

## The taxonomy of *Chimarra* larvae species (Trichoptera: Philopotamidae) based on DNA barcodes with first descriptions of six larvae from Thailand

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**Abstract.** The taxonomy of Trichoptera larvae in Southeast Asia has been poorly studied, with only 39 species in nine families described. In the present study, COI gene sequences were analysed with distance- and tree-based delimitation methods to associate larvae and adults of six known species of *Chimarra*, namely *C. akkaorum* Malicky & Chantaramongkol, 1989; *C. bimbltona* Malicky, 1979; *C. khamuorum* Chantaramongkol & Malicky, 1989; *C. moira* Malicky & Prommi, 2004; *C. okuihorum* Mey, 1998; and *C. usal* Malicky & Chantaramongkol 1998. Species-matched larvae were then further examined for species-diagnostic morphological characters. Larvae of the six species can be distinguished at least by the shapes of their heads, the anterior margin of the frontoclypeal apotome, the submental sclerites, the mandibles, the posterolateral margins of pronota, the presence/absence of a notch anteromesally on the T-shaped labrum, and the position of seta s18. Sharing of certain morphological characteristics between species within the same larval groups supports the early division of adult species groups. In addition, a key to known *Chimarra* larvae of Thailand is provided. Moreover, in this work, we observed some possible cryptic species among the adults. This study demonstrates how DNA can be useful tools for matching caddisfly larvae to adults, facilitating their subsequent description and improving the state of knowledge of regional Trichoptera.

**Key words.** life-stage association, DNA barcoding, *digitata* group, *tsudai* group, *minuta* group

### INTRODUCTION

In terms of species diversity, Trichoptera is one of the largest orders of aquatic insects. For nearly all species, the eggs, larvae, and pupae spend most of their lives in water, emerging as aerial adults into riparian habitats for a few days or weeks to mate and lay eggs in, on, or near the water. Most caddisfly species were described morphologically based on males and especially the male genitalia; the eggs, larvae, pupae, and female adults of their species have been poorly studied taxonomically and most of them are unidentifiable. Traditional approaches to larval and adult association for descriptions of caddis larvae have been laboratory rearing and metamorphotype methods (Wiggins, 1996; Karaouzas,

2014). Recently, DNA sequencing techniques have been used to associate unidentified caddisfly larvae or other life stages with identifiable male adults (Zhou et al., 2007; Vitecek et al., 2015; Hu et al., 2018). In Southeast Asia, larvae of 39 species in nine families of Trichoptera have been described, for example: *Stenopsyche* spp. (Ismail et al., 1996; Hoang & Bae, 2007), *Arctopsyche* spp., *Himalopsyche acharai* Malicky & Chantaramongkol, 1989, *Inthanopsyche trimeresuri* Malicky, 1989 (Thamsenanupap et al., 2005), *Pseudoleptonema* spp. (Prommi et al., 2006a), *Hydatomanicus* spp. (Prommi et al., 2006b), *Potamyia* spp. (Prommi et al., 2006c; Maneechan et al., 2018), *Ugandatrichia* spp. (Laudee, 2008), *Amphipsyche* spp. (Peumwarunyoo & Prommi, 2013), *Hydropsyche* spp. (Prommi, 2016) and *Macrostemum floridum* Navás, 1929 (Valdon et al., 2023), representing less than 1% of the more than 6,000 Oriental species in 28 families (Morse, 2024). Because of the importance of larval identification for discovering species-level functional traits and for assessing water quality, research on and validation of methods to recognise larvae of different Thai caddisfly species are essential.

*Chimarra* is a member of the subfamily Chimarrinae in the family Philopotamidae. In Southeast Asia, over 170 *Chimarra* species have been recorded (Malicky, 2010; Blahnik et al., 2012; Malicky & Laudee, 2019), including 65 species from Thailand (Malicky, 2010; Laudee & Malicky, 2018; Malicky et al., 2018). However, larvae of only four philopotamid

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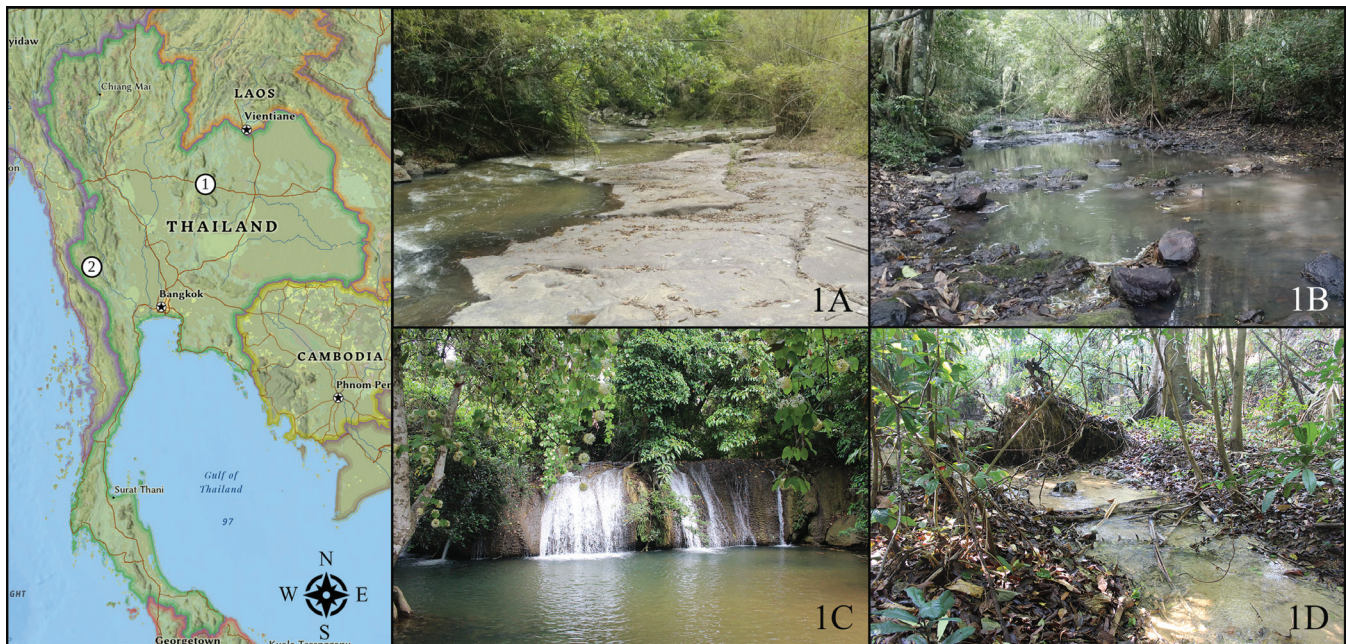


Fig. 1. Collecting sites of *Chimarra* in Thailand. 1. Phetchabun Province (1A, Phromleang stream; 1B, Yakruae stream) 2. Kanchanaburi Province (1C, Saphan Lao waterfall; 1D, Stream near the Dr. Phol Kleepbua Mine).

species are currently identifiable from the Oriental region, including those of *Chimarra paramonorum* Hu, Wang & Sun, 2018; *C. sadayu* Malicky, 1993; *Dolophilodes bellatula* Sun & Malicky, 2002; and *Wormaldia unispina* Sun, 1998, all of them successfully associated with corresponding adults using the mitochondrial cytochrome c oxidase subunit I (COI) gene (Hu et al., 2018, 2020). In this study, we use COI to associate unknown larvae with identifiable adult males of six *Chimarra* species from Thailand and for the first time describe diagnostic morphological characteristics of these species in the larval stage.

## MATERIAL AND METHODS

**Sampling of specimens.** *Chimarra* specimens in this study were collected from Phetchabun and Kanchanaburi Provinces, Thailand from 2021–2023 (Fig. 1). Adults were collected by light traps set overnight using an ultraviolet lamp (15-W fluorescent tube). The final instar larvae were collected by hand picking from in-stream substrate and using a D-frame net. All specimens were preserved in 99% ethanol. More detailed information about the collecting locations are presented in Table 1. The collecting of specimens in Nam Nao National Park was permitted by the Thai Department of National Parks, Wildlife, and Plant Conservation (reference no. 6510302). Voucher specimens from this study are deposited in the Thailand Natural History Museum, Pathum Thani, Thailand (THNHM) and the Aquatic Insects Collection at the Department of Biology, Khon Kaen University, Thailand (KKU-AIC).

**Morphological studies.** Male genitalia preparations were made by dissecting the apex of the abdomen, placing it in warm 10% potassium hydroxide (KOH) solution for 5 min and rinsing off the remaining KOH with distilled water.

The translucent abdomen was then transferred to glycerin for further detailed observation to the species level based on the atlas by Malicky (2010). The larvae were examined with a Nikon C-LEDs microscope and separated into unique morphotypes. Character photos of larvae were taken using a Nikon SMZ 25 stereo microscope and the Nis-Element software (Version 3.22.14; Nikon). Plates were edited, prepared and arranged using Adobe Photoshop 2018 (Adobe Systems). Some diagnostic structures of larvae were digitally drawn from the photographs using Adobe Illustrator CC 2018 (Adobe Systems) and Procreate (Savage Interactive).

For scanning electron microscope (SEM) images, head portions of larvae were dissected and prepared for SEM study by cleaning and dehydration in a graded series of ethanol solutions. The larval heads were then mounted on stubs, air-dried in a dry cabinet for 24 h and coated with gold. After that they were examined and imaged under a high vacuum in a scanning electron microscope (LEO 1450vp, UK). All photographs and SEM images were processed and adjusted in Adobe Photoshop 2018.

**DNA extraction, amplification and sequencing.** A total of 18 adult males (*Chimarra akkaorum*, 5 specimens; *C. Chiangmaiensis*, *C. khamuorum* and *C. okuihorum*, 3 specimens each; *C. moira* and *C. usal*, 2 specimens each) and 13 larvae (*C. sp.1*, 3 specimens; *C. sp.2*, 5 specimens; *C. sp.3*, *C. sp.5*, and *C. sp.6*, 1 specimen each; *C. sp.4*, 2 specimens) were used for molecular study. DNA from each larval morphotype and each adult species were extracted from one leg using the QIAamp Fast DNA Tissue Kit (Qiagen Scientific), following the manufacturer's extraction protocol. Extracted DNA was then amplified for the 658 bp fragment of the COI gene using LCO 1490 (GGTCAACAAATCATAAAGATATTGG) and HCO 2198 (TAACTTCAGGGTGACCAAAAATCA)

Table 1. GPS coordinates of locations of collected specimens.

Province	Location	GPS coordinates	Altitude (m a.s.l.)
Phetchabun	Phromleang stream, Nam Nao National Park	16°38'24.2"N 101°34'52.9"E	720
	Yakruae stream, Nam Nao National Park	16°44'19.7"N 101°34'26.4"E	840
Kanchanaburi	Saphan Lao waterfall, Thong Pha Phum District	14°43'35"N 98°47'28"E	510
	Stream near the Dr. Phol Kleepbua Mine, Thong Pha Phum District	14°45'8"N 98°48'40"E	660

primers (Folmer et al., 1994). Amplification via polymerase chain reactions (PCR) was performed with a 25µl reaction volume, comprising: 15.6µl ddH<sub>2</sub>O, 2.5µl 10x Buffer, 0.5µl dNTPs, 0.3µl Taq, 1µl Primer HCO2198 (2mM), 1µl Primer LCO1490 (2mM), and 3µl template DNA. The PCR mix was preheated at 94°C for 3 min followed by 35 cycles of 94°C for 60 s, 47–51°C for 40 s, and 72°C for 60 s. After 10 min of final extension at 72°C, the products were maintained at 4°C. Successful PCR products were sent to AGCT Corporation, Thailand, for purification and Sanger sequencing.

**Sequence alignment and phylogenetic analyses.** Consensus COI sequences (658 bp) were aligned and edited using MUSCLE, implemented in MEGA X (Kumar et al., 2018). The 31 newly generated COI sequences have been deposited in GenBank (Accession numbers: PX525286–PX525316). An additional 46 available sequences of *Chimarra* species from Southeast Asia and 11 sequences of outgroups (Table 2) were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>) and the Barcode of Life Data System (BOLD) (<https://www.boldsystems.org/>). The final dataset of total 88 COI sequences was used for phylogenetic analyses.

Phylograms were generated from the COI alignments using Maximum Likelihood (ML) and Bayesian inference (BI) approaches, using the online CIPRES Science Gateway server (Miller et al., 2010). Prior to the ML and BI analyses, best-fit models of nucleotide substitution were determined by PartitionFinder2 v. 2.3.4 (Lanfear et al., 2016) using a heuristic search algorithm under the corrected Akaike Information Criterion (AICc). The selected best-fit models for the three COI codon positions in this dataset were GTR+I, F81+I, and GTR+G. Maximum Likelihood analysis was performed in IQ-TREE 2.2.2.7 (Minh et al., 2020) with 10,000 replicates of ultrafast bootstrap (UFBoot) approximation to assess topology bootstrap support. Bayesian analyses were performed with MrBayes 3.2.7 (Ronquist et al., 2012) using the Markov Chain Monte Carlo technique (MCMC), sampling every 1,000 generations for 10,000,000 generations with the first 25% of trees discarded as burn-in. Phylogenetic relationships based on COI were further examined using

the Neighbour-joining (NJ) method implemented in MEGA X (Kumar et al., 2018), with pairwise genetic distances estimated by the Kimura 2-parameter (K2P), and branch support calculated from 1,000 bootstrapping replicates (BS).

For ML nodes with UFBoot values  $\geq 95\%$ , BI posterior probability (PP) values  $\geq 0.95$  and NJ with BS values  $\geq 70\%$ , analyses were considered highly supported (Hillis & Bull, 1993; Huelsenbeck & Rannala, 2004; Mauro & Agorreta, 2010; Hoang et al., 2018). The support values are listed in the order of presentation in the phylogenetic tree.

COI trees were visualised and edited using FigTree v. 1.4 (Rambaut, 2014) and Adobe Photoshop 2018.

**Species delimitation.** Sequences of public databases from GenBank and BOLD that formed a monophyletic group with strong support to known adult species and unidentifiable larvae in the present study were used in species delimitation analyses. Species delimitation analyses for identification of Molecular Operational Taxonomic Units (MOTUs) were conducted using two methods: (i) the distance-based Assemble Species by Automatic Partitioning (ASAP) method (Puillandre et al., 2020) and (ii) the tree-based method Multi-rate Poisson Tree Processes (mPTP) (Kapli et al., 2017). For ASAP, the sequence alignment of COI was uploaded using the ASAP web server available at <https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html> (Puillandre et al., 2020) with the K2P model selected as the distance type with the settings: Kimura (K80) TS/TV 2.0, and Jukes-Cantor distance (JC69) model. The K2P and Jukes-Cantor distance (JC69) models were analysed separately. The mPTP species delimitation analyses used the web service available at <https://mptp.h-its.org> (Kapli et al., 2017) with the phylogenetic input tree obtained from earlier ML analysis.

**Larva-adult association.** If the COI of an unidentified larva was genetically identical to that of adults of known species or nested within a monophyletic group of a known species, these were considered positive larva-adult associations (Zhou et al., 2007).

Table 2. Specimens used in the molecular analyses with GenBank accession numbers.

Species	Code	Stage	Location	GenBank accession number	Source
<i>Chimarra akkaorum</i>	AKM60.01	Adult	Stream near the Dr. Phol Kleepbua Mine	PX525286	This study
	AKM68.37	Adult	Stream near the Dr. Phol Kleepbua Mine	PX525287	This study
	APL68.02	Adult	Phromleang stream	PX525288	This study
	APL68.09	Adult	Phromleang stream	PX525289	This study
	AYK68.01	Adult	Yakruae stream	PX525290	This study
<i>C. chiangmaiensis</i>	APL68.12	Adult	Phromleang stream	PX525297	This study
	AYK60.10	Adult	Yakruae stream	PX525298	This study
	AYK68.11	Adult	Yakruae stream	PX525299	This study
<i>C. khamuorum</i>	AKM68.36	Adult	Stream near the Dr. Phol Kleepbua Mine	PX525300	This study
	AKS98.77	Adult	Saphan Lao waterfall	PX525301	This study
	AYK98.79	Adult	Yakruae stream	PX525302	This study
<i>C. moira</i>	AKS60.04	Adult	Saphan Lao waterfall	PX525304	This study
	AKS68.30	Adult	Saphan Lao waterfall	PX525305	This study
<i>C. okuihorum</i>	APL68.04	Adult	Phromleang stream	PX525308	This study
	AYK68.03	Adult	Yakruae stream	PX525309	This study
	AYK68.14	Adult	Yakruae stream	PX525310	This study
<i>C. usal</i>	AKS60.05	Adult	Saphan Lao waterfall	PX525314	This study
	AKS68.31	Adult	Saphan Lao waterfall	PX525315	This study
<i>C. sp.1</i>	LPL68.07	Larva	Phromleang stream	PX525311	This study
	LPL98.72	Larva	Phromleang stream	PX525312	This study
	LPL98.73	Larva	Phromleang stream	PX525313	This study
<i>C. sp.2</i>	LKS75.13	Larva	Saphan Lao waterfall	PX525291	This study
	LPL68.08	Larva	Phromleang stream	PX525292	This study
	LPL68.18	Larva	Phromleang stream	PX525293	This study
	LYK60.21	Larva	Yakruae stream	PX525294	This study
	LYK68.06	Larva	Yakruae stream	PX525295	This study
<i>C. sp.3</i>	LKS75.14	Larva	Saphan Lao waterfall	PX525316	This study
<i>C. sp.4</i>	LKS60.16	Larva	Saphan Lao waterfall	PX525306	This study
	LKS75.15	Larva	Saphan Lao waterfall	PX525307	This study
<i>C. sp.5</i>	LYK98.82	Larva	Yakruae stream	PX525296	This study
<i>C. sp.6</i>	LYK60.20	Larva	Yakruae stream	PX525303	This study
<i>C. ariadne</i>	KM225401.1	Adult	Vietnam	KM225401.1	Wahlberg & Johanson (2014)
<i>C. akkaorum</i>	KX104811.1	Adult	Chaeson (Jaeson) National Park, Chiang Mai, Thailand	KX104811.1	Zhou et al. (2016)
	KX106510.1	Adult	Muang Pai Resort, Mae Hong Son, Thailand	KX106510.1	Zhou et al. (2016)

Species	Code	Stage	Location	GenBank accession number	Source
	KX291253.1	Adult	Muang Pai Resort, Mae Hong Son, Thailand	KX291253.1	Zhou et al. (2016)
	KX294257.1	Adult	Huai Mae Yen, E von Pai, Mae Hong Son, Thailand	KX294257.1	Zhou et al. (2016)
<i>C. argeia</i>	KX296473.1	Adult	Bolavens Plateau, Laos	KX296473.1	Zhou et al. (2016)
<i>C. atara</i>	KX292359.1	Adult	Doi Suthep, Chiang Mai, Thailand	KX292359.1	Zhou et al. (2016)
<i>C. batukaua</i>	KX140898.1	Adult	Melanting Waterfalls, Munduk, Bali, Indonesia	KX140898.1	Zhou et al. (2016)
	KX142446.1	Adult	Nusa Tenggara Barat, Lombok, Senaru, Indonesia	KX142446.1	Zhou et al. (2016)
	KX142735.1	Adult	Melanting Waterfalls, Munduk, Bali, Indonesia	KX142735.1	Zhou et al. (2016)
	KX294212.1	Adult	Nusa Tenggara Barat, Lombok, Senaru, Indonesia	KX294212.1	Zhou et al. (2016)
<i>C. bimbltona</i>	KX107429.1	Adult	Ban Huai Hia, Mae Hong Son, Thailand	KX107429.1	Zhou et al. (2016)
	KX294150.1	Adult	Huai Mae Yen, E von Pai, Mae Hong Son, Thailand	KX294150.1	Zhou et al. (2016)
	KX225402.1	Adult	Vietnam	KX225402.1	Wahlberg & Johanson (2014)
<i>C. burmana</i>	KX291453.1	Adult	Chiang Mai, Thailand	KX291453.1	Zhou et al. (2016)
<i>C. chiangmaiensis</i>	KX104178.1	Adult	Chaeson (Jaeson) National Park, Lampang, Thailand	KX104178.1	Zhou et al. (2016)
	KX106839.1	Adult	Chaeson (Jaeson) National Park, Lampang, Thailand	KX106839.1	Zhou et al. (2016)
<i>C. demeter</i>	KM225329.1	Adult	Laos	KM225329.1	Wahlberg & Johanson (2014)
<i>C. dulitensis</i>	KM225335.1	Adult	Borneo, Indonesia	KM225335.1	Wahlberg & Johanson (2014)
<i>C. gether</i>	KX291650.1	Adult	Nong Fa (crater lake) Dong Amphan NBCA, Attapu, Laos	KX291650.1	Zhou et al. (2016)
<i>C. haimuoi</i>	KM225395.1	Adult	Laos	KM225395.1	Wahlberg & Johanson (2014)
<i>C. htinorum</i>	KX291978.1	Adult	Doi Suthep, Chiang Mai, Thailand	KX291978.1	Zhou et al. (2016)
<i>C. joliveti</i>	KX104207.1	Adult	Chaeson (Jaeson) National Park, Lampang, Thailand	KX104207.1	Zhou et al. (2016)
	KX105827.1	Adult	Phau Pan, Houaphan, Laos	KX105827.1	Zhou et al. (2016)
	KX106903.1	Adult	Chaeson (Jaeson) National Park, Lampang, Thailand	KX106903.1	Zhou et al. (2016)

Species	Code	Stage	Location	GenBank accession number	Source
	KX291839.1	Adult	Phau Pan, Houaphan, Laos	KX291839.1	Zhou et al. (2016)
<i>C. khamuorum</i>	KM225403.1	Adult	Vietnam	KM225403.1	Wahlberg & Johanson (2014)
<i>C. lannaensis</i>	KX291164.1	Adult	Doi Suthep, Chiang Mai, Thailand	KX291164.1	Zhou et al. (2016)
<i>C. monorum</i>	KX103169.1	Adult	Chiang Dao Wildlife Research Station, Chiang Mai, Thailand	KX103169.1	Zhou et al. (2016)
	KX103622.1	Adult	Muang Pai Resort, Chiang Mai, Thailand	KX103622.1	Zhou et al. (2016)
	KX105667.1	Adult	Muang Pai Resort, Chiang Mai, Thailand	KX105667.1	Zhou et al. (2016)
	KX106043.1	Adult	Huai Mai, Umgebung Pai, Mae Hong Son, Thailand	KX106043.1	Zhou et al. (2016)
	KX291173.1	Adult	Unterlaof des Huai Mae Ya, Mae Hong Son, Thailand	KX291173.1	Zhou et al. (2016)
<i>C. okuihorum</i>	KM225330.1	Adult	Laos	KM225330.1	Wahlberg & Johanson (2014)
<i>C. pipake</i>	KX104404.1	Adult	Chaeson (Jaeson) National Park, Chiang Mai, Thailand	KX104404.1	Zhou et al. (2016)
	KX107082.1	Adult	Chiang Dao Wildlife Research Station, Chiang Mai, Thailand	KX107082.1	Zhou et al. (2016)
	KX107231.1	Adult	Muang Pai Resort, Chiang Mai, Thailand	KX107231.1	Zhou et al. (2016)
<i>C. shanorum</i>	KM225331.1	Adult	Loas	KM225331.1	Wahlberg & Johanson (2014)
<i>C. spinifera</i>	KX106002.1	Adult	Chiang Dao Wildlife Research Station, Chiang Mai, Thailand	KX106002.1	Zhou et al. (2016)
	KX293277.1	Adult	Sekong, Laos	KX293277.1	Zhou et al. (2016)
	KX293899.1	Adult	Muang Pai Resort, Mae Hong Son, Thailand	KX293899.1	Zhou et al. (2016)
<i>C. suadulla</i>	KX103600.1	Adult	Chaeson (Jaeson) National Park, Chiang Mai, Thailand	KX103600.1	Zhou et al. (2016)
	KX106464.1	Adult	Chiang Dao Wildlife Research Station, Chiang Mai, Thailand	KX106464.1	Zhou et al. (2016)
<i>C. suthepensis</i>	KX294921.1	Adult	Doi Suthep, Chiang Mai, Thailand	KX294921.1	Zhou et al. (2016)
<i>C. uppita</i>	KX292733.1	Adult	Mae Charim National Park, Nan, Thailand	KX292733.1	Zhou et al. (2016)
<i>C. argax</i>	KX292400.1	Adult	Mae Charim National Park, Nan, Thailand	KX292400.1	Zhou et al. (2016)

Species	Code	Stage	Location	GenBank accession number	Source
<b>Outgroup</b>					
<i>Dolophilodes torrentis</i>	KX105868.1	Adult	Tsirang Rongchhu, Bhutan	KX105868.1	Zhou et al. (2016)
<i>D. ornatula</i>	KX293523.1	Adult	Sichuan, China	KX293523.1	Zhou et al. (2016)
<i>Kisaura cina</i>	KX293365.1	Adult	Doi Suthep, Chiang Mai, Thailand	KX293365.1	Zhou et al. (2016)
<i>Gunungiella segsafiazga</i>	KX296330.1	Adult	Doi Suthep, Chiang Mai, Thailand	KX296330.1	Zhou et al. (2016)
<i>K. consagia</i>	KX102712.1	Adult	Chiang Dao Wildlife Reserve Station, Chiang Mai, Thailand	KX102712.1	Zhou et al. (2016)
	KX103202.1	Adult	Chiang Dao Wildlife Reserve Station, Chiang Mai, Thailand	KX103202.1	Zhou et al. (2016)
	KX104632.1	Adult	Unterlauf des Huai Mae Ya, Mae Hong Son, Thailand	KX104632.1	Zhou et al. (2016)
	KX107587.1	Adult	Chiang Dao Wildlife Reserve Station, Chiang Mai, Thailand	KX107587.1	Zhou et al. (2016)
	KX291259.1	Adult	Oberlauf des Huai Pang Paek, Mae Hong Son, Thailand	KX291259.1	Zhou et al. (2016)
	<i>K. schimplzichta</i>	KX105271.1	Adult	Vietnam	KX105271.1
<i>K. sura</i>	KX292251.1	Adult	Doi Suthep, Chiang Mai, Thailand	KX292251.1	Zhou et al. (2016)

## RESULTS

**Morphology Classification.** Six *Chimarra* species were identified from adult specimens: *Chimarra akkaorum* Malicky & Chantaramongkol, 1989, *Chimarra chiangmaiensis* Chantaramongkol & Malicky, 1989, *Chimarra khamuorum* Chantaramongkol & Malicky, 1989, *Chimarra moira* Malicky & Prommi, 2004, *Chimarra okuihorum* Mey, 1998, and *Chimarra usal* Malicky & Chantaramongkol, 1989. *Chimarra* larvae were classified into six morphotypes: *Chimarra* spp. 1–6.

**Tree based association.** The ML, BI and NJ trees based on sequences of 77 *Chimarra* adults and larvae yielded species-level monophyletic groupings with strong bootstrap and posterior probability support (UFBoot = 99–100%, PP = 1, BS = 100%) for most species (Fig. 2). The samples included in this study were clearly differentiated into seven monophyletic groups based on COI.

The sequences of larvae *Chimarra* spp. 1–4 were nested into monophyletic groups of adults *C. okuihorum*, *C. akkaorum*, *C. usal*, and *C. moira*, respectively. The sequences of *C. khamuorum* and *C. sp.6* formed a monophyletic group. Moreover, sequences of larvae of *C. sp.5* and public databases

of *C. bimbltona* adults from Thailand (KX107429.1 and KX294150.1) were nested in the same monophyletic group. These six unidentifiable caddisfly larvae and known adult species demonstrated a monophyletic cluster with strong support (UFBoot = 99–100%, PP = 1, BS = 100%), indicating that they were linked.

Some sequences of adult *Chimarra* species obtained from GenBank or BOLD sources nested within clades of other species, with high UFBoot, PP and BS support values, suggesting that at least some of the species were misidentified. For example, a GenBank sequence of a specimen of *C. bimbltona* from Vietnam (VN) (KM225402.1) nested in the *C. pipake* clade and that of *C. khamuorum* from Vietnam (VN) (KM225403.1) nested in the *C. chiangmaiensis* clade. Also, the sequence of *C. okuihorum* from Laos (LA) (KM225330.1) did not group with any clades.

### Pairwise genetic distances and species delimitation.

According to results of the tree-based associations, two separate sets of K2P pairwise divergences values were calculated. The aligned COI sequence data matrix of 30 *Chimarra* adult specimens (16 specimens of this study and 14 public sequences in Southeast Asia) was used to calculate sequence divergences (Table 3). The intra- and inter-specific

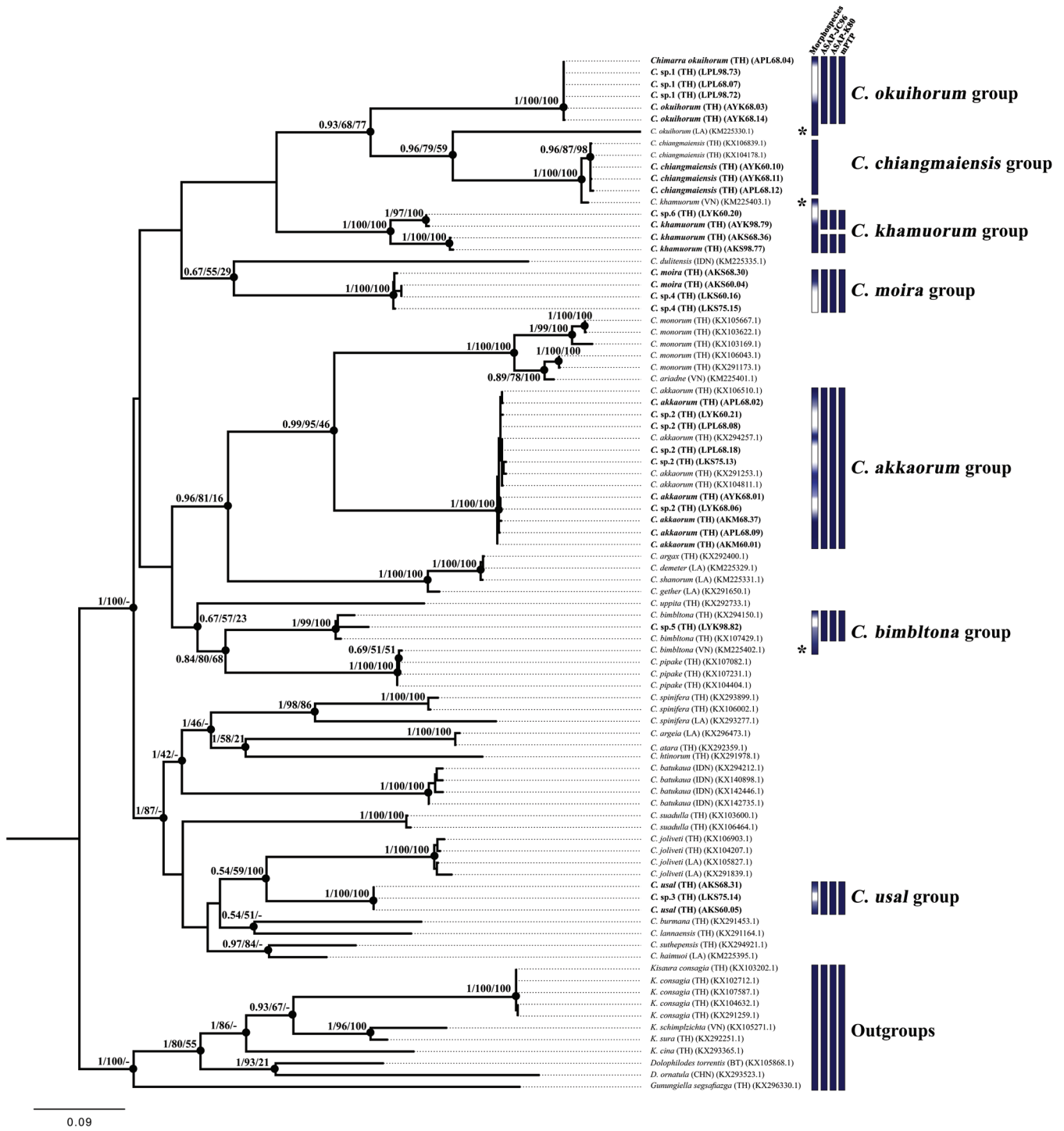


Fig. 2. Phylogenetic tree reconstruction based on Neighbour-joining (NJ), Maximum likelihood (ML) and Bayesian inference (BI) analyses of sequences of the COI gene of *Chimarra* species found in Southeast Asia, larval-adult male associations, and an outgroup. Coloured bars indicate results of species delimitation analyses based on the COI gene using morphospecies, ASAP and mPTP. Numbers on the branches indicate the BI PP values, ML UFboot and NJ BS. The first letter of each specimen label indicates its stage: A = adult male; L = larva. Original country of sequences retrieved from GenBank are shown in parentheses: BT = Bhutan, CHN = China, IDN = Indonesia, LA = Laos, TH = Thailand, VN = Vietnam. Species names in bold indicate species found in this study. Asterisks (\*) indicate anomalous sequences discussed in the text.

genetic distances ranged from 0.0–6.4 and 0.3–22.6%, respectively. The percentage inter-specific genetic distances between *C. bimbltona* (VN) (KM225402.1) and *C. pipake* (TH), *C. chiangmaiensis* (TH) and *C. khamuorum* (VN) (KM225403.1) were 0.3–0.5, and 1.4–1.7, respectively, which were less than the maximum intraspecific genetic distance of 6.4, again suggesting that the Vietnamese specimens were

misidentified. The interspecific genetic distances of other pairs were greater than 14.0%. Therefore, the sequence of *C. bimbltona* (VN) (KM225402.1), *C. khamuorum* (VN) (KM225403.1) were discarded from further species delimitation analysis. In addition, five *C. chiangmaiensis* (TH) sequences that did not match any larvae and a sequence of *C. okuihorum* (LA) (KM225330.1) that did not match

Table 3. Percentages of genetic distance (COI) between sequences of adults of seven species of *Chimarra* using the Kimura 2-parameter (K2P) (- mean single sequence, n = number of specimens).

Species	n	Percent of Kimura 2-parameter (K2P) distances												
		1	2	3	4	5	6	7	8	9	10			
1 <i>C. akkaorum</i> (TH)	9	0.1-0.9												
2 <i>C. bimbltona</i> (TH)	2	16.9-18.3	2.2											
3 <i>C. bimbltona</i> (VN) (KM225402.1)	1	18.5-19.3	14.0-15.2	-										
4 <i>C. chiangmaiensis</i> (TH)	5	20.4-21.4	15.8-16.6	18.3-18.7	0-0.5									
5 <i>C. khamuorum</i> (TH)	3	16.5-17.9	17.7-18.9	21.3-21.9	16.0-17.0	0.5-6.4								
6 <i>C. khamuorum</i> (VN) (KM225403.1)	1	19.7-20.4	17.2-17.6	20.4	1.4-1.7	15.4-16.4	-							
7 <i>C. moira</i> (TH)	2	19.6-20.2	16.5-17.3	16.5-16.9	21.5-22.6	16.5-17.3	20.9-21.3	0.9						
8 <i>C. okuihorum</i> (TH)	3	21.9-22.3	19.4-20.0	17.9-18.3	16.1-16.8	15.4-16.4	16.5-16.7	18.5-19.1	0.2-0.3					
9 <i>C. okuihorum</i> (LA) (KM225330.1)	1	19.1-19.9	17.7-17.9	20.6	14.2-14.8	15.8-16.4	14.0	20.8-21.0	15.0-15.2	-				
10 <i>C. pipake</i> (TH)	3	18.3-19.3	13.8-15.2	0.3-0.5	20.8-21.5	16.9-17.5	17.3-20.0	15.9-16.5	17.7-18.3	20.0-20.2	0-0.2			

any clades as previous mentioned were also discarded from further analysis.

A total of 34 COI sequences of successful *Chimarra* larva-adult associations were compiled for species delimitation analyses. Both distance-based (ASAP- JC96 and K80, p = 0.01) and tree-based (multi-rate PTP) analyses indicated the presence of seven MOTUs for all six adult morphospecies in this study (Fig. 2). The best partition (score=2.00) obtained by ASAP species delimitation analysis grouped all samples of *Chimarra* into seven groups, with a distance threshold of approximately 5%. The intra- and inter-specific genetic divergence of six species of larvae and their associated adults including *C. chiangmaiensis* ranged from 0.0-6.4 and 15.4-26.6%, respectively (Table 4). Morphological identifications agreed with MOTUs for *C. akkaorum*, *C. bimbltona*, *C. moira*, *C. okuihorum*, and *C. usal*. The intraspecific genetic distance of *C. khamuorum* was up to 6.4% which separated their sequences into two MOTUs, corresponding to their locations in Kanchanaburi Province (AKM68.36 and AKS98.77) and Phetchabun Province (AYK98.79 and LYK60.20). Since the larval specimen LYK60.20 was only found in Phetchabun Province, *C. sp.* 6 larva was treated as *C. khamuorum*. The maximum intraspecific genetic distance values (6.4%) were considerably less than minimum interspecific values (15.4%), indicating the existence of barcoding gaps in this study.

**Morphological relationship.** The larvae of the six apparently associated species in the present study showed differentiation by various aspects of their morphology, including the shapes of their heads, the anterior margin of frontoclypeal apotomes, their submental sclerites, their mandibles, the posterolateral margins of their pronota, and the presence/absence of a notch mesally on the T-shaped labrum. A detailed description of each larval species and key to known *Chimarra* larvae of Thailand are provided below.

## TAXONOMY

### Genus *Chimarra* Stephens, 1829

**Diagnosis.** Like all Philopotamidae larvae, the larvae of *Chimarra* spp. are caseless and lack the spacing humps on abdominal segment I, and are characterised by the presence of a membranous, white, T-shaped labrum (often retracted in preserved specimens) with a brush-like anterior margin, short antennae, absence of sclerotised plates on meso- and metanota and abdominal segment IX, and presence of a pair of long anal prolegs each terminated by a stout hook. Among the known philopotamid genera of Southeast Asia (larvae are unknown for *Edidiehlia* Malicky, 1993, and *Gumungiella* Ulmer, 1913), larvae of *Chimarra* are recognised by the anterior margin of the frontoclypeus having a prominent notch asymmetrically to the right of the midline; the paired setae closest to the median suture on the ventral side of the head (setae s18) positioned near the apical angle of the triangular ventral apotome; the mandibles each have two black setae on the convex outer margin and have their mesal margins normally with three regions: the basal region with

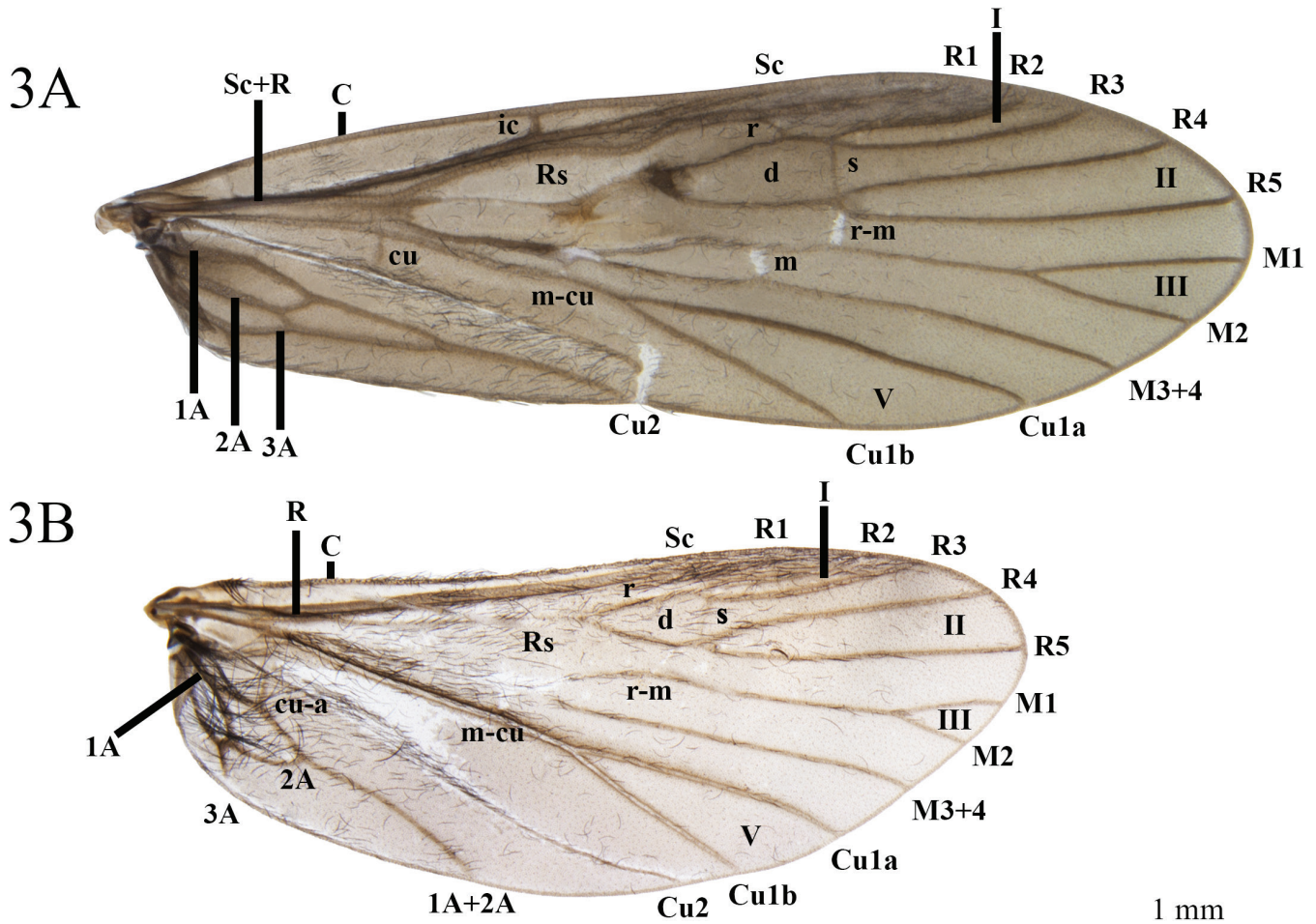


Fig. 3. *Chimarra akkaorum* Malicky & Chantaramongkol, 1989, wings. 3A, forewing; 3B, hind wing. A = Anal veins (as 1A, 2A, 3A); C = Costal vein; Cu = Cubital vein (branched into Cu1a, Cu1b, Cu2); cu = cubital crossvein; cu-a = cubitoanal crossvein; d = discoidal cell; I, II, III = apical forks I and II and III; M = Medial vein (branched into M1–M4); m-cu = mediocubital crossvein; R = Radial vein (branched into R1–R5); Rs = Radial Sector vein (branched into R2–R4); r = radial crossvein; r-m = radiomedial crossvein; SC = Subcostal vein; s = sectoral crossvein; V = apical fork V.

few projecting teeth, the mid-region variably serrate, and the apical region with a variable number of subapical teeth and a large, acute apical tooth. The forecoxae each have a long slender, subapicodorsal, seta-bearing process.

***Chimarra digitata* group**

**Diagnosis.** Adults of the *Chimarra digitata* group share many characteristics of male genitalia and wing venation with the *maginata* group, but the latter group lacks the pair of elongate endotheal spines. The *digitata* group shares some characteristics with the *minuta* group including two sensilla on lateral lobes of tergum X, inferior appendages strong inflection at base, and forewing with m cross-vein proximal to s and r-m cross-veins. The *digitata* group is distinguished from the *tsudai* and the *minuta* groups with the characteristic combination of segment IX bearing well-developed anterodorsal apodemes; segment X with membranous mesal lobes divided apically and without sensilla, two sensilla on sclerite lateral lobes of tergum X, and usually with asymmetrically 1–3 endotheal spines. Forewing each with stem of Rs with enlarged, sclerotised, swollen node at inflection point, discoidal cell about two



Fig. 4. *Chimarra khamuorum* Chantaramongkol & Malicky, 1989, wings. 4A, forewing; 4B, hind wing.

times as long as wide, pigmented s cross-vein; hind wing, 2A vein forked apically.

*Chimarra akkaorum*, *C. chiangmaiensis*, *C. khamuorum*, and *C. okuihorum* have been included in the *Chimarra digitata* group (Blahnik et al., 2009). Wing venations of *Chimarra akkaorum*, *C. khamuorum*, and *C. okuihorum* in

Table 4. Percentages of genetic distance (COI) between sequences of adult and larva of *Chimarra* species including *C. chiangmaiensis* using the Kimura 2-parameter (K2P) (– mean single sequence, n = number of specimens, values in bold indicate minimum pairwise genetic distance of associated larvae and adults).

Species	n	Percent of Kimura 2-parameter (K2P) distances																
		1	2	3	4	5	6	7	8	9	10	11	12	13				
1 <i>C. akkaorum</i> (TH)	9	0.1–0.9																
2 <i>C. bimbltona</i> (TH)	2	16.9–19.1	2.2															
3 <i>C. chiangmaiensis</i> (TH)	5	20.6–21.4	18.1–26.6	0.2–0.5														
4 <i>C. khamuorum</i> (TH)	3	16.5–17.5	16.5–18.8	16.2–17.2	0.5–6.4													
5 <i>C. moira</i> (TH)	2	19.6–20.2	16.5–17.3	21.7–22.8	16.5–17.3	0.9												
6 <i>C. okuihorum</i> (TH)	3	22.1–22.5	18.1–20.1	16.0–16.8	15.6–16.6	18.7–19.3	0.1–0.3											
7 <i>C. usal</i> (TH)	2	16.5–16.9	17.5–17.9	19.7–20.3	15.5–16.7	16.0–16.2	19.0–19.2	0										
8 <i>C. sp.1</i>	3	22.1–22.3	18.3–19.9	16.0–16.6	15.6–16.4	18.9–19.3	<b>0–0.2</b>	19.2	0									
9 <i>C. sp.2</i>	5	<b>0–1.1</b>	17.3–18.9	20.8–21.8	16.7–17.5	19.6–20.2	22.1–22.8	16.5–16.9	22.1–22.8	0.2–0.9								
10 <i>C. sp.3</i>	1	16.5–16.9	17.5–17.9	19.7–20.3	15.5–16.7	16.0–16.2	19.0–19.2	<b>0</b>	19.2	16.5–16.9								
11 <i>C. sp.4</i>	2	19.6–20.2	16.5–17.3	21.9–22.8	16.3–17.3	<b>0–0.9</b>	18.7–19.3	16.0–16.2	18.9–19.3	19.2–20.2	16.0–16.2	0.9						
12 <i>C. sp.5</i>	1	16.7–17.5	<b>3.3–4.0</b>	18.8–19.6	17.8–18.0	15.8–16.1	19.9–20.1	16.7	19.9	17.1–17.7	16.7	15.8–16.1	–					
13 <i>C. sp.6</i>	1	16.7–17.3	17.5–17.8	16.0–16.6	<b>0.3–6.4</b>	16.7–16.9	15.4–15.6	15.1	15.4	17.1–17.7	15.1	16.5–16.9	17.8	–				



Fig. 5. *Chimarra okuihorum* Mey, 1998, wings. 5A, forewing; 5B, hind wing.

this study shown in Figs. 3–5 indicate for their inclusion in the *digitata* group. Photographs of the male genitalia of *Chimarra akkaorum* (Fig. 7F, 7G), *C. khamuorum* (Fig. 9F, 9G) and *C. okuihorum* (Fig. 11F, 11G) are provided.

***Chimarra akkaorum* Malicky & Chantaramongkol, 1989**  
(Figs. 6A–7E)

**Diagnosis.** The larva of *Chimarra akkaorum* appears to be very similar to those of *C. khamuorum* and *C. okuihorum* by having a deeply incised anterior margin of the frontoclypeal apotome, a notch on the center of the anterior margin of the T-shaped labrum, paired setae s18 that are small and located at the level of the tip of the ventral apotome, and foretrochantins with a leaf-like shape. It differs from other described Thai *Chimarra* species in the oval shape of its head capsule; its antennae about ¼ as long as paired seta s9; its pronotum with the posterodorsal margin shallowly impressed and with a broad constriction laterally behind its moderately thick, black hind margin; and each of its anal prolegs with a lateral sclerite about twice as long as tall.

**Description.** Final larval instar (instar V) in alcohol with mean body length 5.4 ± 1.2 mm (n = 10), membranous body white with yellowish- or reddish-brown sclerotised parts (Fig. 6A).

**Head.** Head capsule in dorsal and ventral views (Fig. 6B, 6C) oval with curved anterolateral margins, 1.5 times as long as wide, widest at ⅓ from base, posterodorsal margin round with small mesal notch, posteroventral margin with deep V-shaped notch; in lateral view (Fig. 6D, 6E) slightly tapering anteriorly with smooth surface, black spot-on notch of occipital foramen at each posterolateral margin, paired setae s13 short and small, located on midlength of genae. Frontoclypeal apotome (Fig. 6F) with anterior dark reddish brown and relatively abruptly contrasting with lighter reddish brown posteriorly; its anterior margin appearing as two asymmetrical, triangular projections on either side of deep notch asymmetrically right of midline with small mound in base of notch, left projection longer and broader than right projection; anterolateral margin with three paired setae (setae s1–s3), setae s3 smallest; paired setae s4 closed

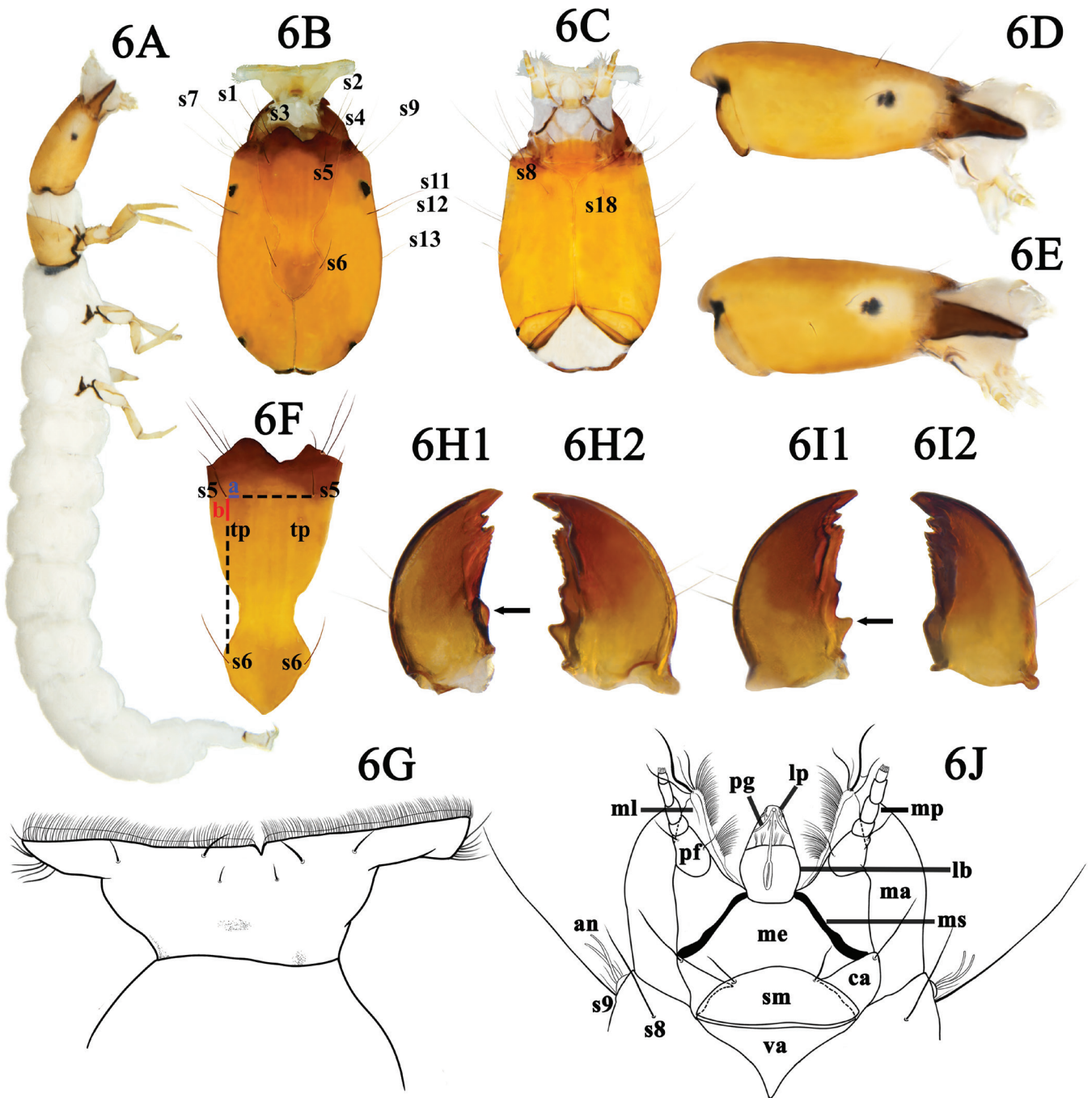


Fig. 6. *Chimarra akkaorum* Malicky & Chantaramongkol, 1989, larva. 6A, habitus, right lateral; 6B, head, dorsal; 6C, head, ventral; 6D, head, right lateral; 6E, head, left lateral (reversed); 6F, frontoclypeus (a, b = transverse and longitudinal distances between tentorial pit and its seta s5, respectively); 6G, labrum with notch, dorsal; 6H1, left mandible with blunt basomesal projection (→), dorsal; 6H2, right mandible, dorsal; 6I1, right mandible with prominent basomesal projection (→), ventral; 6I2, left mandible, ventral; 6J, maxillae and labium, ventral. an = antenna (antennae paired); ca = cardo (cardines, paired); ma = mandible (mandibles, paired); me = mentum; mp = maxillary palp (maxillary palps, paired); ml = maxillary lobe (maxillary lobes, paired); ms = maxillary stipes (maxillary stipites, paired); pf = palpifer (palpifers, paired); pg = palpiger (palpigers, paired); lb = labium; lp = labial palp (labial palps, paired); sm = submental sclerite; s1 = seta 1 (setae 1, paired); s2 = seta 2 (setae 2, paired); s3 = seta 3 (setae 3, paired); s4 = seta 4 (setae 4, paired); s5 = seta 5 (setae 5, paired); s6 = seta 6 (setae 6, paired); s7 = seta 7 (setae 7, paired); s8 = seta 8 (setae 8, paired); s9 = seta 9 (setae 9, paired); s11 = seta 11 (setae 11, paired); s12 = seta 12 (setae 12, paired); s13 = seta 13 (setae 13, paired); s18 = seta 18 (setae 18, paired); tp = tentorial pit; va = ventral apotome.

to anterior edge of frontoclypeus, anterior of tentorial pits with long paired setae s5, transverse distance from s5 to tentorial pits about  $\frac{1}{8}$  distance between setae of s5 (= a in Fig. 6F), longitudinal distance between tentorial pits and s5 about  $\frac{1}{8}$  distance between setae s5 and s6 (= b in Fig. 6F). Ventral apotome triangular, with anterior margin slightly

concave; paired setae s18 relatively small, located at level of posterior tip of ventral apotome (Fig. 6C). Eyes (Fig. 6D, 6E) black, each with two vertically oriented parts, larger part anterior of smaller part, both parts narrowly elliptical, together surrounded by pale yellow halo. Antennae (Figs. 6J, 20A) forked, each resembling two long finger-like

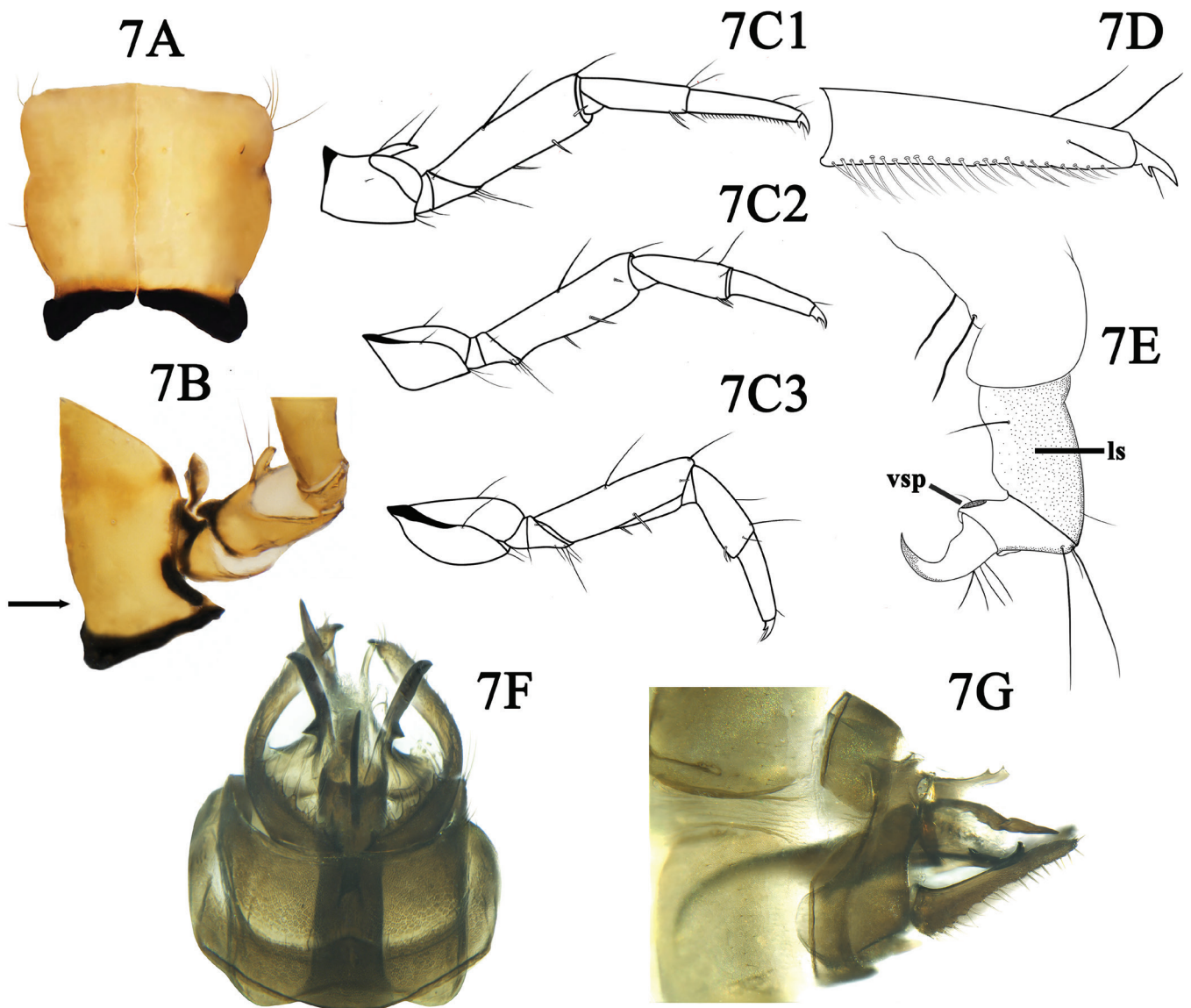


Fig. 7. *Chimarra akkaorum* Malicky & Chantaramongkol, 1989, larva. 7A, pronotum, dorsal; 7B, right side of prothorax with posterodorsal constriction (→), right foretrochantin, and right forecoxa, right lateral; 7C1, right foreleg, posterolateral; 7C2, right mid-leg, posterolateral; 7C3, right hind leg, posterolateral; 7D, tarsus of right foreleg, posterolateral; 7E, left anal proleg, left lateral; 7F, male genitalia, ventral; 7G, male genitalia, left lateral. ls = lateral sclerite; vsp = ventral sole plate.

projections that converge inward from the surface of the cuticle, accompanied by two short fine setae, located near mandibular base, antennal length about  $\frac{1}{2}$  and  $\frac{1}{4}$  length of setae s8 and s9, respectively. Labrum (Fig. 6G) white, membranous, and T-shaped when fully everted, transverse distal part rod-like, translucent, with dense setal fringe along anterior edge interrupted by narrow U-shaped notch mesally; anterolateral margins each with tuft of bristles; thick labral trunk constricted basolaterally. In ventral view (Fig. 6J), labium with submental sclerite (sm) semi-circular, each anterolateral angle with one strong bristle; mentum (me) membranous, trapezoidal; labium (lb) elliptical, palpiger (pg) arched, labial palpi (lp) each 2 segmented, first segment cylindrical, second one triangular. Maxillae (ma) fused with labium (Fig. 6J). Maxillary palpi (mp) each four segmented, segment 3 longest and segment 4 smallest, with 7 apical papilla rods; maxillary lobe (ml) bearing several fine bristles on inner margin and outer subapical margin with small curved bristle and three spine-like bristles. Each cardo (ca)

somewhat cup-like and partially covered by submentum, outer apical angle with one long seta, inner apical angle acute and produced mesad, contiguous with paired oblique sclerites of maxillary stipites (ms), palpifer (pf) large. Mandibles brown, about as dark as anterior portion of frontoclypeus. Left mandible (Fig. 6H1, 6I2) inner margin of basal region with short, broad, blunt projection (Fig. 6H1, arrow); middle region margin straight with seven tiny serrations; apical region with two small subapical teeth below large apical tooth. Right mandible (Fig. 6H2, 6I1) inner margin basal region with two small, blunt subbasal teeth and prominent triangular, basal projection (Fig. 6I1, arrow); middle region with eight tiny serrations on nearly straight edge and blunt or with small teeth apically; apical region with one small subapical tooth below large apical tooth.

**Thorax.** Pronotum subrectangular in dorsal view (Fig. 7A), strongly sclerotised, slightly constricted laterally  $\frac{1}{3}$  distance from anterior margins, more strongly constricted near base

laterally and continuing as shallow and transverse concavity dorsally (Fig. 7B, arrow), and with thick, black hind margin; in lateral view (Fig. 7B), on each side, pronotum with dark spot at convex anterolateral angle and broad dark band on posterior  $\frac{1}{2}$  of posterolateral margin extending from above pleural suture to hind margin, black anteriorly changing to dark brown near hind margin. Anterolateral margin of proepisternum, pleural suture and basodorsal part of forecoxa black, proepimeron triangular. Foretrochantins (Fig. 7B) small, leaf-like (foliaceous). Meso- and metanota membranous. Legs (Fig. 7C1–7C3) yellowish brown, with few scattered bristles on coxae, femora, and tibiae. Forelegs darker and slightly thicker than mid- and hind legs; each with coxa short, subrectangular in posterior view, with long black bristles and sclerotised tubular subapicodorsal process bearing long black bristle apically, mid region with one very short seta, apicoventral margin with one or two setae; trochanter 2-segmented, first segment trapezoidal with one apicoventral seta, second segment triangular with one short dorsal seta at apicodorsal seta, one long and three short apicoventral setae; femur with setae scattered proximal to middle and distal portions, dorsally with two long setae, mid seta about 1.6 times as long as distal one; femur and tibia each bearing two ventral spine-like setae; tibia with distal setae about  $\frac{1}{3}$  as long as tibia, tarsus with two subapicodorsal setae and one subapicolateral seta (Fig. 7C1, 7D), ventral margin with a row of about 22–23 small spine-like setae (Fig. 7D); claw with one thick basal process beside subapical seta; lengths of fore femur: tibia: tarsus = 1.7: 1.1: 1.0. Mid- and hind legs similar to forelegs in overall morphology, but coxae trapezoid in posterior view and without sclerotised seta-bearing process and tarsi without row of small spine-like setae on ventral edge; mid tibia with one middorsal seta half as long as single apicodorsal seta; hind tibia with two dorsal setae, mid-dorsal seta about half as long as distal one; lengths of femur: tibia: tarsus of mid- and hind legs = 1.8: 1.0: 1.0 and 2.0: 1.0: 1.0, respectively.

**Abdomen.** Abdominal segments I–IX white, membranous, with short setae scattered across surface, gills and lateral fringes absent; segment X with tuft of five finger-like gills. Paired long anal prolegs each with hooked anal claw apically (Fig. 7E), each proleg with basal membranous segment about as long as distal segment and with 2 subequally long basoventral setae; distal segment with lateral sclerite about twice as long as tall, with one seta close to ventral base of lateral sclerite, one fine subapicodorsal seta, two stout and subequally long apicodorsal setae about as long as lateral sclerite, and one fine apicodorsal seta about  $\frac{1}{5}$  as long as stout setae; ventral sole plate with one very short ventral seta; anal claw smooth, right angled, and with four fine dorsal setae about  $\frac{1}{3}$  as long as stout setae.

**Material examined.** Phetchabun Province, Nam Nao National Park: Yakruet stream, 16°44'19.7"N 101°34'26.4"E, 840 m a.s.l., 10 larvae, 23 March 2022 (THNHM); 20 larvae, 23 March 2022 (KKU-AIC); 12 larvae, 23 April 2022 (KKU-AIC); 3 larvae, 21 May 2022 (KKU-AIC); 20 larvae, 25 January 2023 (KKU-AIC); Phromleang stream, 16°38'24.2"N 101°34'52.9"E, 720 m a.s.l., 6 larvae, 23 March

2022 (KKU-AIC); 10 larvae, 23 April 2022 (KKU-AIC); 10 larvae, 25 January 2023 (KKU-AIC); Kanchanaburi Province, Thong Pha Phum District: Saphan Lao waterfall, 14°43'35"N 98°47'28"E, 510 m a.s.l., 1 larva, 10 November 2021 (KKU-AIC); 190 larvae, 4 March 2022 (KKU-AIC); all specimens leg. K. Rattanawilai.

*Chimarra khamuorum* Chantaramongkol & Malicky,  
1989  
(Figs. 8A–9E)

**Diagnosis.** The larva of *Chimarra khamuorum* resembles those of *C. akkaorum* and *C. okuihorum*. It can be distinguished from those of other described Thai *Chimarra* species by the oblong head capsule, antennae about half as long as setae s9, the posterodorsal margin of the pronotum with a shallow, narrow, transverse constriction at the moderately thick, black hind margin, and the length of the distal segment of the anal proleg about 1.5 times its width.

**Description.** Final larval instar (instar V) in alcohol with body length  $5.4 \pm 1.0$  mm (n = 10), membranous body white with sclerotised parts yellowish or reddish brown (Fig. 8A).

**Head.** Head capsule in dorsal and ventral views (Fig. 8B, 8C) oblong, almost parallel-sided, with smooth surfaces, 1.6 times as long as wide, tapering in posterior  $\frac{1}{3}$ , posterodorsal margin round with small mesal notch, posteroventral margin with deep V-shaped notch; in lateral view (Fig. 8D, 8E), slightly tapering anteriorly, black spot on notch of occipital foramen on each posterolateral margin. Frontoclypeal apotome (Fig. 8F) dark reddish brown anteriorly gradually transitioning to lighter reddish brown posteriorly; its anterior margin appearing as two asymmetrical, triangular projections separated by deep notch, with left projection shorter and shaped as equilateral triangle, right projection as right triangle, notch right-triangular with small bump in narrow base; each anterolateral margin with paired setae s1–s3, seta s3 thin and smallest; paired setae s4 closed to anterior edge of frontoclypeus, anterior of tentorial pits with long paired setae s5, transverse distance from s5 to tentorial pits about  $\frac{1}{6}$  distance between setae of s5, longitudinal distance between tentorial pits and s5 about  $\frac{1}{6}$  distance between setae s5 and s6 (Fig. 8F). Triangular ventral apotome with straight anterior edge and acute anterolateral angles; paired setae s18 relatively small, located at level of posterior tip of ventral apotome (Fig. 8C). Eyes (Fig. 8D, 8E) black, oval, each with lenses forming single cluster surrounded by pale yellow halo. Antennae (Fig. 8J) located near mandibular base, each consisting of two finger-like projections of equal length, accompanied by two short fine setae, antenna approximately  $\frac{1}{2}$  as long as setae s8 or s9. Labrum (Fig. 8G) white, membranous, and T-shaped when fully everted; transverse distal part rod-like, translucent, with dense setal fringe along anterior edge interrupted by small U-shaped notch mesally; anterolateral margins each with tuft of bristles; thick labral trunk constricted basolaterally. Labium in ventral view (Figs. 8J, 20B) with semicircular submental sclerite bearing one strong bristle on each anterolateral angle; mentum membranous and trapezoidal; labium elliptical,

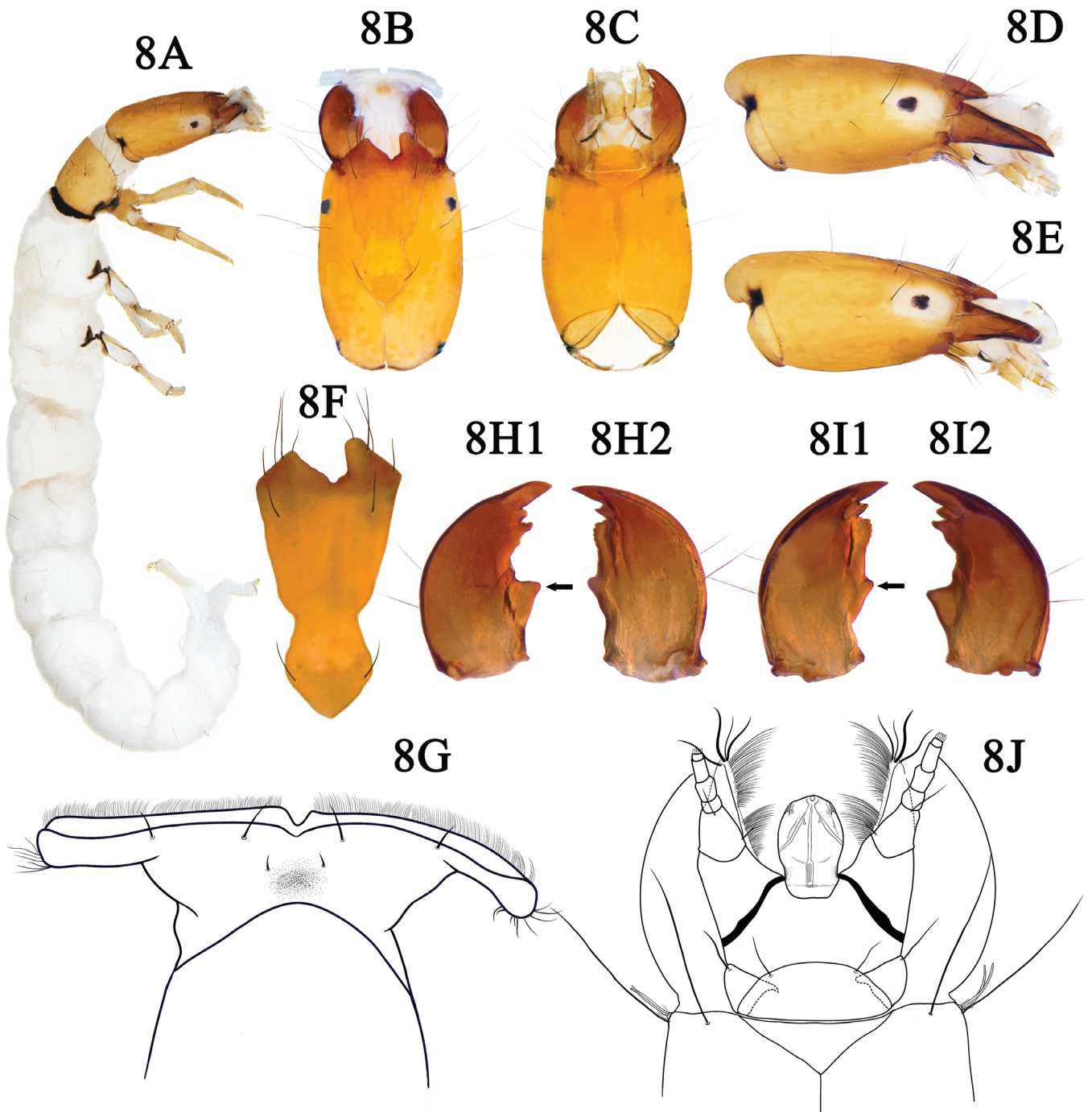


Fig. 8. *Chimarra khamuorum* Chantaramongkol & Malicky, 1989, larva. 8A, habitus, right lateral; 8B, head, dorsal; 8C, head, ventral; 8D, head, right lateral; 8E, head, left lateral (reversed); 8F, frontoclypeus, dorsal; 8G, Labrum with notch, dorsal; 8H1, left mandible with a prominent projection mesally(→), dorsal; 8H2, right mandible, dorsal; 8I1, right mandible with a small projection mesally (→), ventral; 8I2, left mandible, ventral; 8J, maxillae and labium, ventral.

palpiger sclerite arched, each labial palp 2-segmented, basal segment cylindrical, apical segment triangular. Maxillae fused with labium (Fig. 8J) with four segments of maxillary palp, segment 3 longest, segment 4 shortest, and with 7 papilla rods apically; maxillary lobe (Fig. 20C) bearing small curved bristle and three spine-like bristles on outer subapical margin and several fine bristles on inner margin. Submentum covering bases of cup-like cardines, each cardo with one long seta on outer apical angle, inner apical angles acute and produced mesad, adjacent to paired oblique sclerites of maxillary stipites; palpifer large. Mandibles brown, about

as dark as anterior portion of frontoclypeus. Left mandible (Fig. 8H1, 8I2) inner margin of basal region with prominent right-angle projection just below mid-length of mandible (Fig. 8H1, arrow); middle region margin deeply concave; apical region with two small subapical teeth below large apical tooth. Right mandible (Fig. 8H2, 8I1) inner margin basal region with small blunt projection just below mid-length of mandible (Fig. 8I1, arrow); middle region nearly straight with six tiny serrations; apical region with one small subapical tooth below large acute apical tooth.

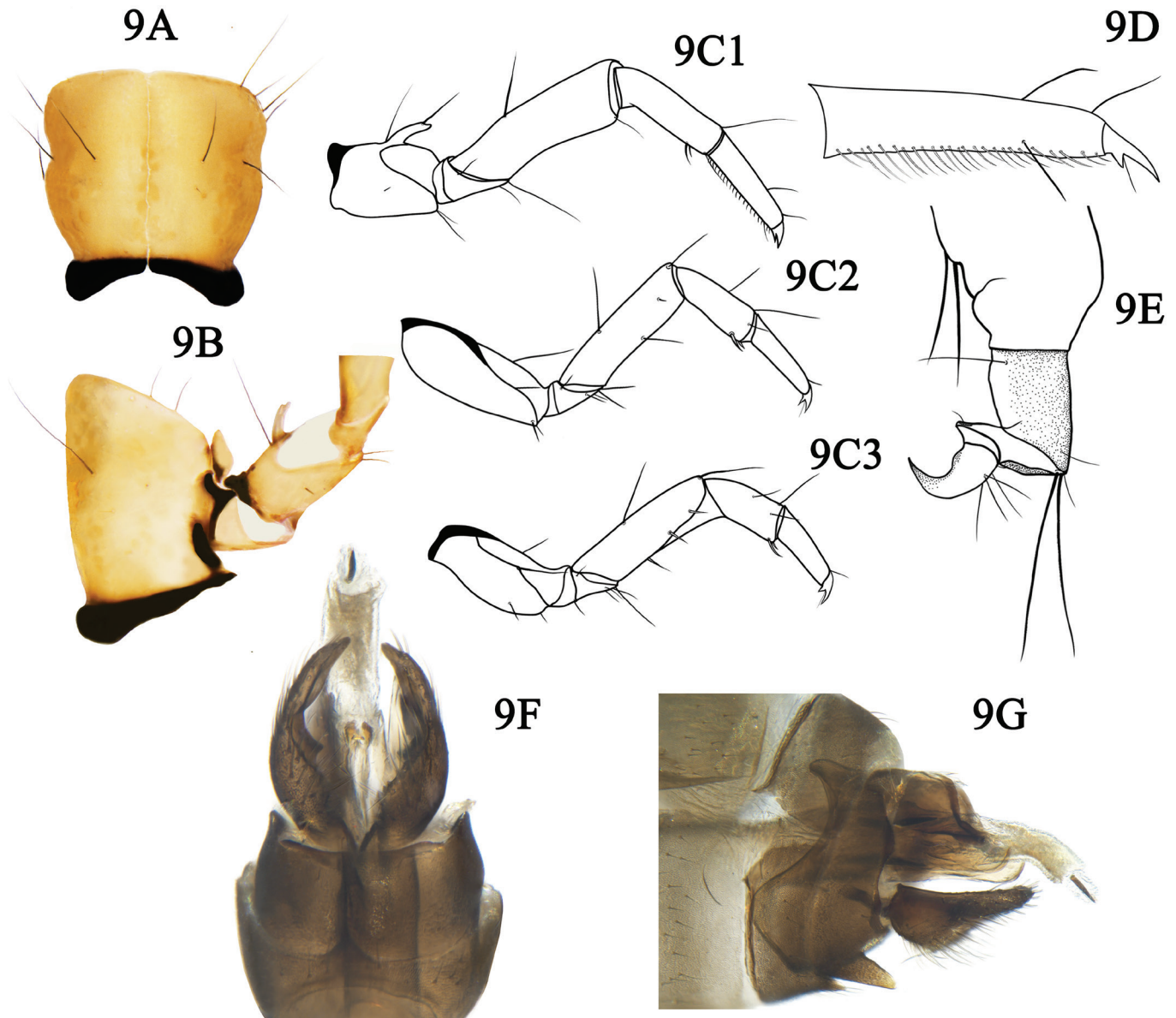


Fig. 9. *Chimarra khamuorum* Chantaramongkol & Malicky, 1989, larva. 9A, pronotum, dorsal; 9B, right side of prothorax, right foretrochantin, and right forecoxa, right lateral; 9C1, right foreleg, posterolateral; 9C2, right mid leg, posterolateral; 9C3, right hind leg, posterolateral; 9D, right tarsus of foreleg, posterolateral; 9E, left anal proleg, left lateral; 9F, male genitalia, ventral; 9G, male genitalia, left lateral.

**Thorax.** Pronotum (Fig. 9A) subrectangular in dorsal view, strongly sclerotised, shallowly constricted laterally at  $\frac{1}{3}$  distance from anterior margins and with deeper lateral constrictions on leading edge of thick, black hind margin, the latter continuing as transverse groove over dorsum; in lateral view (Fig. 9B), each side lacking anterolateral dark spot, with broad black band on posterior  $\frac{1}{2}$  of posterolateral margin extending from above pleural suture to hind margin. Anterolateral margin of proepisternum, pleural suture, and basodorsal part of forecoxa black, proepimeron triangular. Foretrochantins (Fig. 9B) small, leaf-like (foliaceous). Meso- and metanota membranous. Legs (Fig. 9C1–9C3) yellowish brown, with few scattered bristles on coxae, femora, and tibiae. Forelegs darker and slightly thicker than mid- and hind legs; each with coxa in posterior view subrectangular and short, with very short seta at mid region, one or two setae at apicoventral margin, and sclerotised tubular subapicodorsal process bearing long black basodorsal bristle and another

long black bristle subapicodorsally; trochanter 2-segmented, trapezoidal first segment with one apicoventral seta, second segment triangular with short apicodorsal seta, one long and three short apicoventral setae; femur with setae scattered on proximal to middle and distal portion, with two subequally long dorsal setae at midlength and distally; ventral spine-like setae on femur and tibia; setae scattered on distal part of tibia; tarsus with two subapicodorsal setae and one subapicoventral seta (Fig. 9C1), ventral margin with row of about 20–25 small spine-like setae (Fig. 9D); claw with one thick basal process beside subbasal seta; length of fore femur: tibia: tarsus = 1.9: 1.2: 1.0. Mid- and hind legs similar to forelegs in overall morphology, but each with coxa trapezoid in posterior view, sclerotised seta-bearing tubular process absent and without row of small spine-like setae on ventral edge. Moreover, mid-femur with two subequally long dorsal setae at middle and apex, one midventral seta and one short seta on posterior face near distal end; mid-tibia dorsal edge

with one mid-dorsal seta and apicodorsal seta twice as long as middorsal seta; midtarsus with small apicodorsal seta. Hind femora resembling midfemora and each femur bearing three ventral spine-like setae, mid- and hind tibia similar; length of femur: tibia: tarsus of mid- and hind legs = 1.8: 1.0: 1.0 and 2.0: 1.1: 1.0, respectively.

**Abdomen.** Abdominal segments I–IX white, membranous, with short setae scattered across surface, gills and lateral fringes absent; segment X with tuft of five finger-like gills. Paired long anal prolegs right-angle, each with lateral sclerite and hooked anal claw, basal membranous segment of anal proleg about as long as distal segment and with 2 subequally long basoventral setae; distal segment with lateral sclerite about twice as long as tall, one basoventral seta on lateral sclerite, one fine subapicodorsal seta with length subequal to width of distal segment; one fine apicodorsal seta about as long as subapicodorsal seta and two stout apicodorsal setae longer than distal segment; ventral sole plate with one very short ventral seta; anal claw smooth, right angled, and with three fine dorsal setae about  $\frac{1}{3}$  as long as stout setae (Fig. 9E).

**Material examined.** Phetchabun Province, Nam Nao National Park: Yakruae stream, 16°44'19.7"N 101°34'26.4"E, 840 m a.s.l., 10 larvae, 23 March 2022 (THNHM); 39 larvae, 23 March 2022 (KKU-AIC); 31 larvae, 23 April 2022 (KKU-AIC); 8 larvae, 21 May 2022 (KKU-AIC); 11 larvae, 15 December 2022 (KKU-AIC); 350 larvae, 25 January 2023 (KKU-AIC); Phromleang stream, 16°38'24.2"N 101°34'52.9"E, 720 m a.s.l., 5 larvae, 23 March 2022 (KKU-AIC); 2 larvae, 23 April 2022 (KKU-AIC); 20 larvae, 25 January 2023 (KKU-AIC); Kanchanaburi Province, Thong Pha Phum District: Saphan Lao waterfall, 14°43'35"N 98°47'28"E, 510 m a.s.l., 66 larvae, 4 March 2022 (KKU-AIC); stream near the Dr. Phol Kleepbua Mine, 1 larva, 4 March 2022 (KKU-AIC); all of specimens leg. K. Rattanawilai.

### *Chimarra okuihorum* Mey, 1998

(Figs. 10A–11E)

**Diagnosis.** The larva of *Chimarra okuihorum* is similar to those of *C. akkaorum* and *C. khamuorum*, but it differs from those described for other Thai *Chimarra* species by a combination of: (1) antennae about  $\frac{1}{3}$  as long as setae s8, (2) submentum trapezoidal, (3) basal region of inner margin of right mandible with a broad and apically round projection, and (4) posterodorsal margin of pronotum not constricted in lateral view.

**Description.** Final larval instar (instar V) in alcohol. Mean body length  $4.5 \pm 0.4$  mm ( $n = 5$ ), membranous body in alcohol white with sclerotised parts yellowish brown (Fig. 10A).

**Head.** Head capsule oblong, convex laterally, about 1.5 times as long as wide, in dorsal and ventral views, posterodorsal margin round with small mesal notch, posteroventral margin with deep V-shaped notch (Fig. 10B, 10C); in lateral view

(Fig. 10D, 10E), dorsal and ventral surfaces slightly convex and slightly tapering anteriorly, with smooth surfaces, black spot on notch of occipital foramen on each posterolateral margin. Frontoclypeal apotome dark reddish brown in anterior  $\frac{1}{4}$ , abruptly lighter posteriorly, its anterior margin wide, with deep notch asymmetrically slightly right of midline almost parallel-sided, having small bump on right side of notch base and smaller bump near bottom of left edge of notch; each anterolateral margin with setae s1–s3, seta s3 smallest; paired setae s4 closed to anterior edge of frontoclypeus, anterior of tentorial pits with long paired setae s5, transverse distance from setae s5 to tentorial pits about  $\frac{1}{6}$  distance between setae of s5, longitudinal distance between tentorial pits and setae s5 about  $\frac{1}{6}$  distance between setae s5 and s6 (Fig. 10F). Ventral apotome triangular, with anterior margin straight and with each anterolateral angle slightly produced, paired setae s18 relatively small, located at level of posterior angle of ventral apotome (Fig. 10C). Eyes (Fig. 10D, 10E) black, each with both parts oval, each surrounded by pale yellow halo. Antennae (Fig. 10J) each supporting two long finger-like projections and with two fine setae, located at mandibular base, antennae about  $\frac{1}{3}$  as long as setae s8 or s9. Labrum (Fig. 10G) T-shaped when fully everted; white, membranous; transverse distal part rod-like, translucent, its anterior margin notched mesally and with dense setal fringe; each anterolateral margin with tuft of bristles; thick labral trunk constricted basolaterally. Labium in ventral view; consisting of trapezoid submentum with one long black seta at each anterolateral angle (Fig. 10J); labium elliptical, palpiger sclerite arched, labial palpi each two-segmented (Fig. 20D) with first segment cylindrical and second one triangular; mentum membranous. Maxillae combined with labium (Fig. 10J). Maxillary palpi each four-segmented, segment 3 longest and segment 4 shortest with 8 apical sensory papillae (Fig. 20E); maxillary lobe bearing row of several fine bristles on inner margin and small curved bristle and one spine-like bristle on outer subapical margin. Cardine cup-like, partially covered basally by submentum, apical angles acute and produced slightly mesad and each with single subapicolateral seta, contiguous with paired oblique sclerites of maxillary stipites, palpifers large. Mandibles brown, slightly darker than most of head, each with two black setae on convex outer margin. Left mandible (Fig. 10H1, 10I2) inner margin having large, acute apical tooth and two small sharp subapical teeth; middle region with four tiny serrations, basal region with broad, apically truncate projection below middle (Fig. 10H1, arrow). Right mandible (Fig. 10H2, 10I1) inner margin of basal region having broad, apically truncate projection and small, blunt subapical tooth; middle region without serrations and broad, apically round projection subbasally (Fig. 10I1, arrow).

**Thorax.** Pronotum subrectangular in dorsal view (Fig. 11A), strongly sclerotised, with conspicuous lateral constriction  $\frac{1}{3}$  distance from anterior margin and immediately before anterior edge of thick, black hind margin, with neither constriction evident dorsally (Fig. 11B); in lateral view, on each side, pronotum lacking dark spot anterolaterally, posterolateral margin with faded black band above pleural suture changing to light brown posteriorly or not reaching hind margin of

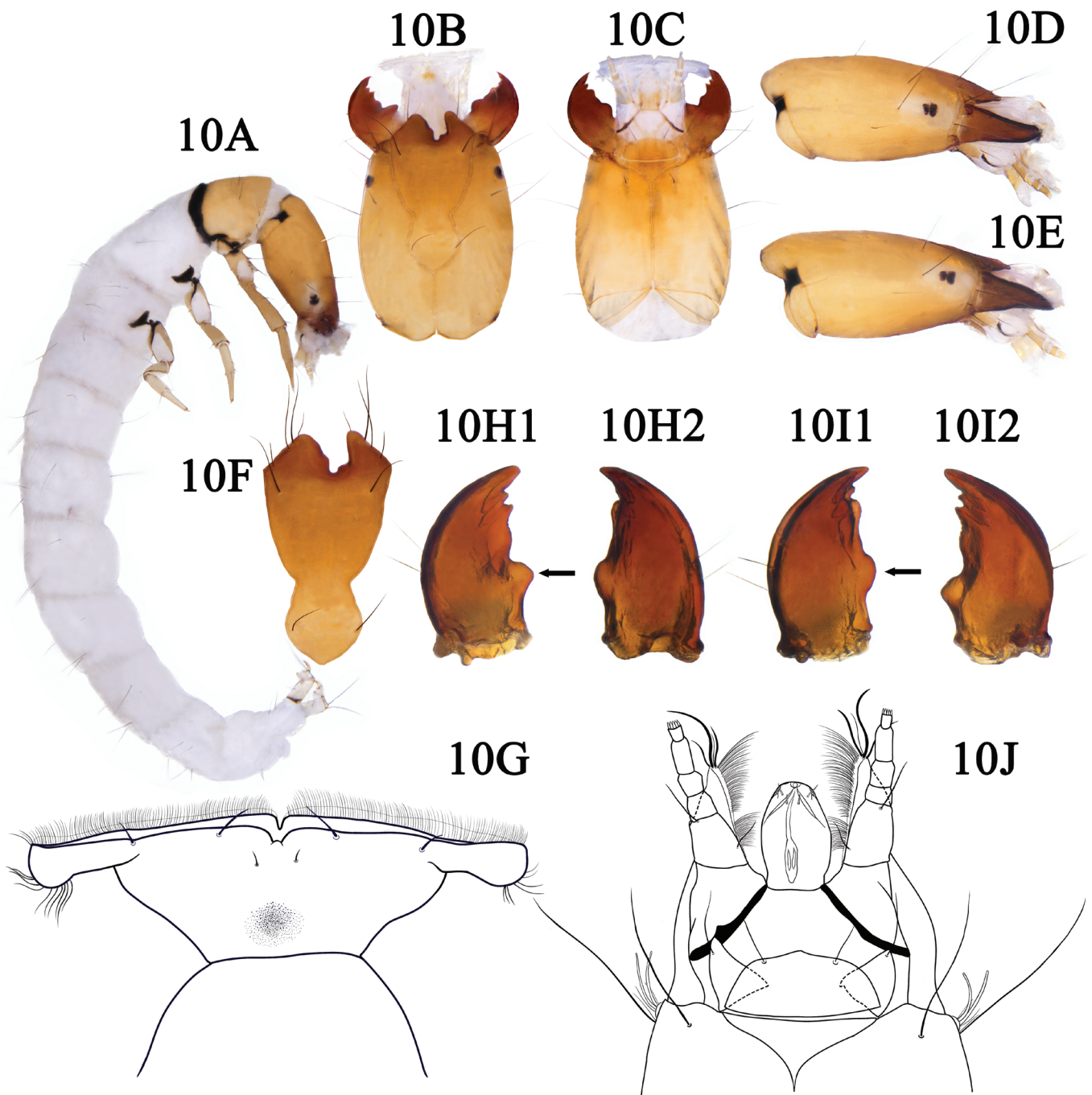


Fig. 10. *Chimarra okuihorum* Mey, 1998, larva. 10A, habitus, right lateral; 10B, head, dorsal; 10C, head, ventral; 10D, head, right lateral; 10E, head, left lateral (reversed); 10F, frontoclypeus, dorsal; 10G, labrum with notch, dorsal; 10H1, left mandible with broad apically truncate basomesal projection (→), dorsal; 10H2, right mandible, dorsal; 10I1, right mandible with broad apically truncate basomesal projection (→), ventral; 10I2, left mandible, ventral; 10J, maxillae and labium, ventral.

pronotum. Anterolateral edge of proepisternum, pleural suture, and basodorsal angle of forecoxa black, proepimeron triangular. Foretrochantins (Fig. 11B) small, leaf-like (foliaceous). Meso- and metanota membranous. Legs (Fig. 11C1–11C3) yellowish brown, with few scattered bristles on coxae, femora, and tibiae. Forelegs slightly thicker and darker than mid- and hind legs; each with coxa short, subrectangular in posterior view, with long black bristles and subapicodorsal sclerotised tubular process with long black apical bristle, mid region with one very short seta, apicoventral margin with one or two setae; trochanter 2-segmented, first segment trapezoidal with one apicoventral seta, second segment

triangular with short apicodorsal seta and one long and three short apicoventral setae; femur with setae scattered on proximal to middle and distal portions, dorsally with two setae of subequal length about midlength and apically; femur and tibia each with two spine-like setae ventrally; tibia with setae scattered on distal part; tarsus subapically with two subapicodorsal setae and a subapicoventral seta (Fig. 11C1), ventral margin with row of about 20–21 small spine-like setae (Fig. 11D); claw with one thick basal process beside subbasal seta; length of femur: tibia: tarsus = 1.7: 1.0: 1.0. Mid- and hind legs similar to forelegs in overall morphology, but each with coxae trapezoidal in posterior

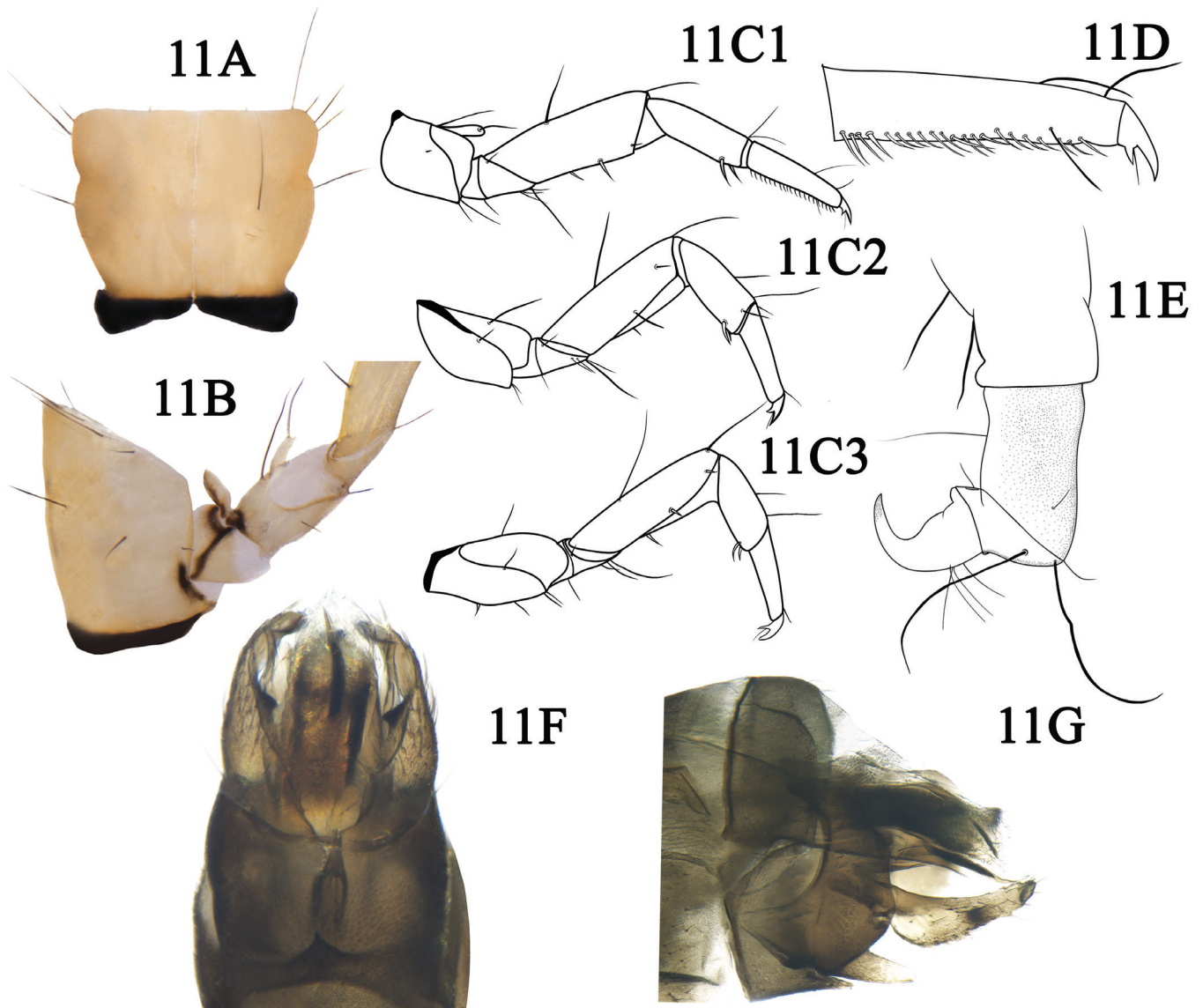


Fig. 11. *Chimarra okuihorum* Mey, 1998, larva. 11A, pronotum, dorsal; 11B, right side of pronotum, right foretrochantin, and right forecoxa, right lateral; 11C1, right foreleg, posterolateral; 11C2, right mid leg, posterolateral; 11C3, right hind leg, posterolateral; 11D, right tarsus of foreleg, posterolateral; 11E, left anal proleg, left lateral; 11F, male genitalia, ventral; 11G, male genitalia, left lateral.

view and lacking both sclerotised tubular seta-bearing process and row of small spine-like setae on ventral edge; moreover, mid-leg with two midventral setae and one subapical seta, tibia with one mid-dorsal seta and one subapicodorsal seta twice as long as middorsal seta; hind leg similar to mid leg but with three ventral spine-like setae on femur; length of femur: tibia: tarsus of mid- and hind legs = 1.8: 1.0: 1.0 and 1.9: 1.0: 1.0, respectively.

**Abdomen.** Abdominal segments I–IX white, membranous, with short setae scattered across surface, gills and lateral fringes absent; segment X with tuft of five finger-like gills; paired long anal prolegs twice as long as wide (Fig. 11D), each with membranous basal segment, right-angled distal segment, and hooked anal claw; basal membranous segment of anal proleg about as long as distal segment and with 2 basoventral setae subequally long; distal segment with one basoventral seta on lateral sclerite, one fine subapicodorsal seta and one fine apicodorsal about equally long, and two stout apicodorsal setae about as long as distal segment; ventral

sole plate with one very short seta; anal claw smooth, right angled, and with four dorsal setae about  $\frac{1}{4}$  as long as stout apicodorsal seta.

**Material examined.** Phetchabun Province, Nam Nao National Park: Phromleang stream,  $16^{\circ}38'24.2''\text{N}$   $101^{\circ}34'52.9''\text{E}$ , 720 m a.s.l., 2 larvae, 25 January 2023 (THNHM); 5 larvae, 25 January 2023 (KKU-AIC), all specimens leg. K. Rattanawilai.

#### *Chimarra tsudai* group

**Diagnosis.** Adult male genitalia of *Chimarra tsudai* group and the *digitata* group bears characteristics of male genitalia with tergum X divided and widely separated mesally into paired, sclerotised lateral lobes. The mesal and lateral lobes of the *tsudai* group have many sensilla while *C. digitata* group has two sensilla on lateral lobe. The *tsudai* group shares the characteristic with the *minuta* group of the anal veins of the forewing lacking an apical fork, thus 2A and 3A both loop to 1A. The *tsudai* group is distinguished from the



Fig. 12. *Chimarra usal* Malicky & Chantaramongkol, 2009, wings. 12A, forewing; 12B, hind wing.

*digitata* and the *tsudai* groups by having unsclerotised dorsal segment IX. The mesal pair of segment X is membranous basally and forms upright projections straddling the phallic apparatus. A photograph of the male genitalia of *Chimarra usal* is shown in Fig. 14F, 14G. Forewings each with curved Rs vein toward anal margin and non-sinuously at inflection point, discoidal cell with swollen basal fork and about three times as long as wide, pigmented s cross-vein, m cross-vein proximal to s and r-m cross-veins, nonlinear s and r-m cross-veins; hind wings each with unfused Sc and R1.

The wing venation pattern of *Chimarra usal* (Fig. 12) indicates *C. usal*'s inclusion in the *tsudai* group.

***Chimarra usal* Malicky & Chantaramongkol, 2009**  
(Figs. 13A–14E)

**Diagnosis.** The larva of *C. usal* is similar to those of *C. akkaorum*, *C. khamuorum*, and *C. okuihorum* by the deep notch on the anterior margin of the frontoclypeal apotome and the tentorial pit being far posterior of setae s5. In addition, *C. usal* shares characters with *C. bimbltona* and *C. moira* by the lack of an anteromesal notch on the T-shape labrum. The larva of *C. usal* can be distinguished from those of the other described Thai *Chimarra* species by a combination of: (1) clearly visible setae s18 located far posterior of the ventral apotome about  $\frac{1}{5}$  the length of the ecdysial line, (2) tentorial pits located longitudinally from its seta s5 about  $\frac{1}{14}$  of distance between setae s5 and s6, (3) the pronotum with a deep transverse posterior constriction, and (4) the curved row of 26–28 small spine-like setae on the posteroventro margin of each foretarsus.

**Description.** Final larval instar (instar V) in alcohol. Mean body length  $6.7 \pm 0.5$  mm ( $n = 5$ ), membranous body in alcohol white with head and pronotum yellowish brown (Fig. 13A).

**Head.** Head capsule elongate-oblong, nearly parallel-sided and tapering in posterior third, about  $\frac{2}{3}$  as wide as long, posterior margin round and anterior margin wider than posterior margin in dorsal and ventral views (Fig. 13B, 13C); in lateral view (Fig. 13D, 13E), dorsal and ventral surfaces slightly convex and slightly tapering anteriorly,

with smooth surfaces, black spot on notch of occipital foramen on each posterolateral margin. Anterior margin of frontoclypeal apotome with wide asymmetrical notch with sinuate base separating right and left projections; its right projection short and with mesal edge almost perpendicular to base and left projection longer, its mesal edge angled  $\sim 45^\circ$ ; anterolateral margin with paired setae s1–s3, seta s3 smallest (Fig. 20G); paired setae s4 closed to anterior edge of frontoclypeus, anterior of tentorial pits with long paired setae s5, transverse distance from s5 tentorial pits about  $\frac{1}{5}$  distance between setae of s5, longitudinal distance between tentorial pits and s5 about  $\frac{1}{14}$  distance between setae s5 and s6 (Figs. 20F). Ventral apotome triangular, with anterior margin slightly concave; paired setae s18 clearly visible and about as long as apotome length, situated anteriorly approximately  $\frac{1}{5}$  distance between ventral apotome and occipital foramen (Fig. 13C). Eyes black, each undivided and circular, surrounded by paler yellow halo. Antennae (Fig. 13J) unusual, located near mandibular base, consisting of two long finger-like projections, accompanied by two short fine setae, antenna length approximately  $\frac{1}{3}$  or  $\frac{1}{4}$  length of setae s8 or s9, respectively. Labrum (Fig. 13G) T-shaped, translucent, white, and membranous; transverse distal section rod-like and its anterior margin with dense setae; bristle fringe along anterior edge without anteromesal notch. Submentum semicircular, each anterolateral angle with one long black seta (Fig. 13J); mentum not sclerotised; labium elliptical, palpifers arched; labial palpi each 2 segmented, with cylindrical basal segment and triangular apical segment. Maxillae fused with labium (Fig. 13J) and with four-segmented maxillary palp, segment 3 longest and segment 4 shortest, with 7 papilla rods apically; maxillary lobe supporting several fine bristles on inner margin and outer subapical margin with small curved bristle. and one spine-like bristle. Cardines somewhat cup-like with their bases covered by submentum, each outer apical angle with one long seta, inner apical angles blunt and produced somewhat mesad, adjacent to paired oblique maxillary stipites, palpifers large. Mandibles brown, slightly darker than head, each with two black setae on convex outer margin. Left mandible (Fig. 13H1, 13I2) inner margin with three apical teeth; first large acute tooth at apex, two subapical teeth and no projection mesally. Right mandible (Fig. 13H2, 13I1) with large acute apical tooth and inner margin with two subapical teeth and deep, V-shaped subbasal concavity.

**Thorax.** Pronotum subrectangular in dorsal view (Fig. 14A), strongly sclerotised, moderately constricted laterally  $\frac{1}{3}$  distance from anterior margins, more strongly constricted near base laterally and continuing as deep transverse concavity dorsally immediately anterior of thick, black hind margin; in lateral view deep transverse concavity especially apparent (Fig. 14B, arrow); on each side, pronotum with dark brown spot on anterolateral angle, posterolateral margin with black mark above pleural suture changing to light brown posteriorly or not reaching hind margin of pronotum. Anterolateral margin of proepisterna, pleural suture, and basodorsal part of forecoxa black, proepimeron triangular. Foretrochantins (Fig. 14B) small, leaf-like (foliaceous). Meso- and metanota membranous. Legs (Fig. 14C1–14C3) yellowish brown, with few scattered bristles on coxae, femora, and tibiae. Forelegs

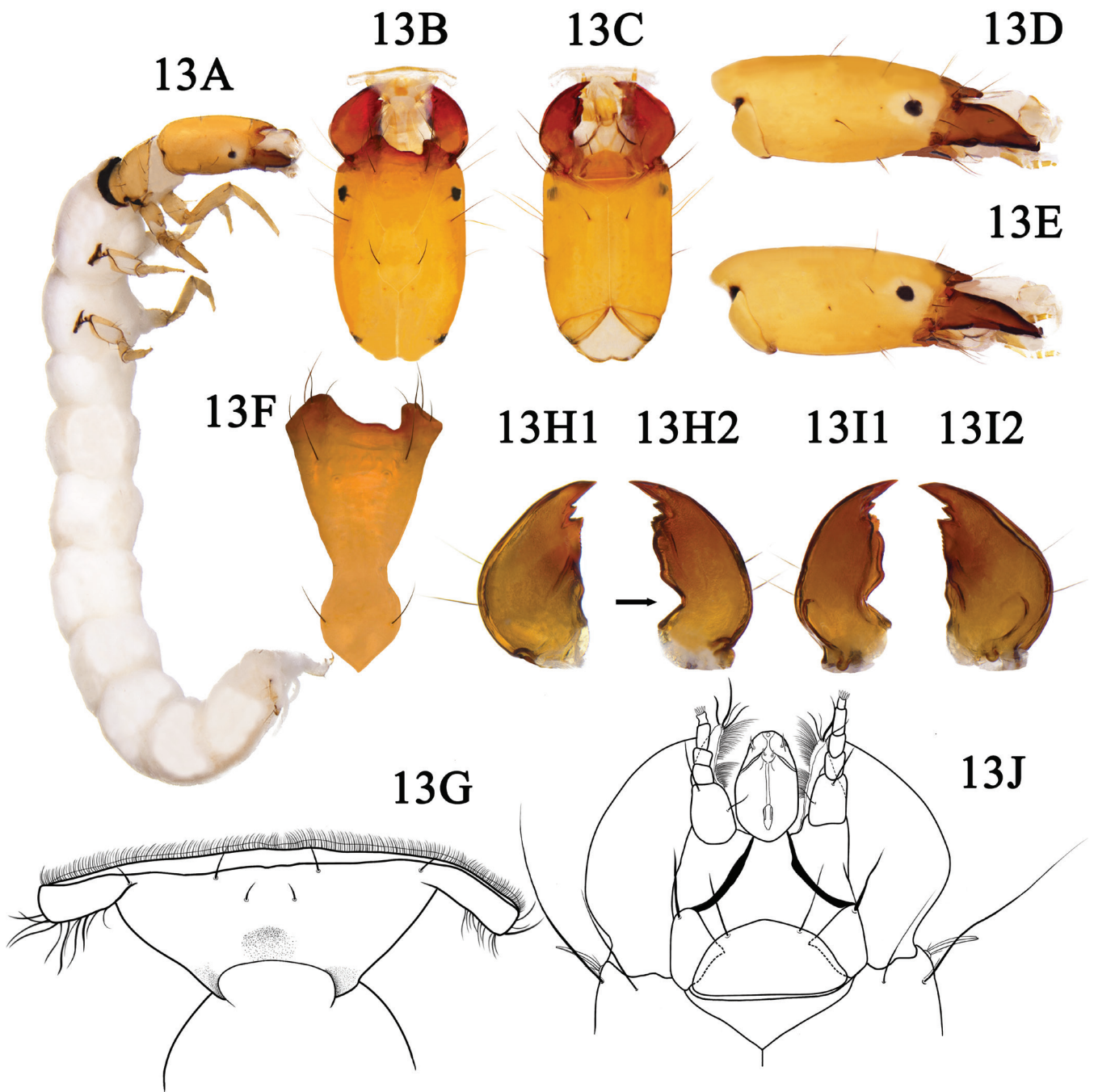


Fig. 13. *Chimarra usal* Malicky & Chantaramongkol, 2009, larva. 13A, habitus, right lateral; 13B, head, dorsal; 13C, head, ventral; 13D, head, right lateral; 13E, head, left lateral (reversed); 13F, frontoclypeus, dorsal; 13G, labrum, dorsal; 13H1, left mandible, dorsal; 13H2, right mandible with deep concavity mesally (→), dorsal; 13I1, right mandible, ventral; 13I2, left mandible, ventral; 13J, maxilla and labium, ventral.

darker and slightly thicker than mid- and hind legs; each with coxa short, subrectangular in posterior view, with long black bristles and sclerotised tubular process bearing long black bristle dorsally, one very short seta on mid region, apicoventral margin bearing one or two setae; trochanter 2-segmented, first segment trapezoidal with one apicoventral seta, second segment triangular with one short apicodorsal seta, apicoventral end with three short and one long setae; femur with setae scattered at proximal to middle and distal portions; with two long dorsal setae, middorsal seta 1.2 times longer than apicodorsal seta, two ventral spine-like setae on each femur and tibia; tibia with setae scattered over distal half; tarsus subapically with two dorsal setae and

one ventral seta (Fig. 14C1), ventral margin with curved row of about 26–28 small spine-like setae (Fig. 14D); claw with one thick basal process beside subbasal seta; length of foreleg femur: tibia: tarsus = 1.8: 1.0: 1.0. Mid- and hind legs similar to forelegs in overall morphology, but each with coxa trapezoidal in posterior view and both sclerotised tubular apicodorsal seta-bearing process and ventral row of small spine-like setae absent; moreover, mid-leg and hind leg each with two ventral spine-like setae and one subapical spine-like seta; mid-femur with one middorsal seta 1.4 times as long as one distal seta, mid-tibia with one middorsal seta half as long as apicodorsal seta; hind femur dorsally with one mid-dorsal seta and one distal seta of subequal length

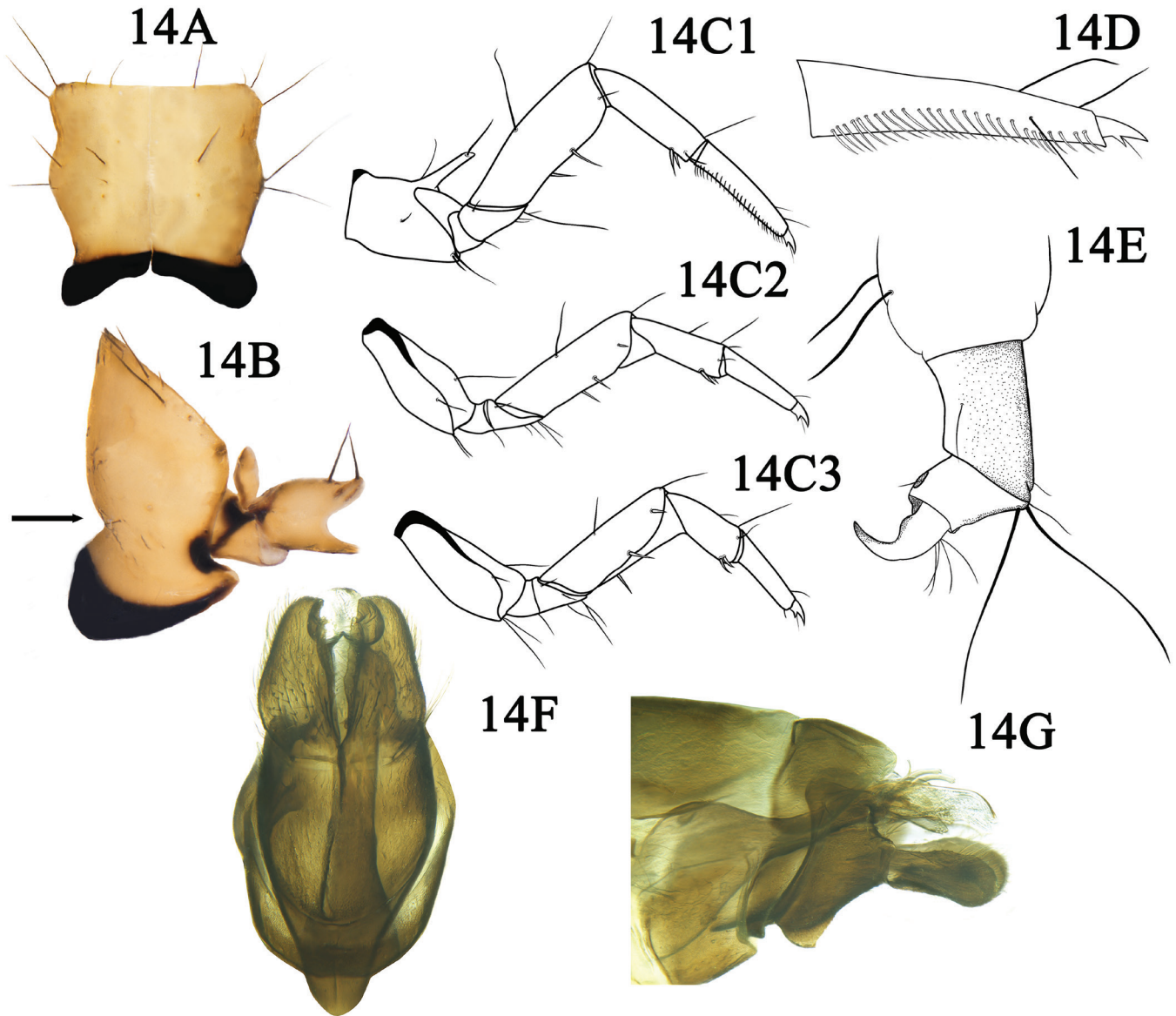


Fig. 14. *Chimarra usal* Malicky & Chantaramongkol, 2009, larva. 14A, pronotum, dorsal; 14B, right side of pronotum with marked posterior constriction (→), right foretrochantin, and right forecoxa, right dorsolateral; 14C1, right foreleg, posterolateral; 14C2, right mid leg, posterolateral; 14C3, right hind leg, posterolateral; 14D, right tarsus of foreleg, posterolateral; 14E, anal proleg, left lateral; 14F, male genitalia, ventral; 14G, male genitalia, left lateral.

and three ventral spine-like setae, hind tibia dorsally with one middorsal seta and one distal seta about  $\frac{3}{4}$  tibial length; length of femur: tibia: tarsus of mid- and hind legs = 1.8: 1.0: 1.0 and 1.9: 1.1: 1.0, respectively.

**Abdomen.** Abdominal segments I–IX white, membranous, with short setae scattered across surface, gills and lateral fringes absent; segment X with tuft of five finger-like gills; paired long anal prolegs each two-segmented, with membranous basal segment and right-angled distal segment and hooked anal claw; membranous basal segment about as long as distal segment and with 2 basoventral setae; distal segment about twice as long as wide, with lateral sclerite having one basoventral seta, one short and fine subapicodorsal seta, one fine apicodorsal seta twice as long as subapicodorsal seta and two stout apicodorsal setae as long as distal segment; ventral sole plate with one very short



Fig. 15. *Chimarra moira* Malicky & Prommi, 2004, wings. 15A, forewing; 15B, hind wing.

seta; anal claw smooth, right angled, and with four dorsal setae about  $\frac{1}{4}$  as long as stout apicodorsal seta (Fig. 14E).

**Material examined.** Kanchanaburi Province, Thong Pha Phum District: Saphan Lao waterfall, 14°43'35"N 98°47'28"E, 510 m a.s.l., 1 larva, 10 November 2021 (KKU-AIC); 10 larvae, 4 March 2022 (THNHM); 83 larvae, 4 March 2022 (KKU-AIC); all of specimen leg. K. Rattanawilai.

### *Chimarra minuta* group

**Diagnosis.** The *Chimarra minuta* group is separated from the *digitata* and the *tsudai* groups by the combination of characteristics of the male genitalia with segment IX anterodorsally without distal apodemes; membranous mesal lobe of tergum X relatively short, sometimes lightly sclerotised, weakly divided apically or not, without sensilla; endotheca with 2 endothecal spines, symmetrical in size and position, membrane not noticeably 'textured' with papillae or small spines. Forewing without a pronounced sinuous inflection of Rs vein, enlarged vein at the base of the discoidal cell, discoidal cell about three times as long as wide, m cross-vein usually proximal to s and r-m cross-veins, hyaline and linear s and r-m cross-veins; hind wing, with fused Sc and R veins.

*Chimarra bimbltona* has been included in the *Chimarra minuta* group (Blahnik et al., 2009). The wing pattern of *Chimarra moira* (Fig. 15) indicates *C. moira*'s inclusion in the *minuta* group. Photographs of the male genitalia of *Chimarra bimbltona* and *C. moira* are shown in Fig. 17F, 17G and Fig. 19F, 19G, respectively.

### *Chimarra bimbltona* Malicky, 1979

(Figs. 16A–17E)

**Diagnosis.** The larva of *C. bimbltona* is similar to that of *C. moira* in the shallow notch on the anterior of the frontoclypeal apotome, the elliptical submentum, the location of the paired setae s18 far behind the ventral apotome (at approximately  $\frac{1}{8}$  the length of the ventral ecdysial line), and tentorial pits positioned close to setae s5. However, the left mandible middle region having eight tiny serrations, the inner margin of the basal region of the right mandible having a broad obtuse ridge subbasally and a blunt mesal projection at mid-length, and transverse distance from s5 to tentorial pit about  $\frac{1}{8}$  distance between setae of s5, longitudinal distance between tentorial pits and s5 about  $\frac{1}{2}$  distance between setae s5 and s6 distinguishes *C. bimbltona* from other described Thai *Chimarra* species.

**Description.** Final larval instar (instar V) in alcohol. Mean body length  $4.8 \pm 0.4$  mm ( $n = 2$ ), membranous body white with sclerotised parts yellowish or reddish brown (Fig. 16A).

**Head.** Head capsule in dorsal and ventral views (Fig. 16B, 16C) somewhat elongate-oblong, almost parallel-sided, 1.9 times as long as wide, posterodorsal margin round with small mesal notch, posteroventral margin with deep V-shaped notch; in lateral view (Fig. 16D, 16E) slightly

tapering anteriorly with smooth surfaces, black spot on notch of occipital foramen at each posterolateral margin. Frontoclypeal apotome (Fig. 16F) with anterior margin dark brown in colour and gradually lighter posteriorly, its anterior margin appearing as two asymmetrical triangular projections on either side of very shallow notch asymmetrically right of midline, left projection much longer and broader than right projection; anterolateral margin with setae s1–s3, setae s3 smallest; paired setae s4 closed to anterior edge of frontoclypeus, anterior of tentorial pits with long paired setae s5, transverse distance from s5 tentorial pits about  $\frac{1}{8}$  distance between setae of s5, longitudinal distance between tentorial pits and s5 about  $\frac{1}{2}$  distance between setae s5 and s6 (Figs. 16F). Triangular anterior ventral apotome with straight anterior margin, each anterolateral angle slightly produced; paired setae s18 relatively small, located anteriorly approximately  $\frac{1}{8}$  length of ecdysial line and separated by  $\frac{1}{3}$  of head width (Fig. 16C). Eyes (Fig. 16D, 16E) black, each with two vertically oriented parts; larger part anterior to smaller part, both parts narrowly elliptical, together surrounded by pale yellow halo. Antennae (Fig. 16J) unusual, each bearing of two long finger-like projections with two short fine setae, located near its mandibular base, antennal length approximately  $\frac{1}{3}$  length of both setae s8 or s9. Labrum (Fig. 16G) white, membranous, and T-shaped when fully everted; transverse distal part rod-like, translucent, with dense setal fringe along anterior edge not interrupted mesally by notch; anterolateral margins each with tuft of bristles; thick labral trunk constricted basolaterally. Labium in ventral view consisting of elliptical submental sclerite with each anterolateral angle having one strong bristle (Fig. 16J); mentum not sclerotised; labium elliptical, palpifers arched, palpi two-segmented with basal segment cylindrical and apical segment triangular. Maxillae fused with labium (Fig. 16J). Maxillary palpi each four-segmented, segment 3 longest and segment 4 shortest with 7 sensory papillae apically; maxillary lobe with several fine bristles on inner margin and small curved bristle and one spine-like bristle on outer subapical margin. Cardines with submentum covering their bases, cardines cup-like, each with one long seta on outer apical angle, inner apical angles round and produced slightly mesad, contiguous with paired oblique sclerites of maxillary stipites, palpifers large. Mandibles brown, about as dark as anterior portion of frontoclypeus. Left mandible (Fig. 16H1, 16I2) with inner margin of basal region having small, triangular projection near mid-length of mandible; middle region with eight tiny serrations; apical region having two small subapical teeth below large apical tooth. Right mandible (Fig. 16H2, 16I1) inner margin basal region with broadly obtuse ridge sub-basally and blunt mesal projection at mid-length of mandible (Fig. 16I1, arrow); middle region with seven tiny serrations on nearly straight margin; apical region having one subapical tooth below large apical tooth.

**Thorax.** Pronotum subrectangular in dorsal view (Fig. 17A), strongly sclerotised, moderately constricted laterally  $\frac{1}{3}$  distance from anterior margin and immediately anterior base, this latter constriction continuing dorsally as shallow, narrow transverse groove immediately before thick, black hind margin (Fig. 17B); in lateral view (Fig. 17B), on each

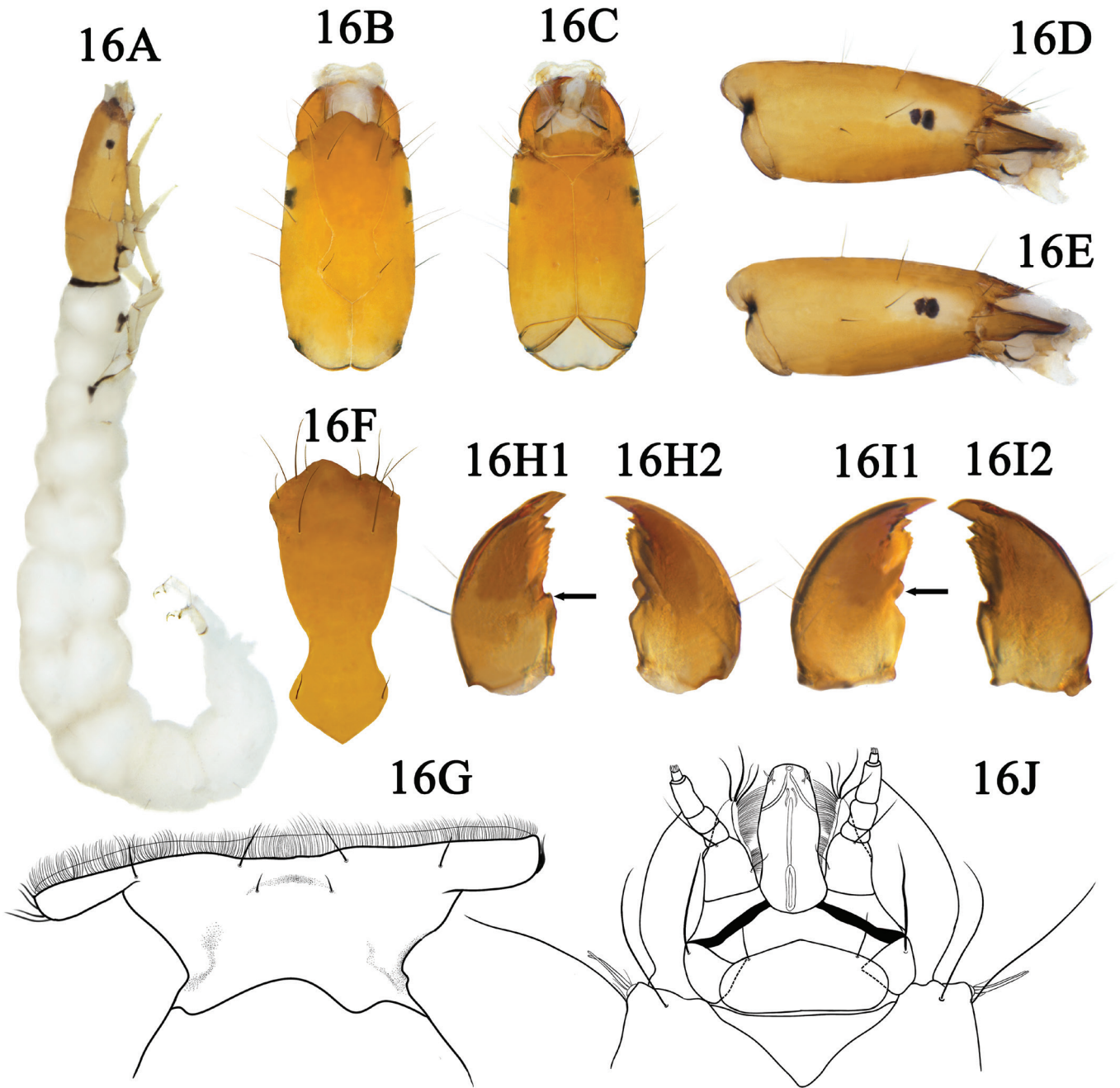


Fig. 16. *Chimarra bimbltona* Malicky, 1979, larva. 16A, habitus, right lateral; 16B, head, dorsal; 16C, head, ventral; 16D, head, right lateral; 16E, head, left lateral (reverse); 16F, frontoclypeus, dorsal; 16G, labrum, dorsal; 16H1, left mandible with small blunt basomesal projection (→), dorsal; 16H2, right mandible, dorsal; 16I1, right mandible with shallow concavity proximal of blunt basomesal projection (→), ventral; 16I2, left mandible, ventral; 16J, maxillae and labium, ventral.

side, pronotum lacking dark spot anterolaterally, with broad dark band on posterior  $\frac{1}{2}$  of posterolateral margin extending from above pleural suture to hind margin, black anteriorly changing to dark brown near hind margin. Anterolateral margin of proepisternum, pleural suture, and basodorsal part of forecoxa black, proepimeron triangular. Foretrochantins (Fig. 17B) each thicker basally, angled dorsad and slender in apical half. Meso- and metanota membranous. Legs (Fig. 17C1–17C3) yellowish brown, with few scattered bristles on coxae, femora, and tibiae. Forelegs slightly thicker and darker than mid- and hind legs; coxae short, subrectangular in posterior view, each with long black bristles and sclerotised tubular apicodorsal process bearing long black bristle, one

very short seta at mid region, and with one or two setae at apicoventral margin; trochanters each 2-segmented, first segment trapezoidal with one apicoventral seta, second segment triangular with one short apicodorsal end seta, with one long and three short apicoventral setae; femora with setae scattered at proximal to middle and distal portions, dorsally with two long setae, first seta at approximately  $\frac{1}{3}$  length of femur and about 1.5 times longer than a apicodorsal seta; femur and tibia with two spine-like seta ventrally and one short seta at distal end; tibia with setae scattered on distal part, with long seta about half as long as tibia; tarsus with two subapicodorsal setae and one subapicalventral seta (Fig. 17C1), ventral margin with row of about 17 small spine-

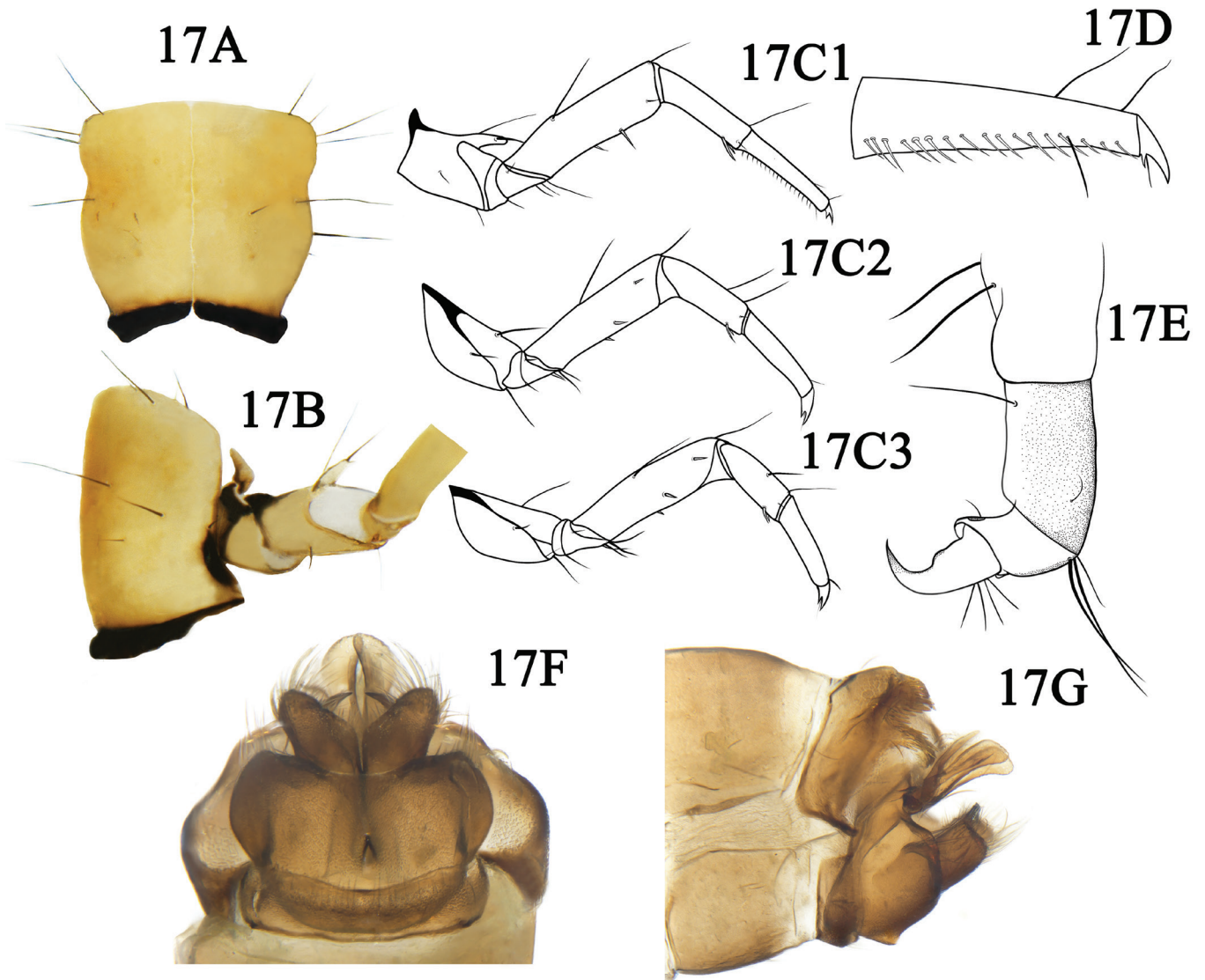


Fig. 17. *Chimarra bimbltona* Malicky, 1979, larva. 17A, pronotum, dorsal; 17B, right side of prothorax, right foretrochantin, and right forecoxa, right lateral; 17C1, right foreleg, posterolateral; 17C2, right mid leg, posterolateral; 17C3, right hind leg, posterolateral; 17D, right tarsus of foreleg, posterolateral; 17E, left anal proleg, left lateral, left lateral; 7F, male genitalia, ventral; 7G, male genitalia, left lateral.

like setae (Fig. 17D); claw with one thick basal process beside subbasal seta; length of foreleg femur: tibia: tarsus = 1.7: 1.0: 1.0. Mid- and hind legs similar to forelegs in overall morphology, but coxae trapezoid in posterior view and without sclerotised tubular apicodorsal seta-bearing process or row of small spine-like setae on ventral edge; moreover, mid leg and hind leg each with femur having two dorsal setae at midlength and distally subequally long, two short ventral spine-like setae and one short subapical spine-like seta; mid-tibia dorsally with two subequal length setae at about  $\frac{3}{4}$  of tibial length and apically, two ventral spine-like setae; hind tibia with two dorsal setae, subapical seta about half as long as distal seta; lengths of femur: tibia: tarsus of mid- and hind legs = 1.6: 1.0: 1.0 and 1.9: 1.0: 1.6, respectively.

**Abdomen.** Abdominal segments I–IX white, membranous, with short setae scattered across surface, gills and lateral

fringes absent; segment X with tuft of five finger-like gills; paired long anal prolegs each with membranous segment, right-angled distal segment, and hooked anal claw, basal membranous segment about as long as distal segment and with 2 basoventral setae; distal segment with one basoventral seta on its lateral sclerite, one subapicodorsal seta; one fine short seta and two stout and long apicodorsal setae; ventral sole plate with one very short seta; anal claw smooth, right angled, and with four dorsal setae about  $\frac{1}{3}$  as long as stout apicodorsal setae; lateral sclerite of distal segment twice as long as tall; anal prolegs more than twice as long as wide (Fig. 17E).

**Material examined.** Phetchabun Province, Nam Nao National Park: Yakruae streams, 16°44'19.7"N 101°34'26.4"E, 840 m a.s.l., 2 larvae, 16 February 2022 (THNHM); 1 larva, 16 February 2022 (KKU-AIC); all specimens leg. K. Rattanawilai.

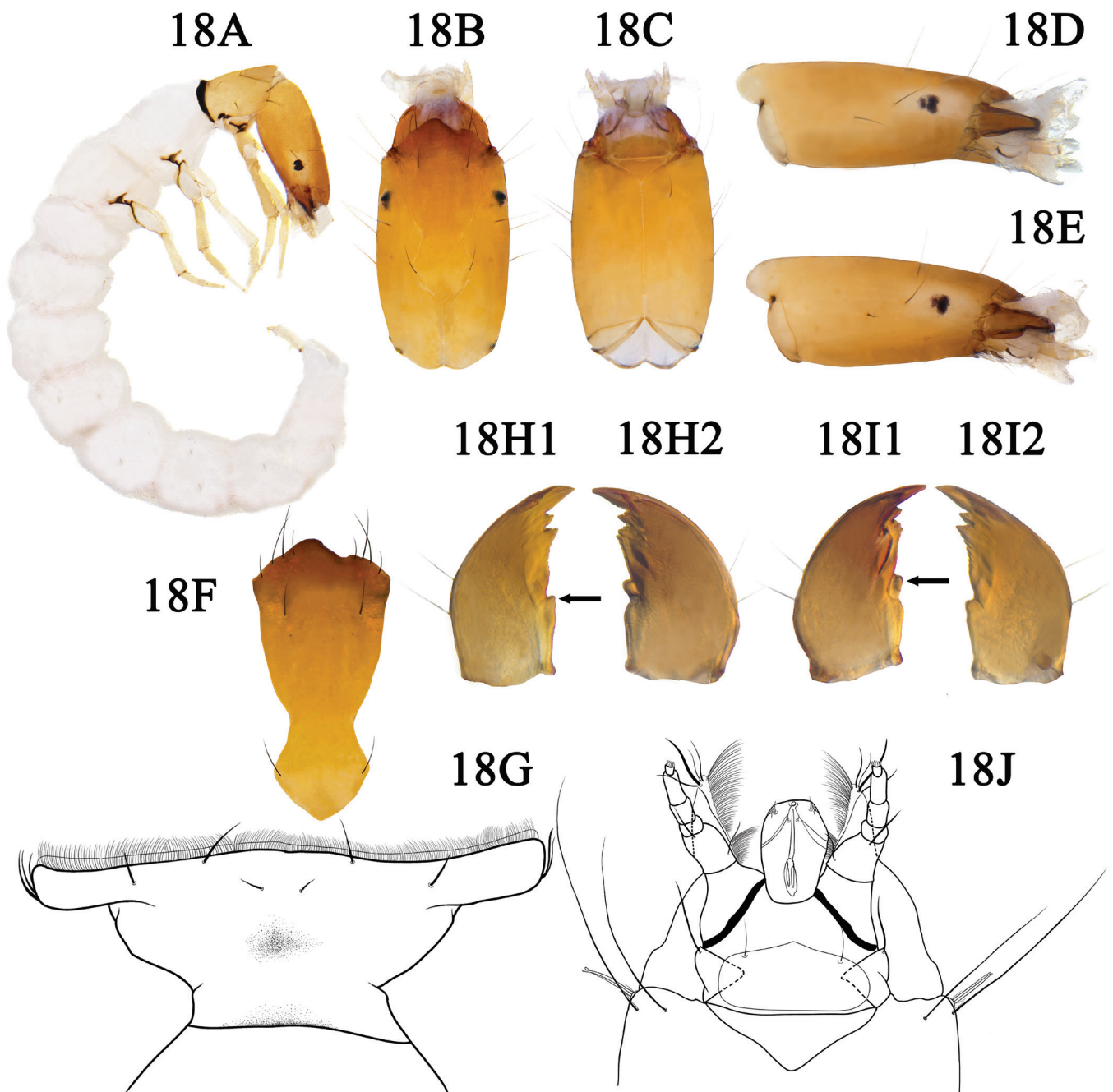


Fig. 18. *Chimarra moira* Malicky & Prommi, 2004, larva. 18A, habitus, right lateral; 18B, head, dorsal; 18C, head, ventral; 18D, head, right lateral; 18E, head, left lateral (reversed); 18F, frontoclypeus, dorsal; 18G, labrum, dorsal; 18H1, left mandible with small basomesal projection (→), dorsal; 18H2, right mandible, dorsal; 18I1, right mandible with shallow concavity just beneath blunt basomesal projection (→), ventral; 18I2, left mandible, ventral; 18J, maxillae and labium, ventral.

***Chimarra moira* Malicky & Prommi, 2004**  
(Figs. 18A–19E)

**Diagnosis.** The larva of *Chimarra moira* is very similar to that of *C. bimbltona*. It differs from those of other described Thai *Chimarra* species by the left mandible's middle region with seven tiny serrations, the inner margin of the basal region of right of the mandible with a shallow concavity basally adjacent to a small blunt projection, and transverse distance from s5 to tentorial pits about  $\frac{1}{20}$  of distance between setae of s5 and longitudinal distance between tentorial pits and s5 about  $\frac{1}{10}$  distance between setae s5 and s6.

**Description.** Final larval instar (instar V) in alcohol. Mean body length  $3.9 \pm 0.4$  mm ( $n = 5$ ), membranous body white with sclerotised parts yellowish or reddish brown (Fig. 18A).

**Head.** Head capsule in dorsal and ventral views (Fig. 18B, 18C) elongate-oblong, nearly parallel-sided, with slight taper posteriorly and 1.9 times as long as wide, with smooth surfaces, having posterodorsal margin round with small mesal notch (Fig. 18B), posteroventral margin with deep V-shaped notch; in lateral view (Fig. 18D, 18E) slightly tapered anteriorly, black spot on notch of occipital foramen on each posterolateral margin. Frontoclypeal apotome (Fig. 18F) with anterior  $\frac{1}{5}$  dark and somewhat abruptly lighter

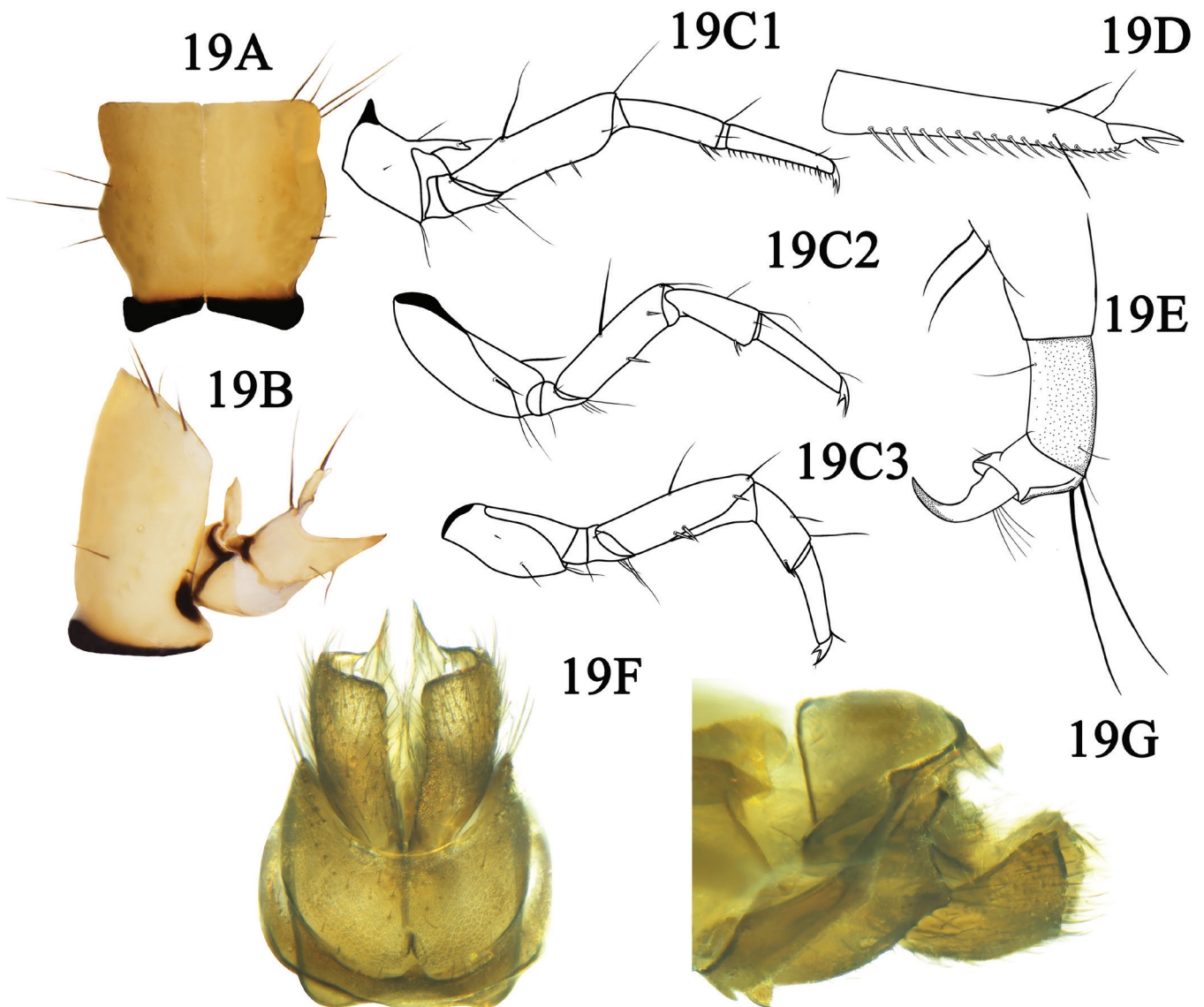


Fig. 19. *Chimarra moira* Malicky & Prommi, 2004, larva. 19A, pronotum, dorsal; 19B, right side of prothorax, right foretrochantin, and right forecoxa, right lateral; 19C1, right foreleg, posterolateral; 19C2, right mid leg, posterolateral; 19C3, right hind leg, posterolateral; 19D, right tarsus of foreleg, posterolateral; 19E, left anal proleg, left lateral; 19F, male genitalia, ventral; 19G, male genitalia, left lateral.

in posterior  $\frac{1}{5}$ , its anterior margin with very shallow notch right of midline, separating two triangular lobes, left lobe much longer and broader than right lobe; each anterolateral margin with setae s1–s3, seta s3 smallest; paired setae s4 closed to anterior edge of frontoclypeus, anterior of tentorial pits with long paired setae s5, transverse distance from s5 to tentorial pits about  $\frac{1}{20}$  distance between setae of s5, longitudinal distance between tentorial pits and s5 about  $\frac{1}{10}$  distance between setae s5 and s6 (Figs. 18F). Ventral apotome triangular, straight anterior margin, each anterolateral angle slightly produced; relatively small setae no. 18, situated anterior approximately  $\frac{1}{8}$  length of ventral ecdysial line and  $\frac{1}{3}$  of head width (Fig. 18C). Eyes (Fig. 18D, 18E) black, each with two vertically oriented parts, much larger oval part anterior of smaller, narrowly elliptical part: surrounded by pale yellow halo. Antennae (Fig. 18J) unusual, located near mandibular bases, consisting of two long finger-like projections accompanied by two short fine setae, antennal length about  $\frac{1}{3}$  length of setae s8 or s9.

Labrum (Fig. 18G) white, membranous, and T-shaped when fully everted; transverse distal part rod-like, translucent, with dense setal fringe along anterior edge continuous, not interrupted; anterolateral margins each with tuft of bristles; thick labral trunk constricted basolaterally. Labium in ventral view; consisting of elliptical submental sclerite with one long black seta at each anterolateral angle (Fig. 18J); mentum membranous; labium elliptical, palpifers apically arched, each labial palp two-segmented, first segment cylindrical, second segment triangular. Maxillae fused with labium (Fig. 18J); maxillary palpi each four segmented, segments 1 and 2 each shorter than segment 3 but longer than segment 4, with 8 papilla rods apically; inner margin of maxillary lobe bearing several fine bristles and with small curved bristle and one spine-like bristle on outer subapical margin. Cardines somewhat cup-like with their bases covered by submentum and each with one long seta on outer apical angle, their inner apical angles blunt and directed anterolaterad, adjacent to pair of oblique sclerites of maxillary stipites, palpifers

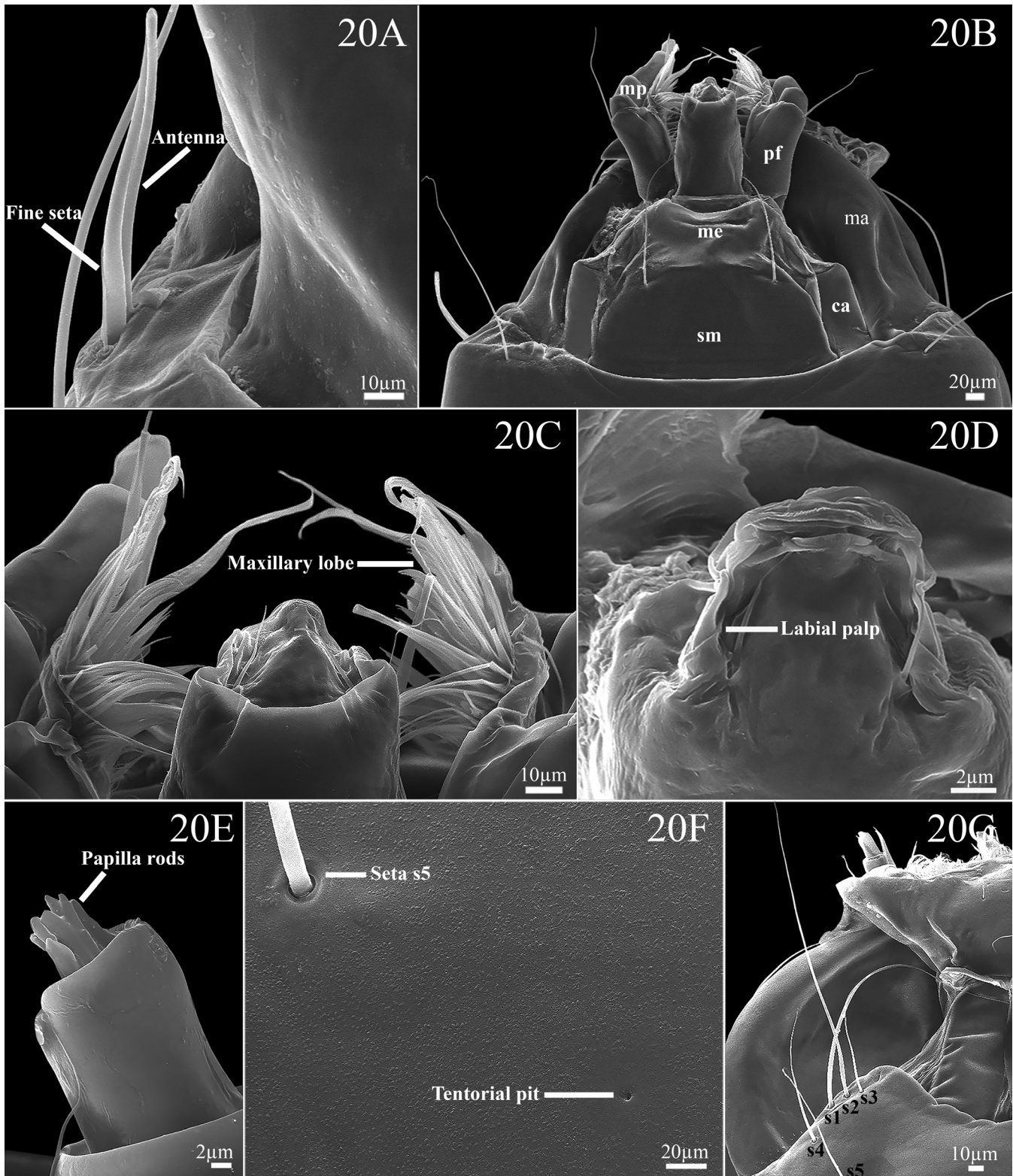


Fig. 20. SEM images of *Chimarra* characters. 20A, antenna and fine seta of *Chimarra akkaorum*; 20B, labium in ventral view of *C. khamuorum*; 20C, maxillary lobe of *C. khamuorum*; 20D, labial palp of *C. okuihorum*; 20E, labial papilla rods of *C. okuihorum*; 20F, seta s5 and tentorial pit of *C. usal*; 20G, left anterolateral margin with three setae (setae s1–s3), seta s4 and seta s5 of *C. usal*. ca = cardo, ma = mandible, me = mentum, mp = maxillary palp, pf = palpifer, sm = submental sclerite, s1 = seta s1, s2 = seta s2, s3 = seta s3, s4 = seta s4, s5 = seta s5.

large. Mandibles brown, about as dark as anterior portion of frontoclypeus. Left mandible (Fig. 18H1, 18I2) inner margin of basal region with small triangular projection (Fig. 18H1, arrow); middle region with seven tiny serrations; apical region having two small subapical teeth and large apical

tooth. Right mandible (Fig. 18H2, 18I1) inner margin basal region with shallow concavity basally adjacent to small blunt projection (Fig. 18I1, arrow); middle region with seven tiny serrations; apical region having one small subapical tooth below large acute apical tooth.

Table 5. Comparison of morphological characters of larvae of eight *Chimarra* species

Characters	<i>C. akkaorum</i>	<i>C. bimblitona</i>	<i>C. khamuorum</i>	<i>C. moira</i>	<i>C. okuihorum</i>	<i>C. paramonorum</i>	<i>C. sadayu</i>	<i>C. usal</i>
<b>Shape of head</b>	Oval with curved anterolateral margin and rounded posterior margin, length 1.5 times width	Elongate-oblong, length 1.9 times width	Oblong, length 1.6 times width	Elongate-oblong, length 2 times width	Oblong, length 1.5 times width	Elliptical, length 1.5 times width	Elongate-elliptical, length 2 times width, posterior margin rounded	Elongate-oblong, length 2 times width, posterior margin rounded and anterior margin wider than posterior margin
<b>Anterior margin of frontoclypeal apotome</b>	Deep, wide notch, without flat base, right side slightly larger than left side	Shallow notch, left lobe much larger than right lobe	Deep, wide notch, without flat base, left side equilateral triangle and acute apically, right side right triangle	Shallow notch, left lobe much larger than right lobe	Deep, wide, U-shaped notch without flat base, left side nearly equilateral triangle and blunt apically, right side right triangle	Deep, elongate, Ω-shaped	Shallow notch, Bilobed, left lobe with anterolateral margin equalling inner margin in length, while right lobe with anterolateral margin longer than inner margin	Deep, very wide notch with flat base, left side longer, obtusely angled, right side short, almost perpendicular to base
<b>Anteromesal notched on T-shaped labrum</b>	Present	Absent	Present	Absent	Present	Absent	Present	Absent
<b>Location of each tentorial pit</b>	Horizontally $\frac{1}{8}$ of distance between pair of setae s5 and longitudinally $\frac{1}{8}$ of distance between setae s5 and s6	Horizontally $\frac{1}{8}$ of distance between pair of setae s5 and longitudinally $\frac{1}{2}$ of distance between setae s5 and s6	Horizontally $\frac{1}{6}$ of distance between pair of setae s5 and longitudinally $\frac{1}{10}$ of distance between setae s5 and s6	Horizontally $\frac{1}{20}$ of distance between pair of setae s5 and longitudinally $\frac{1}{10}$ of distance between setae s5 and s6	Horizontally $\frac{1}{6}$ of distance between pair of setae s5 and longitudinally $\frac{1}{6}$ of distance between setae s5 and s6	Horizontally $\frac{1}{6}$ of distance between pair of setae s5 and longitudinally $\frac{1}{6}$ of distance between setae s5 and s6	Horizontally $\frac{1}{6}$ of distance between pair of setae s5 and longitudinally $\frac{1}{4}$ of distance between setae s5 and s6	Horizontally $\frac{1}{6}$ of distance between pair of setae s5 and longitudinally $\frac{1}{4}$ of distance between setae s5 and s6
<b>Length of antenna/length of seta s8</b>	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$
<b>Length of antenna/ length of seta s9</b>	$\frac{1}{4}$	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{4}$	$\frac{1}{4}$
<b>Submental sclerite</b>	Semicircular	Elliptical	Semicircular	Elliptical	Trapezoidal	Subrectangular	Trapezoidal	Semicircular

Characters	<i>C. akkaorum</i>	<i>C. bimbltona</i>	<i>C. khamuorum</i>	<i>C. moira</i>	<i>C. okaihorum</i>	<i>C. paramonorum</i>	<i>C. sadayu</i>	<i>C. usul</i>
Basal region of inner margin of right mandible	With prominent projection	With small projection	With prominent projection	With small projection	With broad, apically truncate projection	With prominent projection	With prominent projection	With deep concavity
Location of setae s18	At level of posterior point of ventral apotome	At ~ 1/3 distance of ventral ecdysial line	At level of posterior point of ventral apotome	At ~ 1/3 distance of ventral ecdysial line	At level of posterior point of ventral apotome			At ~ 1/3 distance of ventral ecdysial line
Shape of fore trochantin	Leaf-like	Slender	Leaf-like	Slender	Leaf-like	Leaf-like	Leaf-like	Slender
Posterodorsal margin of pronotum	Shallow, broad constriction before moderately thick, black hind margin	Shallow, narrow constriction at moderately thick, black hind margin	Shallow, narrow constriction at moderately thick, black hind margin	Shallow, narrow constriction at moderately thick, black hind margin	Without posterior constriction and thin, black hind margin	Shallow, narrow constriction at moderately thick, black hind margin	Deep, broad constriction before very thick, black hind margin	Deep, broad constriction before very thick, black hind margin
Location of middorsal seta of forefemur	At 1/2 of forefemur length	At 1/3 of forefemur length	At 1/2 of forefemur length	At 1/3 of forefemur length	At 1/2 of forefemur length			At 1/2 of forefemur length
Length of middorsal seta of forefemur/length of its distal seta	1.5	1.5	1	1	1			1.5
Arrangement of spine-like setae on ventro margin of foretarsus	Straight row	Straight row	Straight row	Straight row	Straight row			Curve row
Numbers of spine-like setae on foretarsus	22-23	17	20-25	18	20-21			26-28
Length of middorsal seta of mid femur/length of its apicodorsal seta	2	1	1	1.5	1			1.5
One midventral seta on mid femur	Absent	Absent	Present	Absent	Present			Absent
Location of dorsal seta of mid tibia	At 1/2 of tibial length	At 3/4 of tibial length	At 1/2 of tibial length	At 1/2 of tibial length	At 3/4 of tibial length			At 3/4 of tibial length

Characters	<i>C. akkaorum</i>	<i>C. bimbltona</i>	<i>C. khamtorum</i>	<i>C. moira</i>	<i>C. okuihorum</i>	<i>C. paramonorum</i>	<i>C. sadayu</i>	<i>C. usul</i>
Length of middorsal seta of hind femur/length of its apicodistal seta	1	1	1	1.3	1.3			1.3
Length of anal basoventral seta/height of lateral sclerite	1	1.5	1.5	1.5	1			1
Length of anal proleg stout setae/length of lateral sclerite	1	1	1.5	1.5	1			1.5
Anal proleg lateral sclerite length/height	2	2	1.5	2	2			1.5
References	This study	This study	This study	This study	This study	Hu et al. (2018)	Hu et al. (2020)	This study

**Thorax.** Pronotum subrectangular in dorsal view (Fig. 19A), strongly sclerotised, moderately constricted at  $\frac{1}{3}$  distance from anterior margins and deeper constrictions before leading edge of thick, black hind margin, this posterior constriction continuing transversely across dorsum as very shallow groove (Fig. 19B); in lateral view (Fig. 19B) on each side, pronotum lacking dark spot anterolaterally, with broad dark band on posterolateral margin above pleural suture but this band not continuing to hind margin. Anterolateral margin of proepisternum, pleural suture, and basodorsal part of forecoxa black; proepimeron triangular. Foretrochantins (Fig. 19B) slender, each parallel-sided in basal  $\frac{3}{4}$ , tapering distally to acute apex. Meso- and metanota membranous. Legs (Fig. 19C1–19C3) yellowish brown, with few scattered bristles on coxae, femora, and tibiae. Forelegs darker and slightly thicker than mid- and hind legs; coxae subrectangular in posterior view and short, each with long black bristles and sclerotised tubular apicodorsal process bearing long black bristle apically, mid region with one very short seta, apicoventral margin with one or two setae; trochanters each two-segmented, first segment trapezoidal with one apicoventral seta, triangular second segment with one short apicodorsal seta, apicoventrally with one long and three short setae; femora with setae scattered at proximal to middle and distal portions, dorsal edge with two long setae, proximal seta at about  $\frac{1}{3}$  length of femur about 1.2 times as long as apicodorsal seta; femur and tibia each with two short spine-like setae ventrally and one short seta at near distal end; tibia with setae scattered on distal part; tarsus subapically with two subapicodorsal setae and one subapicoventral seta (Fig. 19C1), its ventral margin with row of about 18 small spine-like setae (Fig. 19D); claw with thick subbasal process before subbasal seta; lengths of foreleg femur: tibia: tarsus = 1.6: 1.0: 1.0. Mid- and hind legs similar to forelegs in overall morphology, but coxae trapezoidal in posterior view, sclerotised tubular apicodorsal seta-bearing process absent and without row of small spine-like setae on ventral edge; moreover, femora of mid- and hind legs each with two ventral spine-like setae and one subapical spine-like seta; mid leg femur with two dorsal setae, mid dorsal seta 1.5 times as long as apicodorsal seta; tibia with apicodorsal seta about 1.5 times mid dorsal seta; lengths of femur: tibia: tarsus = 1.5: 1.0: 1.0; hind leg femur with mid dorsal seta about 1.3 times longer than apicodorsal seta, tibia with mid dorsal seta about  $\frac{2}{3}$  as long as apicodorsal seta; lengths of femur: tibia: tarsus = 1.8: 1.0: 1.0.

**Abdomen.** Abdominal segments I–IX white, membranous, with short setae scattered across surface, gills and lateral fringes absent; segment X with tuft of five finger-like gills; pair of long anal prolegs each with membranous basal segment, right-angled distal segment, and hooked anal claw; basal membranous segment about as long as distal segment with 2 basoventral setae; distal segment with one basoventral seta on its lateral sclerite, one fine short subapicodorsal seta, apicodorsally with one fine short seta and two stout setae as long as distal segment; ventral sole plate with one very short seta; anal claw smooth, right angled, and with four dorsal setae about  $\frac{1}{4}$  as long as stout apicodorsal setae; lateral sclerite on distal segment length twice as long as wide (Fig. 19E).

**Material examined.** Kanchanaburi Province, Thong Pha Phum District: Saphan Lao waterfall, 4°43'35"N 98°47'28"E, 510 m a.s.l., 10 larvae, 4 March 2022 (THNHM); 76 larvae, 4 March 2022 (KKU-AIC); Stream near the Dr. Phol Kleepbua Mine, 14°45'8"N 98°48'40"E, 660 m a.s.l., 1 larva, 10 November 2021 (KKU-AIC); all specimens leg. K. Rattanawilai.

**Key to known *Chimarra* larvae of Thailand**

1. Anterior margin of frontoclypeal apotome with shallow notch (Figs. 16F, 18F); T-shaped labrum without anteromesal notch (Figs. 16G, 18G); submental sclerite elliptical (Figs. 16J, 18J); basomesal region of inner margin of right mandible with broad basal ridge separated from small projection (Figs. 16I1 arrow, 18I1 arrow) by small excision; trochantins slender (Figs. 17B, 19B); forefemur with middorsal seta located at distance about 1/3 of femur length from base (Figs. 17C1, 19C1).....2
- Anterior margin of frontoclypeal apotome with deep notch (Figs. 6F, 8F, 10F, 13F); T-shaped labrum with (Figs. 6G, 8G, 10G) or without (Fig. 13G) anteromesal notch; submental sclerite semicircular (Figs. 6J, 8J, 13J) or trapezoidal (Fig. 10J); basomesal region of inner margin of right mandible with prominent projection (Fig. 8I1 arrow) or broad apically round projection (Fig. 10I1 arrow) or with deep cavity (Fig. 13H2 arrow); trochantins leaf-like (Figs. 7B, 9B, 11B, 14B); forefemur with middorsal seta located at distance about 1/2 of femur length (Figs. 7C1, 9C1, 11C1, 14C1).....3
2. Left mandible middle region with eight tiny serrations; right mandible with inner margin of basal region with broad obtuse sub-basal ridge separated from blunt mesal projection (Fig. 16H2, 16I1 arrow) by small excision; tentorial pits located horizontally from its seta s5 about 1/3 of distance between paired setae s5 and longitudinally from its seta s5 about 1/3 of distance between setae s5 and s6 (Fig. 16B, 16F); fore-femora each with middorsal seta about 1.5 times as long as distal seta (Fig. 17C1); meso-femora each with middorsal seta and apicodorsal seta subequal in length (Fig. 17C2); anal prolegs each with stout setae about as long as lateral sclerite (Fig. 17E).....*Chimarra bimbltona*
- Left mandible middle region with seven tiny serrations; right mandible with inner margin of basal region with narrow ridge separated from small blunt projection (Fig. 18H2, 18I1 arrow) by very shallow concavity; tentorial pits located horizontally very close to setae s5, about 1/20 of distance between paired setae s5 (Fig. 18B, 18F); fore-femora each with middorsal seta about as long as distal seta (Fig. 19C1); meso-femora each with middorsal seta about 1.5 times as long as distal seta (Fig. 19C2); anal prolegs each with stout setae about 1.5 times as long as lateral sclerite (Fig. 19E).....*Chimarra moira*
3. Membranous T-shaped labrum with anteromesal notch (Figs 6G, 8G, 10G); setae s18 short and located at level of tip of ventral apotome (Figs. 6C, 8C, 10C); row of small spine-like setae on fore tarsus straight (Figs. 7D, 9D, 11D).....4
- Membranous T-shaped labrum without anteromesal notch (Fig. 13G), setae s18 clearly visible and located far posterior of ventral apotome (Fig. 13C), curved row of small spine-like setae on foretarsus (Fig. 14D).....*Chimarra usal*
4. Submentum sclerite semicircular (Figs. 6J, 8J); basal region of inner margin of right mandible with prominent projection (Figs. 6I1, 8I1); antenna length about half length of seta s8 (Figs. 6J, 8J).....5
- Submentum sclerite trapezoidal (Fig 10J); basal region of inner margin of right mandible with broad, apically truncate projection (Fig. 10H2, 10I1 arrow), antenna about 1/3 as long as seta s8 (Fig. 10J).....*Chimarra okuihorum*

5. Transverse posterodorsal constriction of pronotum shallow, broad before moderately thick, black margin (Fig. 7B arrow); head capsule oval (Fig. 6B, 6C); antennae about 1/4 as long as setae s9 (Fig. 6J), fore-femora each with middorsal seta about 1.5 times as long as apicodorsal seta (Fig. 7C1); meso-femora each with middorsal seta about 2 times as long as apicodorsal seta (Fig. 7C2); anal proleg distal segment about 2 times as long as thick (Fig. 7E).....*Chimarra akkaorum*
- Transverse posterodorsal constriction of pronotum very shallow, narrow before moderately thick, black margin (Fig. 9B); head capsule oblong (Fig. 8B, 8C); antennae about 1/3 as long as setae s9 (Fig. 8J); fore-femora each with middorsal seta and apicodorsal seta subequally long (Fig. 9C1); meso-femora each with middorsal seta about apicodorsal seta subequally long (Fig. 9C2); anal proleg distal segment about 1.5 times as long as thick (Fig. 9E).....*Chimarra khamuorum*

**DISCUSSION**

**Molecular larva-adult association.** Results from our molecular data analysis added six new larva-male associations of *Chimarra* species and facilitated the morphological descriptions of the larvae, bringing the number of Oriental species for which philopotamid larvae have been described from four (Hu et al., 2018, 2020) to ten. This is the first time that philopotamid larvae have been described from Thailand, which are 5.88% and 9.23% of all known Thai philopotamid and *Chimarra* species, respectively. The six newly described larvae are *C. akkaorum*, *C. bimbltona*, *C. khamuorum*, *C. moira*, *C. okuihorum*, and *C. usal*.

The phylogram showed that the *C. chiangmaiensis* clade was not associated with any larval specimens, but sequences of three males of *C. chiangmaiensis* formed a monophyletic group with two sequences of adults of Zhou et al. (2016) from Lampang, Thailand, obtained from BOLD. More sampling in the future may capture larval stages of this species.

The gene trees resulting from our study, based on the COI fragment of 34 sequences of *Chimarra* larvae and adults with ML, BI and NJ showed six monophyletic clades with strong support (UFBoot = 99–100%, PP = 1, BS = 100%), while distance-based and tree-based species delimitation showed seven MOTUs, with high statistical support (Fig. 2). In this study, *Chimarra akkaorum*, *C. moira*, *C. okuihorum*, and *C. usal* had low intraspecific genetic distances of 0.0–0.9 (Table 4) as expected, less than the 2% general intraspecific threshold of COI in Philopotamidae (Hogg et al., 2009) and other Trichoptera (Hogg et al., 2009; Orfinger et al., 2022). Notably, however, we found intraspecific values for *C. bimbltona* and *C. khamuorum*, exceeding the general threshold at 2.2% and 6.4% respectively. This may not be surprising considering that intraspecific genetic distances up to a maximum of 3.08 % have been reported in *Ecnomus mammus* (Ecnomidae) from Thailand (Jaroenchaiwattanachote et al., 2025). The high intraspecific genetic distances in *C. khamuorum* suggest either the probable existence of cryptic species (Hogg et al., 2009) or the presence of different geographical populations within the same species (Ge et al., 2021). Acceptance thresholds could be higher for some species compared with

the general threshold because of genetic differentiation between populations in the latter situation. COI intraspecific distance thresholds greater than 5% have been used for species delimitation of some Trichoptera (e.g., *Pycnocentria evecta* (Conoesucidae): Hogg et al., 2009; *Cheumatopsyche campy* (Hydropsychidae): Zhou et al., 2010; *Eoneureclipsis* spp. (Psychomyiidae): Peng et al., 2023). Furthermore, in the family Polycentropodidae, genetic divergences based on COI of *Polycentropus rickeri* (4–5%) were more than twice the general threshold value of 2% within species belonging to the family (Orfinger et al., 2022).

**Taxonomy of *Chimarra* larvae.** The principal results of our study are the first descriptions of *Chimarra* larvae in Southeast Asia. Currently, larvae of eight *Chimarra* species are known from the Oriental region, including six species in our study and two Chinese species, *C. paramonorum* Hu, Wang, & Sun, 2018, and *C. sadayu* Malicky, 1993 (by Hu et al., 2020). The six larval species in this study could be differentiated into three groups based on their unique morphological characteristics. Further investigation of larvae of more species will improve confidence for diagnostic characteristics of each species and species group. Our findings of certain shared morphological characters within larval groups strongly support previous groupings based on adult *Chimarra*.

**Possible misidentifications of BOLD sequences.** Our molecular analyses indicated some potential misidentifications of species on BOLD. The nested position of the Vietnamese *C. khamuorum* (KM225403.1) within the *C. chiangmaiensis* clade (Fig. 2) in ML, BI and NJ trees with strong support suggests a very close relationship between these two species, and the pairwise genetic distances between them, 1.4–1.7, are less than the 5% ASAP-scored interspecific threshold and the 2% general intraspecific threshold (de Pietro et al., 1997; Hogg et al., 2009; Orfinger et al., 2022) suggesting that all of these specimens represent the same species. On the other hand, the *C. khamuorum* clade (sequences of the other three males and a larva of *C. khamuorum*) in this study was clearly separated from the *C. chiangmaiensis* clade. Therefore, it is likely that the specimen of *C. khamuorum* from Vietnam (KM225403.1) was misidentified as *C. khamuorum*, and is in fact *C. chiangmaiensis*.

A similar situation was observed between a Vietnamese specimen of *C. bimbltona* (KM225402.1) and the *C. pipake* clade. So, it is likely that these Vietnamese specimens of *C. bimbltona* are in fact *C. pipake*. Moreover, a Laotian specimen of *C. okuihorum* (KM225330.1) did not cluster with any clades, including *C. okuihorum*, suggesting that this so-called *C. okuihorum* (KM225330.1) specimen was likely also misidentified. The ambiguity of these sequences deposited in BOLD suggests that species identities of many sequences in BOLD are questionable and potentially require correction.

**Outlook.** While COI barcoding allows for faster and more accurate species identification, barcodes of many freshwater insects remain unknown and/or unavailable. Currently, COI

from only about 30% of known Trichoptera species are available in public databases such as BOLD (Zhou et al., 2016). In addition, larval and female populations of many freshwater insect species cannot be identified based on morphological characteristics alone. Female trichopterans are often overlooked due to difficulties in their identification, despite their often more abundant occurrences (than males) in traps and consequent easier detection. As with larva-male matching—which proved highly successful in this study—associating unknown females to known male species based on COI or other DNA barcodes will likely help improve taxonomic resolution of female trichopterans in this region. Barcoded specimens grouped into putative molecular species units can be examined to establish more taxonomically-informative morphological characters for species identification or diagnosis. The association between known male species and unidentified females or larvae based on DNA can thus greatly enhance taxonomic knowledge of female and larval stage of all freshwater insects including Trichoptera.

Climate change, pollution, and habitat degradation impact the biodiversity of freshwater insects globally. In Asia, rapid urbanisation, industry, mining, and increased hydroelectric dam construction have led to increased water pollution and habitat degradation, affecting both humans and aquatic life. Therefore, assessing the taxonomic and functional diversities of freshwater insects are crucial as these represent proxies of environmental condition and ecosystem health. Accurate identification of freshwater insects such as Trichoptera larvae not only facilitates studies of their life histories, biology, and ecology, but is also important for practical biomonitoring applications such as assessing water quality.

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## LITERATURE CITED

- Blahnik RJ, Arefina-Armitage TI & Armitage BJ (2012) The genus *Chimarra* Stephens (Trichoptera: Philopotamidae) in Vietnam. *Insecta Mundi*, 0229: 1–25.
- Blahnik RJ, Holzenthal RW & Huisman J (2009) *Chimarra* of Sabah and Sarawak, northern Borneo (Trichoptera: Philopotamidae). *Tijdschrift voor Entomologie*, 152(1): 109–166.

- de Pietro R, Lombardo BM & Viglianisi F (1997) Genetic diversity in some species of *Hydropsyche* (Trichoptera, Hydropsychidae) in central and southern Italy. *Italian Journal of Zoology*, 64(1): 31–39.
- Folmer O, Black M, Hoch W, Lutz R & Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5): 294–299.
- Chantaramongkol P & Malicky H (1989) Some *Chimarra* (Trichoptera: Philopotamidae) from Thailand (Studies on caddisflies from Thailand, No. 2. *Aquatic Insects*, 11(4): 223–240.
- Ge Y, Xia C, Wang J, Zhang X, Ma X & Zhou Q (2021) The efficacy of DNA barcoding in the classification, genetic differentiation, and biodiversity assessment of benthic macroinvertebrates. *Ecology and Evolution*, 11(10): 5669–5681.
- Hillis DM & Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, 42(2): 182–192.
- Hoang DH & Bae YJ (2007) Vietnamese species of *Stenopsyche* McLachlan (Trichoptera: Stenopsychidae). *Zootaxa*, 1624(1): 1–15.
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ & Vinh LS (2018) UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35(2): 518–522.
- Hogg ID, Smith BJ, Banks JC, Dewaard JR & Hebert PD (2009) Testing use of mitochondrial COI sequences for the identification and phylogenetic analysis of New Zealand caddisflies (Trichoptera). *New Zealand Journal of Marine and Freshwater Research*, 43(5): 1137–1146.
- Huelsenbeck JP & Rannala B (2004) Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology*, 53(6): 904–913.
- Hu YL, Tsring S, Wang BX & Sun CH (2020) Descriptions of larvae of three Philopotamidae species from China (Insecta, Trichoptera). *Zootaxa*, 4731(4): 509–521.
- Hu YL, Wang BX & Sun CH (2018) A new species of *Chimarra* from China (Trichoptera, Philopotamidae) with description of its larva. *Zootaxa*, 4504(2): 253–260.
- Ismail AR, Edington JM & Green PC (1996) Descriptions of the pupae and larvae of *Stenopsyche siamensis* Martynov, 1931 (Trichoptera: Stenopsychidae) with notes on larval biology. *Aquatic Insects: International Journal of Freshwater Entomology*, 18(4): 241–252.
- Jaroenchaiwattanachote C, Pramual P, Wangwasit K, Bunchalee P & Thanee I (2025) Integrative taxonomy and DNA barcoding of Thai Caddisflies (Trichoptera), with the description of a new Species. *Zootaxa*, 5613(2): 307–322
- Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A & Flouri T (2017) Multirate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics*, 33(11): 1630–1638.
- Karaouzas I (2014) Description of the larva of *Philopotamus achemenus* Schmid 1959 (Trichoptera: Philopotamidae) and a larval key for species of *Philopotamus* in Greece. *Zootaxa*, 3815(3): 428–434.
- Kumar S, Stecher G, Li M, Knyaz C & Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35(6): 1547–1549.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T & Calcott B (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 34(3): 772–773.
- Laudee P (2008) Larval morphology and diagnosis of the giant microcaddisfly species, *Ugandatrichia* spp. (Hydroptilidae: Trichoptera) in Thailand. *Zootaxa*, 1825(1): 29–39.
- Laudee P & Malicky H (2018) Five new species of caddisflies (Trichoptera: Insecta) from Upper Tenasserim Range, Thailand and Myanmar. *Zootaxa*, 4524(3): 351–358.
- Malicky H (1979) Neue Köcherfliegen (Trichoptera) von den Andamansen-Inseln. *Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen*, 30: 97–109.
- Malicky H (1989) Odontoceridae aus Thailand (Trichoptera). *Entomologische Berichte Luzern*, 21: 95–122.
- Malicky H (1993) Neue asiatische Köcherfliegen (Trichoptera: Rhyacophilidae, Philopotamidae, Psychomyiidae and Polycentropodidae). *Entomologische Berichte Luzern*, 29: 77–88.
- Malicky H (2010) Atlas of Southeast Asian Trichoptera. Biology Department, Science Faculty, Chiang Mai University, pp. 71–97.
- Malicky H & Chantaramongkol P (1989) Einige *Chimarra* (Trichoptera: Philopotamidae) aus Thailand. *Aquatic Insects*, 11(4): 223–240.
- Malicky H & Chantaramongkol P (1998) Beiträge zur Kenntnis thailändischer *Chimarra* (Trichoptera, Philopotamidae) (Arbeit Nr. 24 über thailändische Köcherfliegen). *Linzer biologische Beiträge*, 30(2): 767–793.
- Malicky H & Laudee P (2019) Neue Köcherfliegen (Insecta, Trichoptera) aus Laos und Kambodscha. *Linzer Biologische Beiträge*, 51(2): 1395–1408.
- Malicky H & Prommi T (2004) Neue Köcherfliegen aus Thailand (Trichoptera) (Arbeit Nr. 37 über thailändische Köcherfliegen). *Linzer biologische Beiträge*, 36(1): 287–304.
- Malicky H, Suwannarat N & Laudee P (2018) Köcherfliegen (Trichoptera) aus dem Süden Thailands. mit der Beschreibung von vier neuen Arten. *Linzer Biologische Beiträge*, 50(2): 1319–1328.
- Maneechan W, Kruttha P & Prommi TO (2018) The larva and pupa of *Potamyia flavata* (Banks 1934) (Trichoptera: Hydropsychidae): Description, life cycle, and notes on its biology. *Zootaxa*, 4394(3): 395–406.
- Mauro DS & Agorreta A (2010) Molecular systematics: a synthesis of the common methods and the state of knowledge. *Cellular & Molecular Biology Letters*, 15(2): 311–341.
- Mey W (1998) Beitrag zur Köcherfliegenfauna von Vietnam. 2. Die Gattung *Chimarra* Stephens, 1829 (Trichoptera, Philopotamidae). *Entomofauna*, 19(33): 537–548.
- Miller MA, Pfeiffer W & Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings: 2010 Gateway Computing Environments Workshop (GCE)*. IEEE, New Orleans, pp. 1–8.
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A & Lanfear R (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37: 1530–1534.
- Morse JC (2024) Trichoptera World Checklist. <https://trichopt.app.clemson.edu/> (Accessed 13 December 2024).
- Navás L (1929) *Insecta Orientalia*. *Memorie della Pontificia Accademia Romana dei Nuovi Lincei, Series 2*, 12: 33–56.
- Orfinger AB, Morse JC & Hix RL (2022) Associating life stages and sexes of Nearctic *Polycentropus* Curtis, 1835 (Trichoptera: Polycentropodidae) using mitochondrial DNA barcoding. *Ecology and Evolution*, 12(3): e8741.
- Peng L, Zang H, Sun C, Wang L & Wang B (2023) Four new species of the genus *Eoneureclipsis* (Trichoptera: Psychomyiidae) from China inferred from morphology and DNA barcodes. *Insects*, 14(2): 158.
- Peumwarunyoo P & Prommi TO (2013) Larvae of *Amphipsyche* species (Trichoptera: Hydropsychidae) from Thailand. *Zootaxa*, 3635: 251–260.

- Prommi TO (2016) Descriptions of larvae of four species of *Hydropsyche* (Hydropsychidae: Trichoptera) from Thailand. *Zootaxa*, 4158(4): 577–59.
- Prommi T, Permkam S & Malicky H (2006a) The immature stages of *Pseudoleptonema quinquefasciatum* Mart. and *P. supalak* Malicky & Chantaramongkol (Trichoptera: Hydropsychidae). *Braueria*, 33: 26–30.
- Prommi T, Permkam S & Malicky H (2006b) Description of larvae and pupae of *Hydatomanicus klanklini* Malicky & Chantaramongkol and *H. adonis* Malicky & Chantaramongkol (Trichoptera: Hydropsychidae) from southern Thailand. *Braueria*, 33: 31–36.
- Prommi TO, Permkam S & Sites RW (2006c) Description of the larva and pupa of *Potamyia phaidra* Malicky and Chantaramongkol (Trichoptera: Hydropsychidae) from southern Thailand. *Zootaxa*, 1357(1): 21–29.
- Puillandre N, Brouillet S & Achaz G (2020) ASAP: Assemble species by automatic partitioning. *Molecular Ecology Resources*, 21(2): 609–620.
- Rambaut A (2014) FigTree. Version 1.4.4. <http://tree.bio.ed.ac.uk/software/figtree/> (Accessed 15 August 2024).
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA & Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61(3): 539–542.
- Stephens JF (1829) *A Systematic Catalogue of British Insects: Being an Attempt to Arrange All the Hitherto Discovered Indigenous Insects in Accordance with Their Natural Affinities*. Baldwin and Cradock, London, 318 pp.
- Sun CH (1998) New species of the genus *Wormaldia* from China (Trichoptera: Philopotamidae). *Jiangsu Journal of Agricultural Sciences*, 14(1): 40–44.
- Sun CH & Malicky H (2002) 22 neue Köcherfliegen aus China (Trichoptera). *Linzer biologische Beiträge*, 34(1): 521–540.
- Thamsenanupap P, Chantaramongkol P & Malicky H (2005) Description of caddis larvae (Trichoptera) from northern Thailand of the genera *Himalopsyche* (Rhyacophilidae), *Arctopsyche* (Arctopsychoidea). *Braueria*, 32: 7–11.
- Ulmer G (1913) Über einige von Edw. Jacobson auf Java gesammelte Trichopteren. *Zweiter Beitrag*. Notes from the Leyden Museum, 35: 78–101.
- Valdon SB, Pongtippatee P & Laudee P (2023) Description of the larva of *Macrostemum floridum* (Navás 1929) (Trichoptera: Hydropsychidae) and its feeding habits in Kaengkrung National Park, southern Thailand. *Zootaxa*, 5293(3): 581–595.
- Vitecek S, Previšić A, Kučinić M, Bálint M, Keresztes L, Waringer J & Graf W (2015) Description of a new species of *Wormaldia* from Sardinia and a new *Drusus* species from the Western Balkans (Trichoptera, Philopotamidae, Limnephilidae). *ZooKeys*, 496: 85–103.
- Wahlberg E & Johanson KA (2014) The age, ancestral distribution and radiation of *Chimarra* (Trichoptera: Philopotamidae) using molecular methods. *Molecular Phylogenetics and Evolution*, 79: 433–442.
- Wiggins GB (1996) *Larvae of the North American caddisfly genera (Trichoptera)*. Second Edition. University of Toronto Press, Toronto, pp. 150–157.
- Zhou X, Frandsen PB, Holzenthal RW, Beet CR, Bennett KR, Blahnik RJ & Kjer KM (2016) The Trichoptera barcode initiative: a strategy for generating a species-level Tree of Life. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1702): 20160025.
- Zhou X, Jacobus LM, DeWalt RE, Adamowicz SJ & Hebert PD (2010) Ephemeroptera, Plecoptera, and Trichoptera fauna of Churchill (Manitoba, Canada): insights into biodiversity patterns from DNA barcoding. *Journal of the North American Benthological Society*, 29(3): 814–837.
- Zhou X, Kjer KM & Morse JC (2007) Associating larvae and adults of Chinese Hydropsychidae caddisflies (Insecta: Trichoptera) using DNA sequences. *Journal of the North American Benthological Society*, 26(4): 719–742.