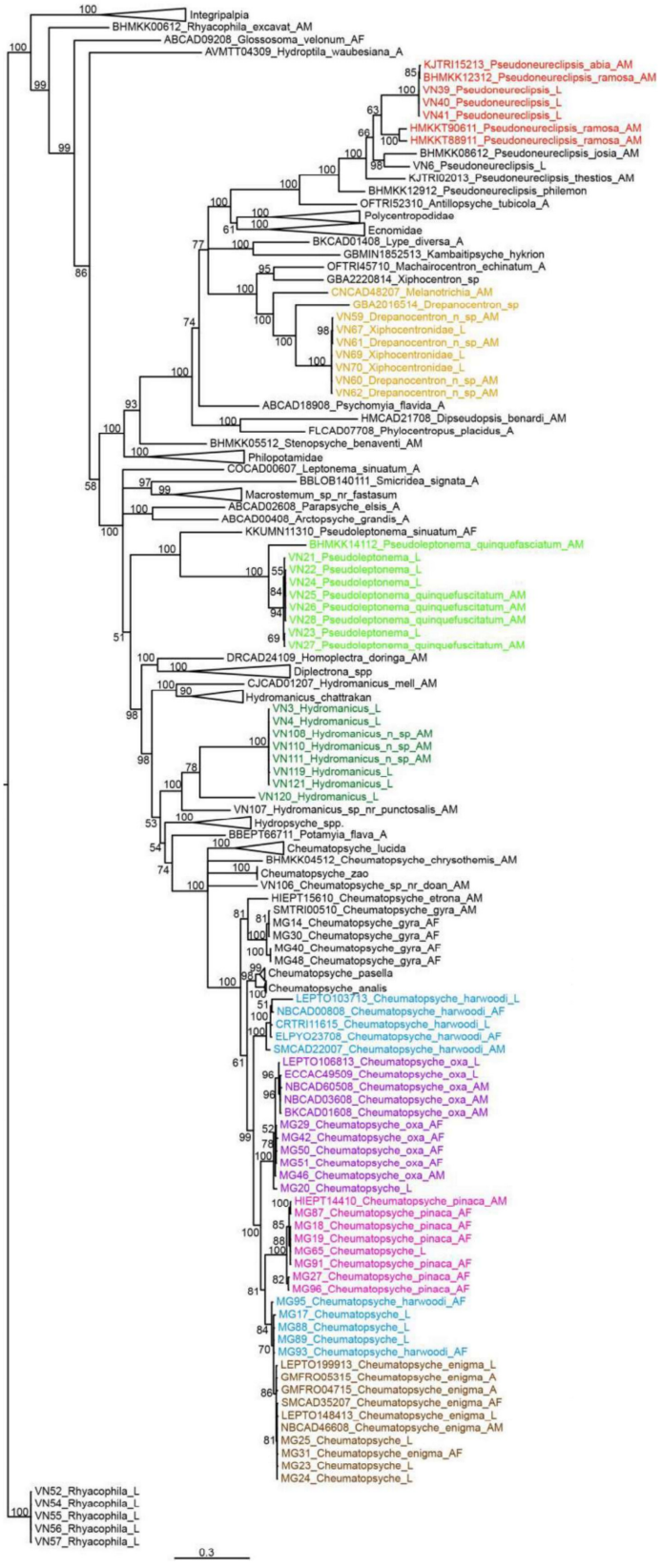


FIGURE 3. Phylogram of majority rule consensus tree assembled from post burn-in Bayesian Analysis. Posterior probabilities are included on branches. Extraction numbers beginning with VN indicate the specimen was collected in Vietnam, MG indicates it was collected in North or South Carolina (USA). BOLD process ID's are included for outgroups. A = adult specimen, L = larva, M = male, and F = female.

Maximum Parsimony analysis was run with PAUP 4.0® (Swofford 2002), using random starting trees and 1000 replicates, and, for branch swapping, saving no more than 1000 trees for each replicate. The trees were rooted using *Rhyacophila* Pictet 1834. The resulting trees were used to create a 50% majority rule consensus tree in PAUP (Fig. 1).

Maximum Likelihood analysis was performed in RAxML version 8.2.9 (Stamatakis 2014), using Mesquite's Zephyr package (Maddison and Maddison 2015). We used 100 search replicates and 1000 bootstrap replicates to test congruence for the phylogenetic inferences. The trees were rooted using *Rhyacophila*. The resulting trees were used to create a 50% majority rule consensus tree in Mesquite® (Fig. 2).

Bayesian analysis was run twice for 10,000,000 generations each to ensure the best topology had been reached (nchains=4). The resulting trees were then rooted with *Rhyacophila* using PAUP 4.0 (Swofford 2002). A majority-rule consensus tree was created using only the post-burn-in trees (the first 25% of trees were discarded). A phylogram was created using PAUP® (Fig. 3). This tree was edited using FigTree v1.4.3 to show posterior probabilities and increase text size and condense branches we don't discuss in this publication.

Associating Larvae and Adults

We considered larvae or adults to be a species according to the Phylogenetic Species Concept (Eldredge & Cracraft 1980, i.e., according to whether each group of larval or adult specimens represented the smallest aggregation of populations diagnosable by a unique combination of character states). The larvae and adults were considered to be the SAME species only if all trees (from Parsimony, Maximum Likelihood, and Bayesian analyses) supported the same larvae and adult specimens as a monophyletic group, and the larvae nested among the adults (Zhou *et al*. 2007) and if there was less than a 2% sequence difference (uncorrected 'p' distance) between a larva and a named adult. This 2% threshold is admittedly arbitrary, but is a common starting point for assessing whether divergences among sequences may be taxonomically significant (Hebert *et al*., 2003).

Describing Newly Associated Larvae and Adults

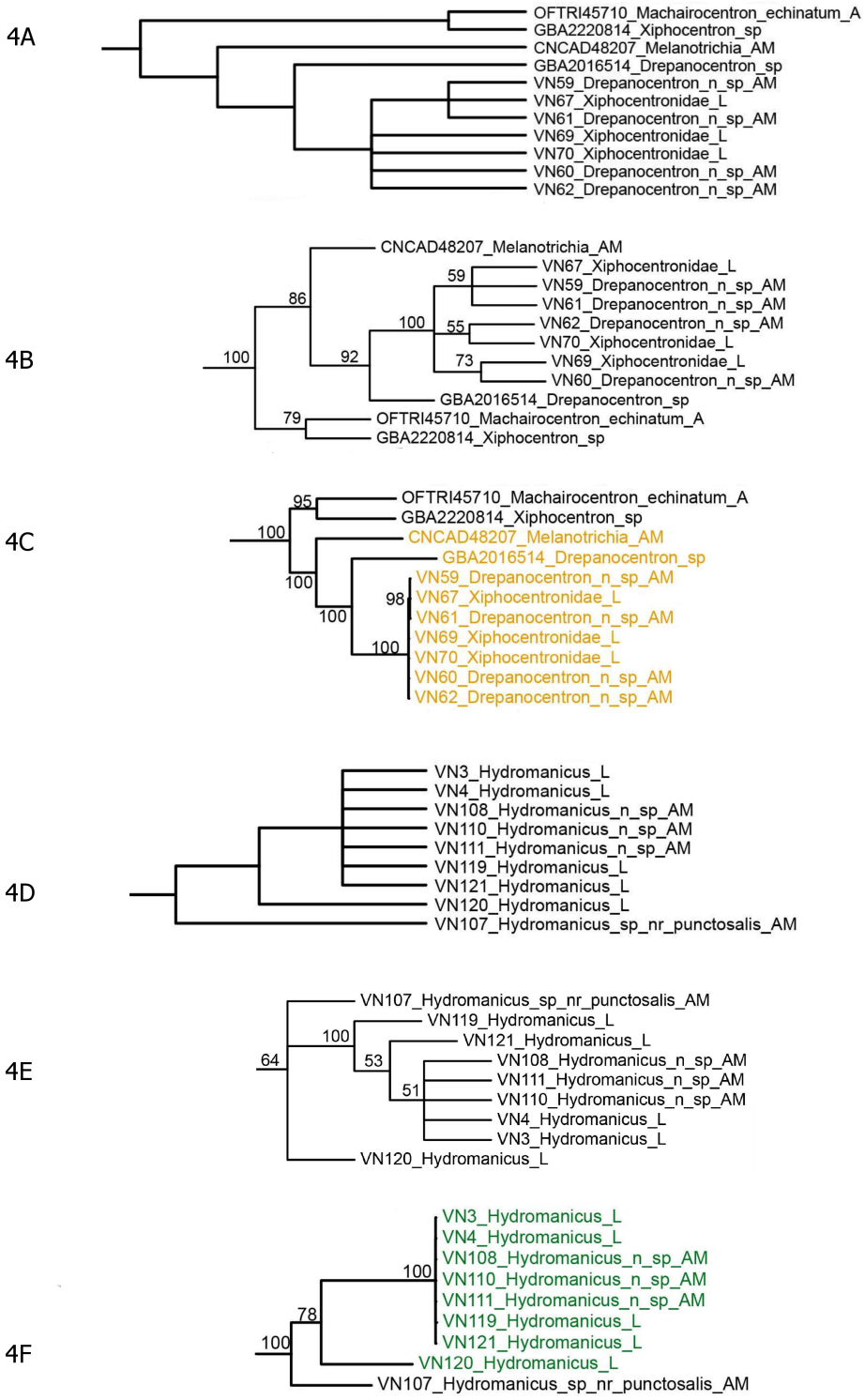
For each newly associated larva, we observed and hypothesized a set of morphological character states to distinguish each species from its congeners. To be potentially diagnostic, each character state must be distinct for its respective species, without intermediates, and any particular set of character states must always occur in the same combination. Larvae were compared to other larvae in this study, as well as to larval forms described in the literature.

Illustrations were created using methods modified from those of Holzenthal (2008, 2015) and Genco & Morse (2017). Pencil templates for illustrations were prepared using a gridded eyepiece in a Wild® M5D (Wild Heerbrugg, Switzerland) dissecting microscope. Digital drawings were prepared with Abode Illustrator® (versionCC2015; Adobe Systems, San Jose, California) and Adobe Photoshop® (version CC 2015; Adobe Systems). Krita® (version 4.2) was used to make small adjustments to finalize figures (minor adjustment to text, arrows, etc.) and to assemble them into a plate following the first round of revisions. For very small specimens, a doubler objective and 20X eyepieces were also used, allowing 200X magnification.

Alternatively, for some illustrations, a photograph was taken through a microscope lens using a smartphone camera and the photograph was used as a template in Adobe Illustrator. The specimen was maintained under the microscope for comparison while drawing. Setal brushes created for use in Illustrator® by Dr. Ralph Holzenthal were used. For lateral drawings, as per convention in trichopterology, the left side was used for genitalia; the right side was used for all other structures unless it was damaged, in which case the opposite side was used and then the drawing was reflected in Adobe Illustrator® to show the conventional orientation. Terminology for warts conforms with that of Ivanov (1990). Terminologies for male genitalia of *Drepanocentron* spp. and *Hydromanicus* spp. conform with those of Schmid (1982) and Oláh & Johanson (2008a, 2008b), respectively, except where indicated. Terminology for structures of larvae conforms with that of Wiggins (1996).

RESULTS

We attempted to extract DNA from 125 specimens from Vietnam, including at least 26 species. Of these, we were able to extract and amplify DNA from 54 specimens. From 49 specimens, we were able to obtain mtCOI sequences for at least 10 species.



FIGURES 4A–4F. Pruned branches from cladogram and phylograms (Figs. 1–3) of *Drepanocentron* spp. and *Hydromanicus* spp. showing concordant monophyly, and thus probable identity, of larvae and adults of new species. Extraction numbers beginning with "VN" indicate the specimen was collected by the authors in Vietnam, BOLD process ID's are included for outgroups. 4A–4C, *Drepanocentron* spp.: 4A, Parsimony analysis; 4B, Maximum Likelihood analysis; 4C, Bayesian analysis. 4D–4F, *Hydromanicus* spp.: 4D, Parsimony analysis; 4E, Maximum Likelihood analysis; 4F, Bayesian analysis. A = adult specimen, L = larva, M = male, and F = female.

The three majority-rule consensus trees (from Parsimony, Maximum Likelihood, and Bayesian analyses, Figs. 1, 2, and 3) are similar and monophyletic for larvae and adults of each of the following species and equivocally for two other species that are to be investigated further in the future (for more details see Genco 2018). We have also provided pruned trees (Fig. 4) to show the relationships of the taxa in question more easily.

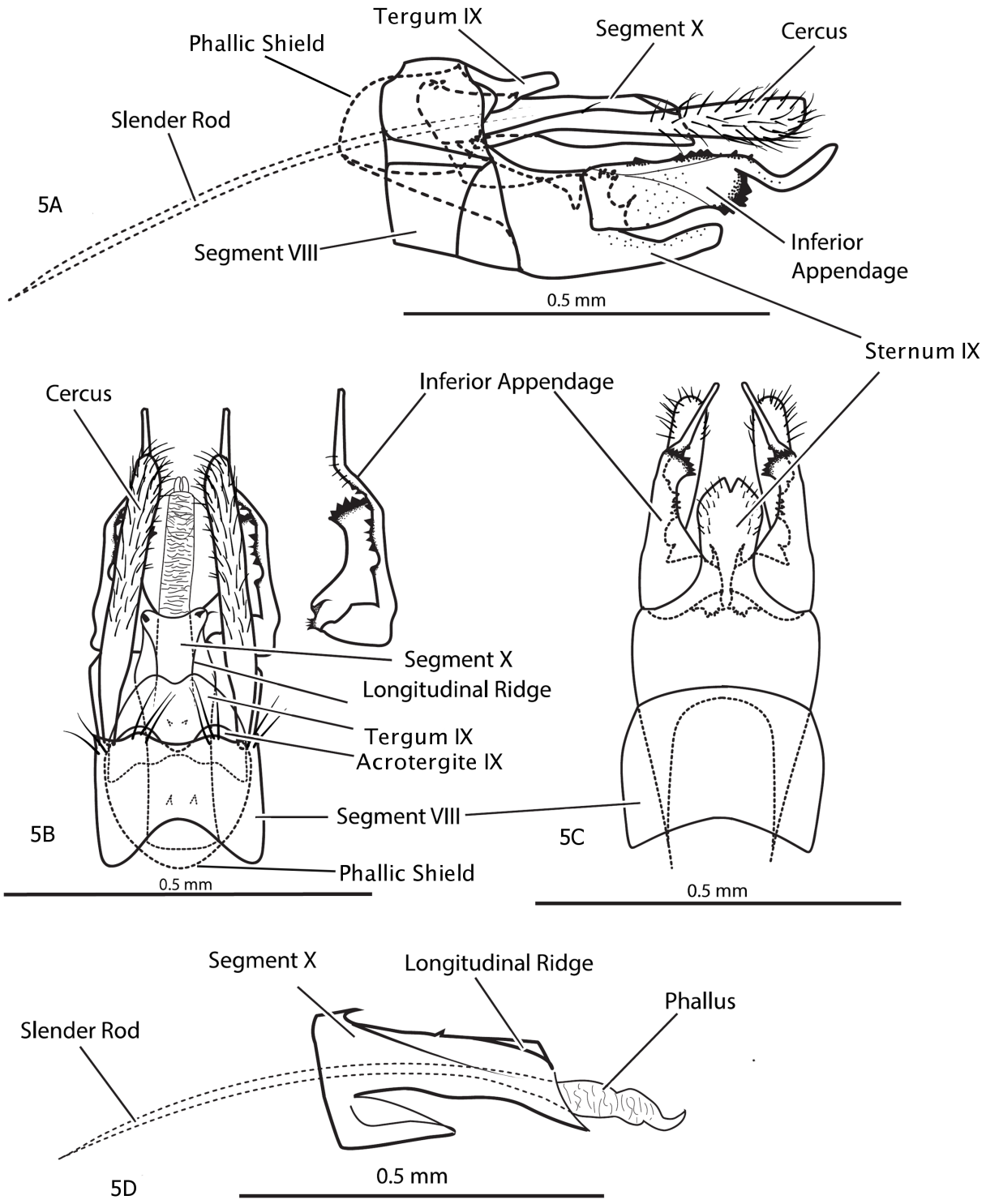


FIGURE 5. *Drepanocentron dentatum* n. sp., male genitalia. 5A, left lateral; 5B, dorsal, with inset of left inferior appendage; 5C, ventral; 5D, phallus, left lateral.

- *Drepanocentron* n. sp.: 4 adult males and 3 larvae formed a monophyletic group, with P-distances of all sequences less than 0.16% different from each other; thus our data support the hypothesis that they are the same species. The adult male (Fig. 5) and larva (Fig. 6) are described below. The larva was described from only two specimens as one specimen (extraction number VN67) was destroyed during extraction.
- *Hydromanicus* n. sp.: 3 adult males formed a monophyletic group with 4 larvae. Larval sequence P-distances are all between 0% and 0.15% different from adult sequences; thus our data support the hypothesis that they are the same species. The adult male (Fig. 7) and larva (Fig. 8) of this presumed species are described below. One additional *Hydromanicus* larva (VN120) appeared sister to the rest, with a longer branch length in the phylogram; its P-distance sequence is 16% to 17% different from the rest, suggesting that it is a different species in the same genus.

SPECIES DESCRIPTIONS

Drepanocentron dentatum, n. sp. (Xiphocentronidae)
Figs. 5, 6.

Holotype, male (VN62): VIETNAM: Bach Mã National Park: Krem Stream, upstream of culvert at road (16.1958°N, 107.8490°E, elev: 1159 m) by J.C. Morse, K. Murray-Stoker, M.S. Genco, Nguyễn T.M., Hugnh, D.H., and Nguyễn V.H.; deposited in the Vietnam National Museum of Nature, Hanoi, Vietnam.

Paratypes: Three adult male specimens (VN59, VN60, and VN61) and two larvae (VN69 and VN70), same data as for holotype; VN69 deposited in the Vietnam National Museum of Nature, Hanoi, Vietnam; all others deposited in the Clemson University Arthropod Collection, Clemson, South Carolina, USA.

Additional Material Examined: Dr. Hans Malicky kindly loaned an adult male paratype of *Drepanocentron vercius* (Thailand, Ohuket, Tonesai waterfall, 100 m, 8°02'N, 98°22'E, 4 March 1990, leg. Chantaramongkol) for comparison.

Description of Adult Male: Maxillary palps 5-segmented with dark setae on basal 3 segments. Labial palps 3-segmented. Pair of large kidney-bean-shaped anterolateral warts anterior to antennae and much smaller ovoid pair of antennal warts posterior to antennae; medial frontal wart on dorsum of head with rounded anterior margin and pointed posterior margin; pair of ocellar warts and pair of occipital warts, all elongate-transverse, posteriorly on head on either side of mesal line. Pair of medial prothoracic warts brown, bulbous, and touching medially. Mesoscutal warts large, brown, ovoid, flattened (plate-like), and touching medially. Wings brown in alcohol. Spur formula: 2-4-3. Each pair of spurs of mesothoracic legs with mesal spur distinctly longer than lateral spur. Distal tibial spur of each hind leg much thicker than other spurs, similar in shape to that of *Drepanocentron vercaius* Malicky 1992, but much longer (about 4 times as long as wide) and with slender apical spine.

Description of Male Genitalia (Fig. 5): Tergite VIII overlapping base of segment IX and its acrotergite, with 2 long setae in each posterolateral corner and 3 pairs on convex submesal portions of posterodorsal margin. In dorsal view, Tergum IX shield-like, with concave posterior margin, this concavity sometimes slight; its anterior acrotergite expanded with posterior margin convex submesally and concave mesally; pleural regions of segment IX each consisting of very slender sclerotized connection between dorsal (tergal) and ventral (sternal) regions; ventrolateral portion of sternum IX on each side slender and extended internally as spine-like apodeme tapering anteriorly in lateral view, and extending anteriorly beyond segment VIII; in ventral view sternum IX with nearly parallel-sided posteromesal expansion, about twice as long as wide in ventral view, and concealing most or all of phallus, its posterior margin round except for small mesal notch; in lateral view, tergum IX appearing shelf-like, with posterior portion narrowed, and jutting over part of segment X. Phallic shield with anterior margin round in dorsal and lateral views. Cerci (appendices préanaux of Schmid 1982) slender, nearly parallel-sided in dorsal view, gradually broader toward rounded apices in lateral view, nearly reaching ends of inferior appendages. Segment X telescoped into segments VIII and IX, long, originating beneath tergum VIII and extending beyond tergum IX; tube-like, ventral margin of tube V-shaped in caudal view (not shown); 1 or 2 spines present basally on dorsal side of segment X beneath tergite VIII, and pair of spines at midlength beneath dorsum IX; segment X with longitudinal diagonal ridge on each side dorsolaterally, this

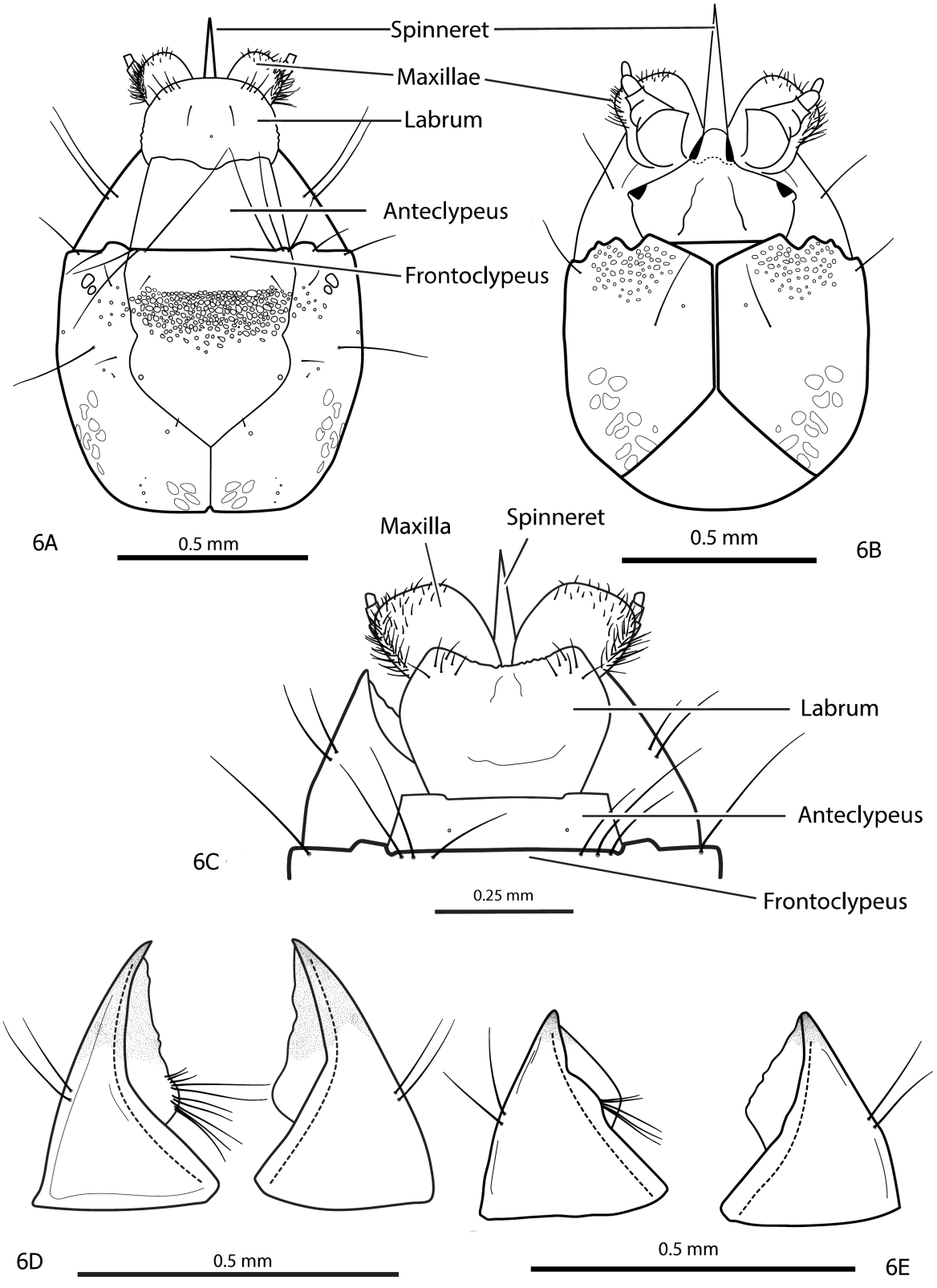


FIGURE 6A–6E. *Drepanocentron dentatum* n. sp., larva, presumably mature. 6A, head, dorsal; 6B, head ventral; 6C, anterior portion of head of different specimen, dorsal; 6D, 6E, mandibles of two different specimens, dorsal.

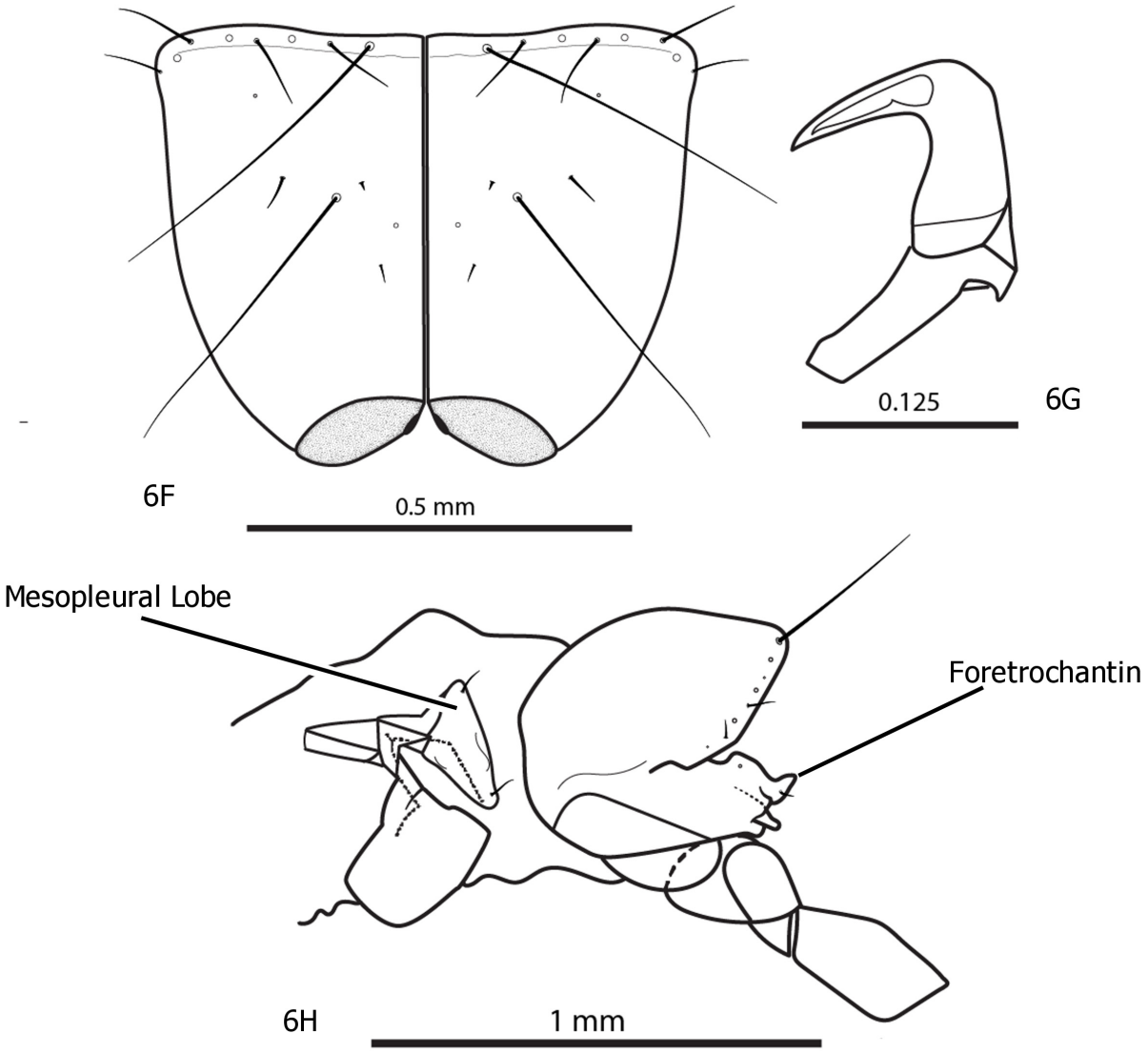


FIGURE 6F–6H. 6F, pronotum, dorsal; 6G, right anal proleg, right lateral; 6H, pro- and mesothorax, right lateral.

ridge forming plate with concave ventral margin and convex posterior margin and with spine on each apicolateral corner. Internally, slender rod of phallus [likely Schmid's (1982) phallotheca] longer than rest of genitalia, sclerotized anteriorly, posteriorly inserted through segment X with diameter expanding and becoming indistinguishable from membranous remainder of phallus [Schmid's (1982) endotheca], exiting tubular segment X. Phallus (edéage of Schmid 1982) subapically with scarcely apparent, roughened portion and apically with pair of fleshy, pointed lobes or rods. Inferior appendages each with anteromesal corner having single large hooked spine and group of smaller spines; middle portion cupped on mesal surface; black spines present dorsomesally along thin dorsal margin and dense patch of black spines subapicoventrally; apicodorsal margin extending as slender projection beyond rest of inferior appendage and bent ventromesad and then dorsocaudad.

Diagnosis of Male: The male of this new species is most similar to that of *Drepanocentron vercaius* Malicky & Chantaramongkol 1992 (from Thailand) in the general shape of the inferior appendages. This new species has spines on the dorsal margins of the inferior appendages, but dorsal spines are absent from the inferior appendages of *D. vercaius*. Additionally, the new species can be distinguished because it has a more nearly parallel-sided ventromesal projection of segment IX with a rounded posterior margin having a mesal notch;

D. vercaius has a more nearly triangular venter IX with a blunt and unnotched posterior margin. The apex of segment X is concave in dorsal view and with a spine on each apicolateral corner in *D. dentatum* n. sp. but is blunt and without spines in *D. vercaius*.

Etymology: The species is named *D. dentatum*, with "*dentatum*" meaning "toothed" in Latin, referring to the prominent toothy spines on the inferior appendages.

Description of Presumed-Mature Larva (Fig. 6): Mandibles triangular, with slight convex curvature to lateral margins or at tips. Tips of mandibles black. Thin mesal margin of each mandible with nearly smooth edge without teeth. Fan of setae situated mesally near base of left mandible. Each mandible with two setae near lateral margin about 1/3 to 1/2 length of mandible from base. Maxillae large, inflated, and pillow-like, with fine hair along lateral and apical margins. Spinneret long, extending beyond mandibles and labrum, similar to, but not as long as that of Dipseudopsidae. Labrum membranous and nearly circular or with distal margin concave. Labrum connected to frontoclypeus by expanded, membranous anteclypeus, extending labrum beyond apical tips of mandibles. Frontoclypeus with apical margin straight; wide transverse band of circular markings dorsally at 2/3 distance from posterior apex. Anterior margins of genae each with apical expansion just lateral of lyre-shaped frontoclypeal suture on each side; posterior half of genae with very faint ovoid muscle scars. Ventrally, mentum and submentum membranous with no clear division, tiny sclerotized corners of mentum possibly remnants of cardo. Ventral apotome broad and triangular. Apical margin of head capsule on each side of ventral apotome conspicuously notched and with patch of circular markings similar to those on frontoclypeus; 1 pair of fine, long setae (setae #8 of Wiggins 1996) near anterior margin; very faint ovoid muscle scars on posterior half of head. Pronotum similar to that of larva of *Xiphocentron masapus* (Wiggins 1996, fig. 11.1B) with dark flat areas on posterior margin. All legs with tibiae and tarsi fused. Mesopleural lobes fan-like, each with short, fine seta at each corner; each lobe associated with sclerotized, invaginated pocket. Anal hooks of prolegs without teeth or spines, and abruptly curved at angle greater than 90°.

Diagnosis of Larva: Females and immature stages of species of *Drepanocentron* have not been described previously, so that the larval description above is the first for a species in the genus. Larvae of only 2 other species of Xiphocentronidae have been described previously. The larva of *Xiphocentron messapus* Schmid 1982, was described by Edwards (1961, as *Xiphocentron mexico* Ross 1949, according to Wiggins 1996) and Wiggins (1996). The larva, pupa, and adult male of *Cnodocentron yavapai* Moulton & Stewart 1997 were described originally, with its authors observing that the larva is "morphologically indistinguishable" from that of *X. messapus* described by Wiggins (1996). Among these 3 genera, we recognize the following potentially diagnostic larval characters based on comparisons of *D. dentatum* n. sp. with the above-referenced descriptions:

(1) Xiphocentron messapus:
- *a.* Mandibles with mesal margins without teeth, slightly sinuous
- *b.* Mandibles each with cluster of four lateral setae
- *c.* Anteclypeus long
- *d.* Lateral margins of frontoclypeus straight
- *e.* Frontoclypeus with scattered circular markings, but no transverse band
- *f.* Pronotum posterior margin transverse, straight or nearly so, with flat areas; central setae present

(2) Cnodocentron yavapai:
- *a.* Mandibles with mesal margins toothed
- *b.* Mandibles each with two lateral setae
- *c.* Anteclypeus not shown, possibly short or indistinguishable
- *d.* Lateral margins of frontoclypeus sinuous
- *e.* Frontoclypeus apparently without scattered circular markings
- *f.* Pronotum posterior margin convex with median notch, without dark flat areas; central setae not shown

(3) Drepanocentron dentatum:
- *a.* Mandibles with mesal margins without teeth, slightly sinuous
- *b.* Mandibles each with two lateral setae

c. Anteclypeus long
d. Lateral margins of frontoclypeus sinuous
e. Frontoclypeus with transverse band of circular markings
f. Pronotum posterior margin convex with median notch, with dark flat areas; central setae present

Discussion: Xiphocentronidae Ross 1949 was described originally with 3 species in the type genus *Xiphocentron* Brauer 1870. Ross (1949) described adults of the family in detail and provided a key to distinguish them from those of other then-known families of suborder Annulipalpia. Schmid (1982) revised the family, and distinguished two subfamilies: Xiphocentroninae which included most genera (*Melanotrichia* Ulmer 1906*, Cnodocentron* Schmid 1982*, Machairocentron* Schmid 1982*, Xiphocentron* Brauer 1870*, Drepanocentron* Schmid 1982*,* and *Abaria* Mosely 1948) except for *Proxiphocentron* Schmid 1982 which he placed in its own subfamily, Proxiphocentroninae basal to other xiphocentronids. Xiphocentronidae and Psychomyiidae are sister groups, with Dipseudopsidae sister to them (Kjer *et al.* 2016).

The known larvae of these 3 xiphocentronid genera-species are most similar to those of Psychomyiidae, but without hatchet-shaped foretrochantins. We hypothesize that the mesopleural lobes and fused tibiae-tarsi mentioned by Wiggins (1996), the expanded pillow-like maxillae, and the longer spinneret are family-level characters for Xiphocentronidae larvae, as they are present in all 3 genera for which larvae are now known.

Hydromanicus calyx, n. sp. (Hydropsychidae)
Figs. 7, 8.

Holotype, male (VN108). VIETNAM: Bach Mã National Park, Vietnam, Pheasant Falls and its tributary (16.2287°N, 107.8486°E, elev: 159 m) on 30 June 2017 by J.C. Morse, K. Murray-Stoker, M.S. Genco, Nguyễn T.M., Hugnh D.H., and Nguyễn V.H.; deposited in the Vietnam National Museum of Nature, Hanoi, Vietnam.

Paratypes: Two adult males (VN110 and VN111), three mature larvae (VN4, VN3, and VN121), and one younger instar (VN119), same data as for the holotype; VN4 deposited in the Vietnam National Museum of Nature; all others deposited in the Clemson University Arthropod Collection, Clemson, South Carolina, USA.

Additional Material Examined: Dr. Hans Malicky kindly loaned us representative specimens from two morphologically similar species for comparison, including three adult male specimens of *Hydromanicus abiuid* (Thailand, Doi Suthep, 500 m 18°49'N, 95°55'E, 3 May 1996, leg. Malicky) and three adult male specimens of *Hydromanicus serubabel* (Thailand, Phuket, Tonesai waterfall, 100 m, 8°02'N, 98°22'E, 28 February 1990, leg. Chantaramongkol).

Description of Adult Male: Maxillary palps 5-segmented. Labial palps 3-segmented. All warts on head and prothorax hairy. Pair of irregularly shaped anterolateral warts anterior to antennae, with excisions, points, or bumps along mesal margins. Single ovoid hypomedial wart at anterior margin of head; posterior of antennae, 1 nearly circular central frontal wart (anterior margin sometimes notched), 1 pair of transverse (3 times wider than long), parallel-sided antennal warts, 1 pair of ovoid ocellar warts, and pair of large tear-drop-shaped occipital warts (pointed ends medial) near posterior margin. Pronotum with pair of medial pronotal warts, touching or nearly so medially, and tapering laterally. Each anterolateral corner of mesothorax with bulbous tegulum. Mesoscutum without warts. Mesoscutellum with large medial wart, nearly circular, but constricted in posterior half. Spur formula 2-4-4.

Description of Male Genitalia (Fig. 7): In lateral view, segment IX with notch on each posterior margin, continuing anteriorly as internal sclerotized carina; dark setae present along posterior margins dorsal and ventral of notch. Dorsum of segment X divided, paired apical lobes having rounded apices with tiny sensilla dorsally. Cerci (preanal appendages) slender basally, enlarged and convex subapically, covered with fine setae with large alveoli. Gonopods (inferior appendages) each two-segmented, long, extending beyond preanal appendages and segment X; long setae present subdorsally at apex of coxopodite (basal segment), giving cuticle bumpy appearance; harpago (distal segment) distinctly cupped mesally. In lateral view, basal half of phallic apparatus (phallus) enlarged, constricted near midlength, with small vertical "dewlap" subapicoventrally, apical lobes darker in color than rest of phallic apparatus.

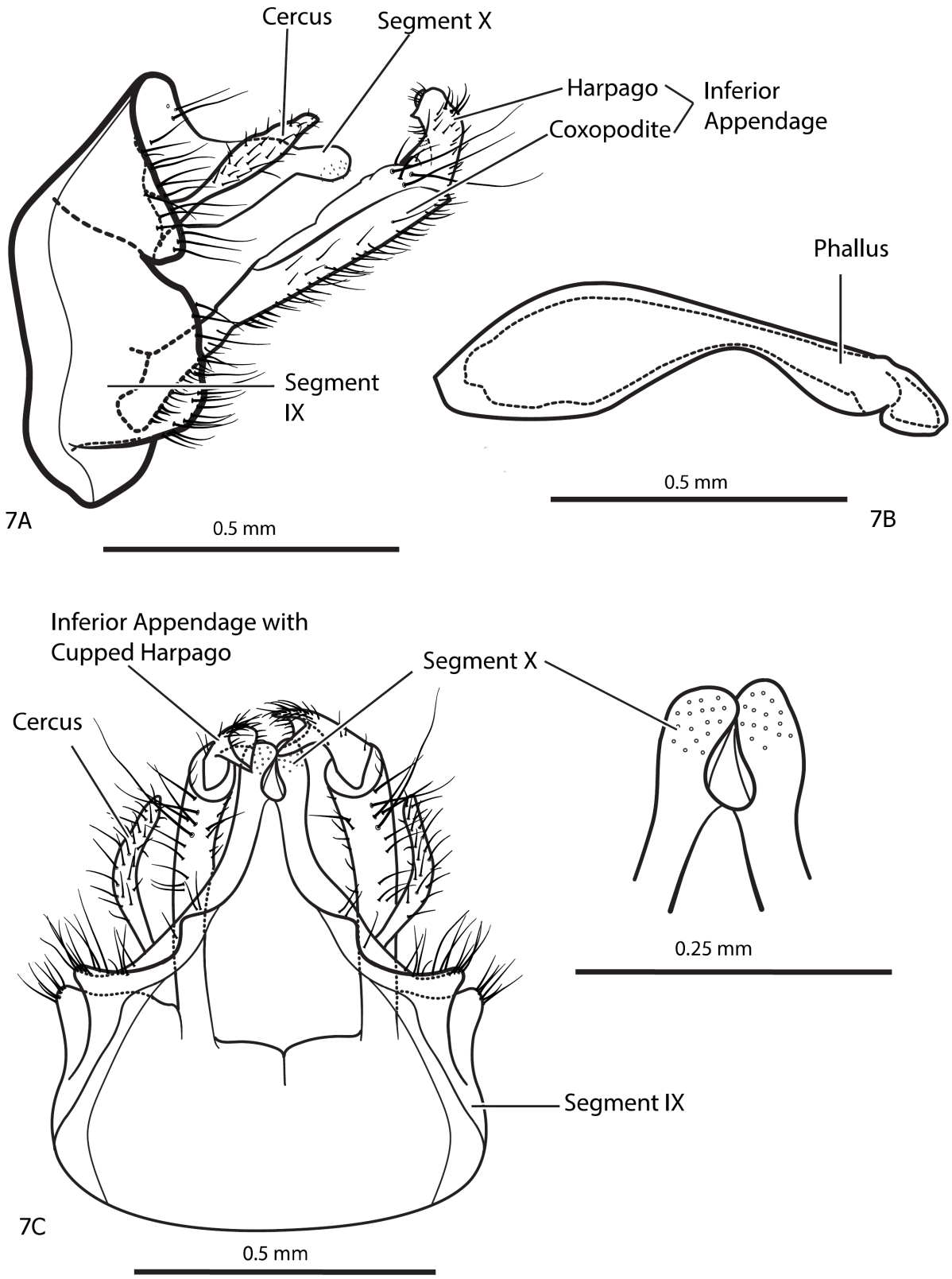


FIGURE 7. *Hydromanicus calyx* n. sp., male genitalia. 7A, left lateral; 7B, dorsal, with inset of segment X; 7C, phallic apparatus, left lateral.

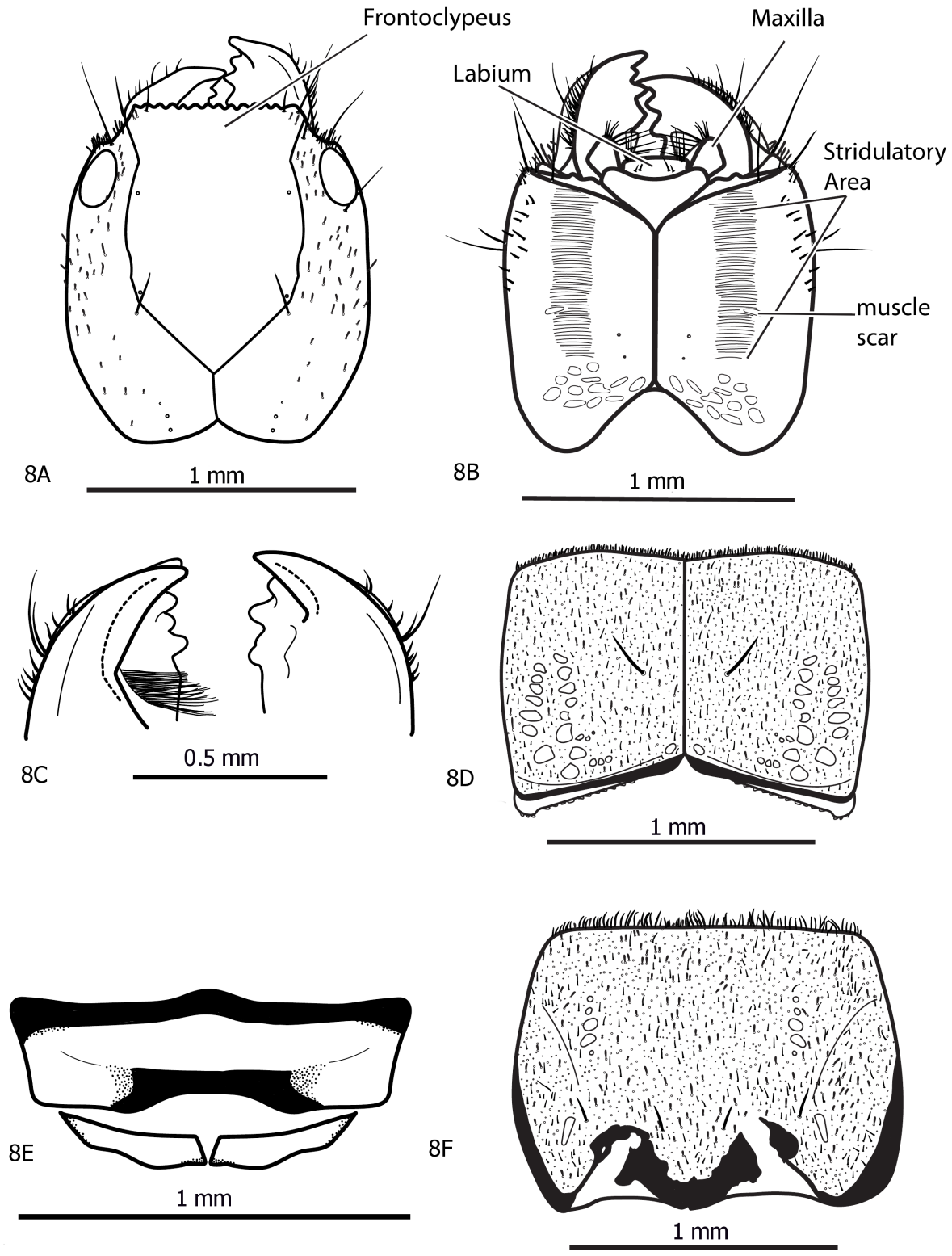


FIGURE 8A–8F. *Hydromanicus calyx* n. sp., larva, presumably mature. 8A, head, dorsal; 8B, head, ventral; 8C, mandibles, dorsal; 8D, pronotum, dorsal; 8E, prosternum and intersegmental sclerites, ventral; 8F, mesonotum, dorsal;

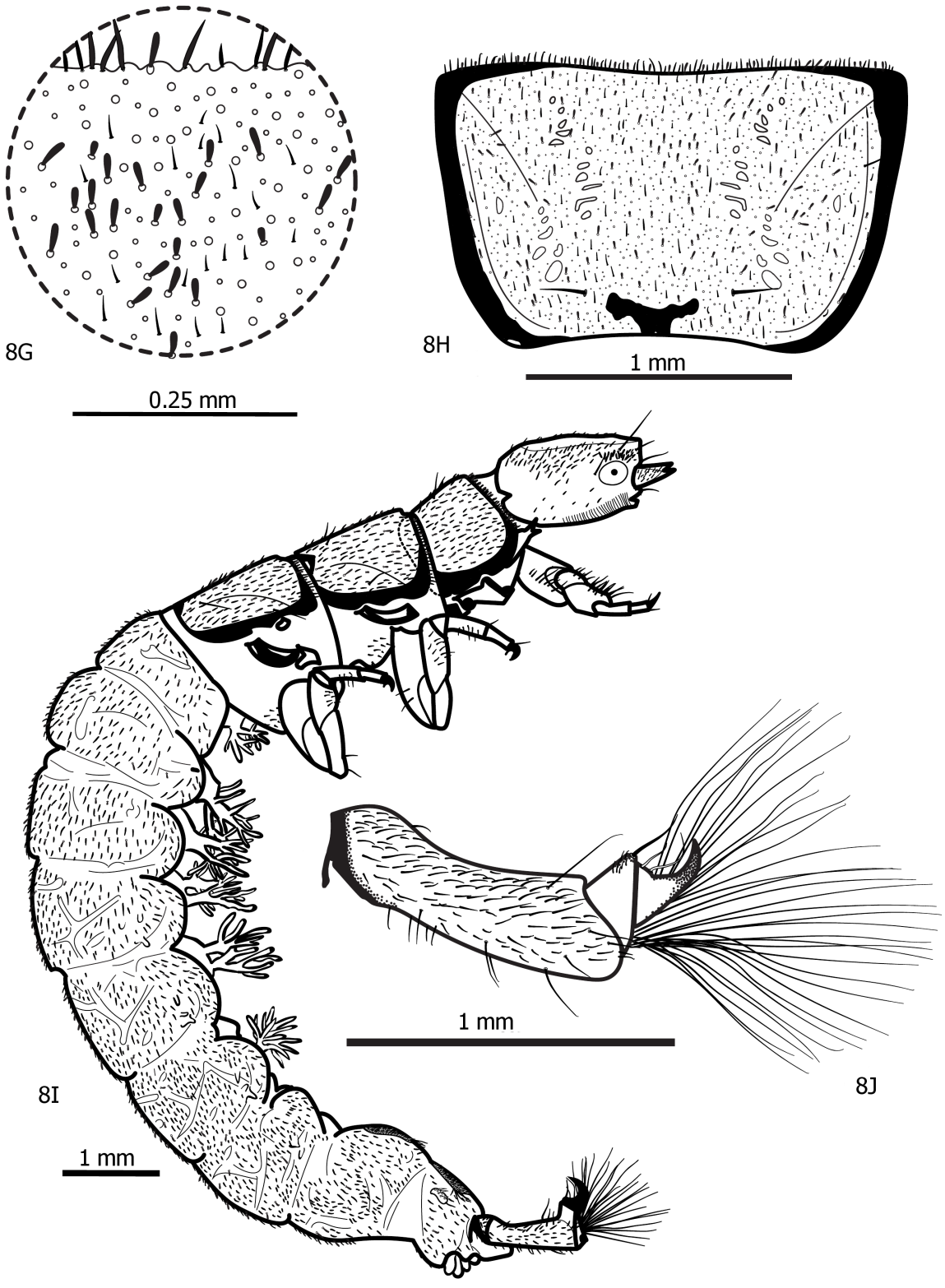


FIGURE 8G–8J. 8G, magnified view of anterior margin of mesonotum, dorsal; 8H, metanotum, dorsal; 8I, Habitus, right lateral; 8J, right anal proleg, right lateral.

Diagnosis of Male: The genitalia of this new species are morphologically most similar to those of *Hydromanicus nieuwenhuisi* Ulmer 1951, *Hydromanicus abiud* Malicky & Chantaramongkol 1993, and *Hydromanicus serubabel* Malicky & Chantaramongkol 1993 in general appearance. Unlike these three *Hydromanicus* species, the new species has cupped harpagones. It also lacks the upturned basomesal point on the dorsum of segment X, visible in lateral views of *H. nieuwenhuisi* and *H. abiud*. The warts on the head and pronotum of *H. calyx* are conspicuously hairy, whereas the warts on *H. serubabel* are less densely covered.

Description of Larva (Fig. 8): Each mandible approximately 3/4 as broad as long with large rounded teeth along mesal margin; left mandible with brush of setae near mesal margin; in lateral view (not shown), each mandible with flattened triangular region bearing setae. Frontoclypeus with crenulated anterior margin; secondary setae absent on frontoclypeus, sparse on parietals. Corners of anterior margins of genae near eyes with thick blunt setae. Maxillae with long, thick setae on mesal margins. Labium with two pairs of setae. Ventrally, stridulatory regions spanning anterior 3/4 of head, not conspicuously tapered posteriorly; lateral margins of stridulatory areas each interrupted by single faint muscle scar; faint muscle scars present on head posterior to stridulatory areas. All three nota and abdomen densely covered with mix of fine setae and stubble-like scale hairs; setae evenly distributed, except not covering faint pattern of muscle scars on thoracic nota (alveolae visible if setae broken). Anterior margin of each thoracic notum with fringe of thick hair, margin finely crenulate (visible only with very high magnification), and crenulations obscured by hair at margin. Posterior margin of pronotum with black band and with bead-like sculpturing along edge (visible only with high magnification). Mesonotum with black "M" pattern on posterior margin and black edges continuing laterally before tapering to near midlength. Metanotum with black "T" pattern posteromesally and black lateral margins tapering onto anterolateral and posterolateral margins. Large prosternal sclerite anterior to intersegmental sclerites with black anterior margin and black patch mesally along posterior margin. Prosternal-mesosternal intersegmental sclerites angular and somewhat tapered at lateral ends. Abdomen with ventral gills as in other hydropsychids; segments IV–VII each with 3 very small nub-like lateral gills on each side; segments VIII and IX with paired pupal-hook-plate-like sclerites (similar to those shown by Prommi and Permkam, 2015, figs 18 and 52); anal gills present posteriorly, varying in length from specimen to specimen or variably retracted into anus. Anal prolegs each with large fan of long setae (if setae broken, black sclerite bearing alveolae present caudally on mesal margin of proleg); anal claws each with single, thick, black seta near ventral base extending towards tip of claw; two lightly colored fine setae present between black seta and claw, seta nearest claw bent away from claw.

Diagnosis of Larva: The *Hydromanicus calyx* larva lacks color patterns on the dorsal side of the head, unlike those of *H. klanklini* and *H. adonis*. The larva of the new species has a straight anterior margin of the frontoclypeus, but the anterior margin of that of *H. malayanus* is convex mesally, that of *H. canaliculatus* is asymmetrical, and that of *H. abiud* is slightly asymmetrical with a convexity to the right. Of the 5 species with previously described larvae, the larva of *H. calyx* is morphologically most similar to that of *H. inferior* and *H. umbonatus.* but the new species can be distinguished by the slightly more-slender intersegmental sclerites and by the presence of faint muscle scars interrupting the lateral margins of the stridulatory areas on the ventral side of the head.

Our data tentatively reject the hypothesis that the species is indistinguishable from known *Hydromanicus* species. However, because there are only 6 Oriental species for which the larvae have been described in the genus, we cannot compare the *H. calyx* larva to all possible *Hydromanicus* larval forms and cannot eliminate the possibility that other undescribed larvae in the genus are indistinguishable from this larval form. Additionally, according to publications by Zhou (2007) and Geraci *et al.* (2010), *Hydromanicus* and *Hydatopsyche* may be paraphyletic. Further morphological research and comparison of DNA sequences may help resolve the relationships as more larval forms are associated with adults.

In our phylogram, larval specimen VN120 is sister to *H. calyx*, but it likely represents a different species. It is nearly identical morphologically to *H. calyx* but may possibly be separated as it seems to have a slightly shorter trunk to the T-shape on the posteromesal margin of the metanotum. Additionally, this specimen (VN120) could possibly be *H. inferior* if *H. inferior* has a faint muscle scar, not noted by Prommi *et al.* (2006), interrupting the lateral margin of each stridulatory area on the ventral side of the head. Our specimens were cleared during the DNA extraction process and this procedure may make this character easier to see.

DISCUSSION AND CONCLUSIONS

There are numerous adult specimens that we collected in Vietnam but that still are awaiting identification, and several new species that we intend to describe. There were also many larvae that were collected, but not yet sequenced; these could be associated with adults in the future.

Some initial research into surface water quality in Vietnam has been explored in the Nhue and Day sub-river systems, including chemical parameters such as dissolved oxygen, nutrients, and fecal coliform (Hanh *et al.* 2010). As the freshwater fauna in Vietnam continues to be studied, Vietnamese scientists can begin to use insects and other macroinvertebrates to monitor water quality in their country. The benefits of using benthic macroinvertebrates for this purpose are well-known and allow for a more long-term reflection of the health of the system than what chemical tests alone can provide (Lenat & Penrose 1996; Morse *et al*. 2007; Resh & Unzicker 1975).

All Vietnamese caddisfly collecting for this project was conducted within two National Parks, Bach Mã and Cúc Phýõng. All of the streams we sampled fell into the optimal or suboptimal categories when using EPA habitat assessment protocols (Barbour *et al*. 1999); this was because we were targeting streams the Vietnamese scientists could use in the future as reference sites, and because we wanted to visit streams with high macroinvertebrate diversity. However, there were several streams in Cúc Phýõng for which we did not collect specimens or conduct habitat assessments because we could visually see the water quality was impaired (algal blooms, trash, manure smell, etc.). It is our hope that this study, among others, will inspire continued research of Trichoptera in Vietnam. The more specimens are collected and barcoded, the more progress can be made for discovering species relationships and morphological distinctions, hopefully leading to future implementation of macroinvertebrate bioassessment in the region.

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