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Integrative taxonomy and DNA barcoding of Thai Caddisflies (Trichoptera), with the description of a new Species

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Abstract

Caddisflies (Trichoptera) are abundant and diverse aquatic insects. Their immature stages inhabit a wide range of aquatic environments, making them ideal candidates for water quality biomonitoring. However, the limited morphological characteristics available for species identification in the immature stages pose a significant challenge to their application in biomonitoring. In this study, we evaluated the effectiveness of DNA barcoding, based on mitochondrial cytochrome c oxidase I (COI) sequences, for species identification of caddisflies in Thailand. A total of 1,487 adult specimens were collected and morphologically identified into 13 species across 8 genera and 4 families. From these taxa, 88 COI sequences were generated from representative specimens. Maximum intraspecific genetic divergence ranged from 0% to 3.08%. Only three species were successfully matched to COI sequences in the BOLD database, while nine species are reported here for the first time, and one species remained ambiguous. Integrating COI barcoding sequences with morphological data revealed that one species, morphologically similar to *Triplectides indicus* (Walker 1852), represents a novel species, *Triplectides buengkanensis* **sp. nov.** We provide a detailed description, illustrations, diagnostic features, and DNA barcoding sequences for this new species.

Key words: COI, cryptic species, integrative taxonomy, long-horned caddisflies, *Triplectides buengkanensis* sp. nov.

Introduction

The caddisflies, or Trichoptera, constitute one of the most diverse groups of aquatic insects, with more than 17,000 species reported globally (Morse 2024). However, it has been estimated that the number of world species could be >29,000 (Zhou *et al*. 2016) or even 50,000 (Schmid 1984). Based on these figures, only approximately 59% or 34% of the global Trichoptera species have been described, respectively. Caddisflies play an essential role in the nutrient turnover in aquatic ecosystems because they are usually biomass-dominant macroinvertebrates that link nutrients in algae and detritus to predators (Wiggins 1996; Holzenthal *et al*. 2007, 2015; Morse *et al*. 2019). Because most caddisfly species may be intolerant of certain environmental pollutants, such as various pesticides, nutrients, low oxygen concentrations, high or low pH, and sediment (Wallace & Webster 1996) they are commonly used as biological indicators for monitoring water quality (Prommi *et al*. 2014; Ab Hamid & Md Rawi 2017).

Accurate identification of the aquatic larvae is necessary for developing the use of these macroinvertebrates as effective water quality indicator species (Morse *et al*. 2007;). In other words, the main limitation of using caddisflies as bioindicators is the incomplete taxonomic knowledge and difficult species identification of the immature stages (Johanson 2007; Zhou *et al*. 2007; Orfinger *et al*. 2022). The adult male's characteristics are mainly used for species identification, whereas the larvae of many caddisfly species remain unidentifiable. (Zhou *et al*. 2007, 2016). To overcome this taxonomic impediment, several studies have used DNA barcoding based on the mitochondrial

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cytochrome c oxidase I (COI) gene to associate unknown immature stages with known adult males (Johanson 2007; Zhou *et al*. 2007; Waringer *et al*. 2008; Barcelos-Silva *et al*. 2018; Orfinger *et al*. 2022). Furthermore, DNA barcoding is valuable for revealing hidden diversity among morphologically cryptic species. (Pauls *et al*. 2010; Previšić *et al*. 2014; Wickson *et al*. 2014).

In Thailand, caddisfly diversity has been studied for over three decades (Malicky 1987) with 1,005 species recorded across the country (Chantaramongkol *et al*. 2010). Research has addressed various aspects of Trichoptera, including taxonomy and diversity (Malicky 1987; Chantaramongkol & Malicky 1997; Laudee & Malicky 2014), ecology and distribution (Malicky & Chantaramongkol 1993; Thapanya *et al*. 2004), and applications in biomonitoring (Chaibu 2000; Cheunbarn 2002; Laudee 2002; Prommi & Thamsenanupap 2015). However, despite extensive study in these areas, DNA barcoding data for Trichoptera in Thailand remains scarce.

This study evaluated DNA barcoding sequences for species identification of caddisflies collected in northeastern Thailand. In addition, we discovered a novel caddisfly species, for which we provide descriptions, illustrations, and diagnostic morphological characters. The DNA barcoding sequences of this new species from the type locality serve as a valuable reference for future research.

Material and methods

Specimen collection, identification, and morphological study

Adult caddisfly specimens were collected from six sampling sites in Thailand (Table 1 and Fig. 1) using a light trap (black light tubes 12V, 10W). The traps were placed on the ground near the ponds, lakes, or streams after sunset (usually after 18.30 hrs) and operated for 3 to 4 hours. All insect specimens collected by the light traps were preserved in 80% ethanol. Adult specimens of caddisflies were sorted under a stereomicroscope, preserved in 80% ethanol, and stored at -20 °C in a freezer until they were analyzed. The specimens are deposited in the Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham, Thailand.

To observe male genital structures, the abdominal segments IX and X were removed and cleared using hot 10% KOH as detailed by Malicky (2010). After clearing, the abdomen was examined with a Zeiss Stemi 508 stereomicroscope. Photographs were taken using a stereomicroscope (Zeiss Stemi 508 equipped with an Axiomcam 208 camera) and compound microscope (Zeiss PrimoStar 3 light microscope, Carl Zeiss, Germany). Resulting images of the genitalia were used as templates for illustrations. Specimens were identified using the Atlas of Southeast Asian Trichoptera (Malicky 2010) and its supplement (Malicky 2023). The morphological terminology used for male and female genitalia follows Holzenthal (1988).

TABLE 1. Sampling locations and numbers of Trichoptera collected from six sampling sites in northeastern Thailand between October 2022 and April 2023.

Location (Code)	Collection Date	Coordinates	Elevation (m)	Species	N	
					Male	Female
Bueng Khong Long, Bueng Kan (BK)	13 Oct 2022 & 4 Feb 2023	18.0233 N/ 104.0157 E	168	*Dipseudopsis* sp. A	61	9
				Oecetis bengalica Martynov, 1836	3	1
				Oecetis biramosa Martynov 1936	22	28
				Oecetis angkor Malicky, Melnitsky & Ivanov 2014	42	99
				Triplectides buengkanensis **sp. nov.**	27	42
				Leptocerus posticus Banks, 1911	137	76

.....continued on the next page

TABLE 1. (Continued)

Location (Code)	Collection Date	Coordinates	Elevation (m)	Species	N	
					Male	Female
				Ecnomus argonautos Laudee & Malicky, 1999	86	-
				Cheumatopsyche lucida (Ulmer 1907)	5	5
				Cheumatopsyche schwendingeri Malicky & Chantaramongkol, 1997	3	4
				Aethaloptera sexpunctata Kolenati, 1859	1	1
				Macrostemum dione Malicky & Chantaramongkol, 1998	2	6
				Ecnomus spp.[1]	-	526
Ban Yang, Kantharawichai, Maha Sarakham (MK)	7 Dec 2022	16.2905 N/ 103.1834 E	194	*Dipseudopsis* sp. A	6	-
Waritchaphum, Sakhon Nakhon (SK)	11 Dec 2022	17.2415 N/ 103.5745 E	202	*Dipseudopsis* sp. A	7	22
Bang Sai Yai, Mukdahan (MD)	1 Jan 2023	16.5981 N/ 104.7319 E	143	*Cheumatopsyche schwendingeri* Malicky & Chantaramongkol, 1997	5	5
Chatturat, Chaiyaphum (CP1)	4 Mar2023	15.6834 N/ 101.9809 E	188	*Aethaloptera sexpunctata* Kolenati, 1859	4	6
Phu Khiao, Chaiyaphum (CP2)	8 Apr 2023	16.4005 N/ 102.1295 E	210	*Ecnomus obtusus* Ulmer, 1910	100	-
				Ecnomus mammus Malicky & Chantaramongkol, 1993	18	-
				Ecnomus spp.[1]	-	128
Total					529	958

[1]Female specimens unidentified to species level.

Molecular study

DNA was extracted from a single leg from each adult specimen using the GF-1 Nucleic Acid Extraction Kit (Vivantis Technologies Sdn. Bhd, Shah Alam, Malaysia). The primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer *et al*. 1994) were used to amplify a fragment of the cytochrome c oxidase I (COI) gene using the reaction conditions as described in Tangkawanit *et al*. (2018). PCR products were checked using 1% agarose gel electrophoresis, staining with 1X Novel Juice loading dye (GenDirex®, Taiwan, China). Successful PCR products were purified using a PureDireX PCR CleanUp & Gel Extraction Kit (Bio-Helix, Taiwan, China) following manufacturing instruction protocol. The purified PCR products were sent for DNA sequencing at ATCG Company Limited [Thailand Science Park (TSP), Pathumthani, Thailand] using the same primers as for PCR.

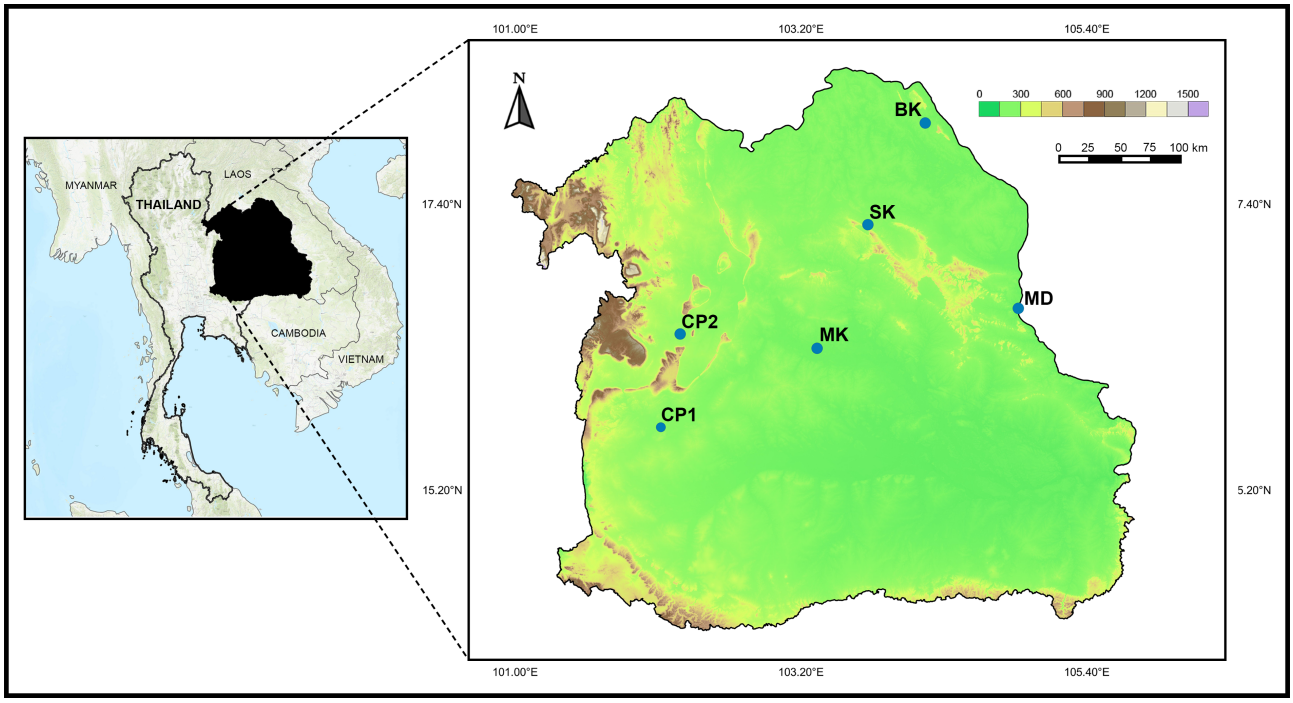

FIGURE 1. Sampling locations of caddis flies from Thailand used in this study.

Data analysis

The obtained COI sequences were checked for quality using the "Edit/View sequencer file" in MEGA X (Kumar *et al*. 2018). and compared with existing data using the Basic Local Alignment Search Tool (BLAST) option in GenBank. Sequences were then aligned using align options in MEGA X (Kumar *et al*. 2018). In total, sequences from 13 species were obtained in the present study (Table 2). A total of 22 sequences from conspecific and closely related species, as identified in the BOLD and NCBI GenBank public databases, were retrieved and included in the analyses. The BOLD sequences included those for the following species: *Triplectides misakianus* (n = 1; AB778888); *T. indicus* (n = 7; KX142733, KX293522, KX293699, KX106412, KX103118, KX141674, KX104257); *Aethaloptera sexpunctata* Kolenati 1859 (n = 1; KY983360); *Cheumatopsyche* XZ sp. CN (n = 1; KX106328); and *C. lucida* Ulmer 1907 (n = 12; KX292952, KX292815, KY983357, KX144323, HQ958920, HQ578287, HQ578288, HQ578289, HQ578291, HQ578299, HQ578300, HQ578301). These sequences were analyzed along with sequences from our specimens (PQ009780–PQ009783).

Intraspecific and interspecific genetic divergence was calculated using uncorrected p-distance in TaxonDNA (Meier *et al*. 2006). We evaluated the effectiveness of COI sequences for species identification with the best-match (BM) and best close match (BCM) methods in TaxonDNA (Meier *et al*. 2006). Successful identification with the BM method occurs when all conspecifics show the smallest distance to the query, while for the BCM method, the distance must be within the 95th percentile of overall intraspecific genetic distance (Meier *et al*. 2006). We also used the BOLD identification system (https://www.boldsystems.org/index.php/IDS_OpenIdEngine) (Ratnasingham & Hebert 2007) and assessed species differentiation of caddisflies in Thailand using Assemble Species by Automatic Partitioning (ASAP) (Puillandre *et al*. 2021) and the multi-rate Poisson tree process (mPTP) (Kapli *et al.* 2017). The ASAP analysis was conducted via a web server (https://bioinfo.mnhn.fr/abi/public/asap/#) (accessed on 18 June 2024), and the mPTP analysis was performed on another server (https://mptp.h-its.org/#/tree) (accessed on 18 June 2024).

Genetic relationships between species were inferred using sequences obtained in this study and those from conspecific and closely related species available in public databases (BOLD and NCBI GenBank). Two phylogenetic inference methods, neighbor-joining (NJ) and maximum likelihood (ML) were used. The NJ tree was calculated in MEGA X (Kumar *et al*. 2018) using the Kimura 2-parameter for genetic distance estimation. Branch support was calculated based on 1,000 bootstrapping replications. The ML tree was also inferred in MEGA X using general

time reversible with gamma distribution and invariant sites (GTR+G+I) model. Branch support was calculated based on bootstrapping with 1,000 replications. FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize and prepare graphics of the NJ tree.

Results

Species diversity

In total, 1,487 adult caddisfly specimens (529 males and 958 females) were collected (Table 1). Morphological identification revealed 13 species belonging to 8 genera and four families (Table 1). The most abundant species was *Leptocerus posticus* Banks 1911 (n = 213), followed by *Oecetis angkor* Malicky, Melnitsky & Ivanov 2014 (n = 141), while the least common species was *Oecetis bengalica* Martynov 1836 (n = 4). The site with the highest abundance and diversity of caddisflies was Bueng Khong Long in Bueng Kan Province (BK), northeastern Thailand, where 1,186 specimens (389 males and 797 females) were collected.

Genetic variation and DNA barcoding

Representative specimens of the morphological species were used for DNA barcoding analysis. A total of 88 COI sequences (accession nos. PQ009818-PQ009829; PQ009746-PQ009817; PQ009830-PQ009833) were obtained. Among these, 13 were females that currently cannot be morphologically identified to species level. These unidentified females were associated with the known males using phylogenetic analyses and all of them were successfully linked. Therefore, these female specimens were also included in the genetic variation analyses. In total, DNA barcoding sequences were obtained from 13 species from five families of the Trichoptera; the sequences of eight of these (*Dipseudopsis* sp. A, *Cheumatopsyche schwendingeri* Malicky & Chantaramongkol 1997, *Macrostemum dione* Malicky & Chantaramongkol 1998 (in Malicky 1998), *Ecnomus argonautos* Laudee & Malicky 1999*, E. mammus* Malicky & Chantaramongkol 1993, *E. obtusus* Ulmer 1910, *Leptocerus posticus* and *Oecetis angkor*) are here reported for the first time.

Maximum intraspecific genetic divergence ranged from 0% in *E. obtusus* to 3.08% in *E. mammus* (Table 2). The inclusion of sequences from public databases revealed extremely high intraspecific genetic divergence in *Cheumatopsyche lucida* (13.61%). Three additional species with publicly available conspecific sequence data also exhibited relatively high maximum genetic divergence (Table 2), suggesting that at least some of the species were misidentified or that there are additional morphologically cryptic species to be discovered. Interspecific genetic divergence varied from 7.76% between *E. argonautos* and *E. obtusus* to 18.11% between *Oecetis angkor* and *O. biramosa* (Table 2). The species identifications of all sequences obtained in this study were concurrent for both the BM and BCM methods. Species identification using the BOLD identification engine successfully identified three species: *Aethaloptera sexpunctata*; *Oecetis bengalica*; and *O. biramosa*. The remaining specimens either yielded ambiguous results (*Cheumatopsyche lucida* and *Triplectides* nr. *indicus*) or had no matching sequences (*Dipseudopsis* sp. A, *C. schwendingeri, Macrostemum dione*, *Ecnomus argonautos*, *E. mammus*, *E. obtusus*, *Leptocerus posticus,* and *Oecetis angkor*) (Table 2).

Phylogenetic analysis demonstrated that all species were monophyletic with strong statistical support (>90%) (Fig. 2). However, two species, *Cheumatopsyche lucida* and *Triplectides indicus*, exhibited deeply divergent lineages. Three genetically distinct lineages were identified in *C. lucida*, with a minimum genetic differentiation of 9.43%. Specimens collected in this study formed a genetically divergent lineage alongside a specimen from Mae Hong Son province, northern Thailand, reported by Zhou *et al*. (2016). The other two lineages of *C. lucida* were represented by single sequences: one from Nan province, northern Thailand (also reported by Zhou *et al*. (2016)), and another from Indonesia. Similarly, three lineages were identified for *Triplectides indicus*. One lineage comprised a sequence from Indonesia, another consisted of a sequence from Laos, and a third lineage included five sequences from Thailand. Specimens identified as *T.* nr. *indicus* in this study formed a distinct clade separated from *T. indicus* but clustered with *T. mikianus* (Fig. 2). A minimum genetic differentiation between *T. indicus* and *T.* nr. *indicus* + *T. mikianus* clades was 11.27%.

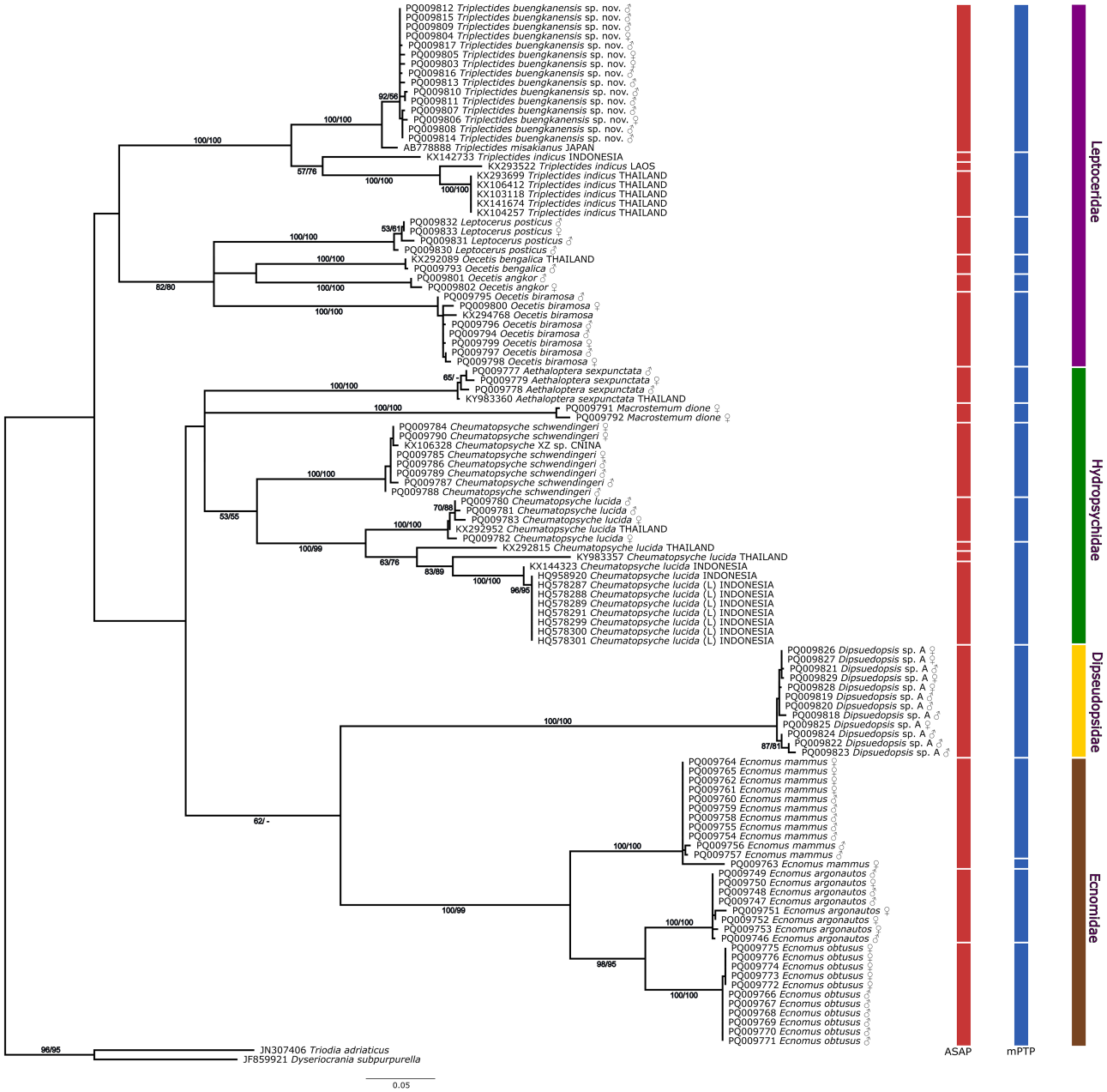

FIGURE 2. Maximum likelihood tree based on 113 COI sequences from 13 species of Trichoptera collected in Thailand during this study, along with conspecific sequences retrieved from public databases. Bootstrap values for both maximum likelihood (ML) and neighbor-joining (NJ) trees are indicated above or below the branches.

Species delimitation using the ASAP method was consistent with the phylogenetic tree (Fig. 2). All sequence-delimited species aligned with morphological identifications, except for *C. lucida* and *T. indicus*, which exhibited divergent lineages in the ML tree. The ASAP analysis delimited four species within *C. lucida*: (i) specimens collected in this study plus one sequence from Thailand, (ii) a second sequence from Thailand, (iii) a third sequence from Thailand, and (iv) sequences from Indonesia. For *T. indicus*, three species were resolved: (i) a sequence from Indonesia, (ii) a sequence from Laos, and (iii) five sequences from Thailand. Specimens identified as *T.* nr. *indicus* in this study, along with a sequence of *T. mikianus*, were delimited as a distinct species separate from *T. indicus*. The mPTP method resolved a total of 16 species, 10 of which agreed with morphological identifications. Two morphologically identified species, *C. lucida* and *E. mammus*, were each split into two species (Fig. 2). *Triplectides* nr. *indicus* and *T. mikianus* were treated as conspecific but distinct from *T. indicus*.

Morphological examinations revealed that *T*. nr. *indicus* is morphologically distinct from other closely related species. Those morphological differences, together with the molecular genetic data from COI sequences, support a hypothesis that *T*. nr. *indicus* represents a novel species. Consequently, a detailed morphological description of this species is provided.

TABLE 2. Minimum and maximum intraspecific genetic divergent and BOLD identification of the Trichoptera in Thailand based on COI barcoding sequences.

Family/ Species	GenBank accession number	Min–Max. p-distance (%) (this study) (n)	Min.–Max. p-distance (included public data) (n)	BOLD identification
Dipseudopsidae				
Dipseudopsis sp. A	PQ009818–PQ009829	0–1.45 (12)	No record	No match
Macronematinae				
Aethaloptera sexpunctata	PQ009777–PQ009779	0.54–1.45 (3)	0.54–1.45 (4)	*A. sexpunctata*
Hydropsychinae				
Cheumatopsyche lucida	PQ009780–PQ009783	0.36–1.09 (4)	0–13.61 (16)	*C. cognita*/*C. lucida*
Cheumatopsyche schwendingeri	PQ009784–PQ009790	0–2.36 (7)	No record	*Cheumatopsyche* XZ sp. CN16
Macrostemum dione	PQ009791–PQ009792	1.27 (2)	No record	No match
Ecnomidae				
Ecnomus argonautos	PQ009746–PQ009753	0–1.99 (8)	No record	No match
Ecnomus mammus	PQ009754–PQ009765	0–3.08 (12)	No record	No match
Ecnomus obtusus	PQ009766–PQ009776	0 (11)	No record	No match
Leptoceridae				
Leptocerus posticus	PQ009830–PQ009833	0–1.45 (4)	No record	No match
Oecetis bengalica	PQ009793	NA (1)	0.18 (2)	*O. bengalica*
Oecetis biramosa	PQ009794–PQ009800	0.18–1.09 (7)	0.18 – 1.63 (8)	*O. biramosa*
Oecetis angkor	PQ009801–PQ009802	0.18 (2)	No record	No match
Triplectides nr. *indicus* (= *T. buengkanensis* **sp. nov.**)	PQ009803–PQ009817	0–1.09 (15)	No record	*T. misakianus*

Taxonomy

Family Leptoceridae Leach 1815

Subfamily Triplectidinae Ulmer 1906

Genus *Triplectides* Kolenati 1859

Triplectides buengkanensis sp. nov. Jaroenchaiwatthanachote, Pramual, & Thanee

Figs. 3–6

Diagnosis. *Triplectides buengkanensis* **sp. nov.** is similar to *T. misakianus* (Matsumura 1931) (Kuranishi 1999; Katsuma & Kuranishi 2016), *T. nessimiani* Desidério & Pes (Desidério *et al*. 2020), and *T. indicus* Walker 1852 in many characteristics including those of the forewings and hind wings and the shapes and lengths of the preanal appendages and of the basoventral and basomesal lobes and apically acute second articles of the inferior appendages.

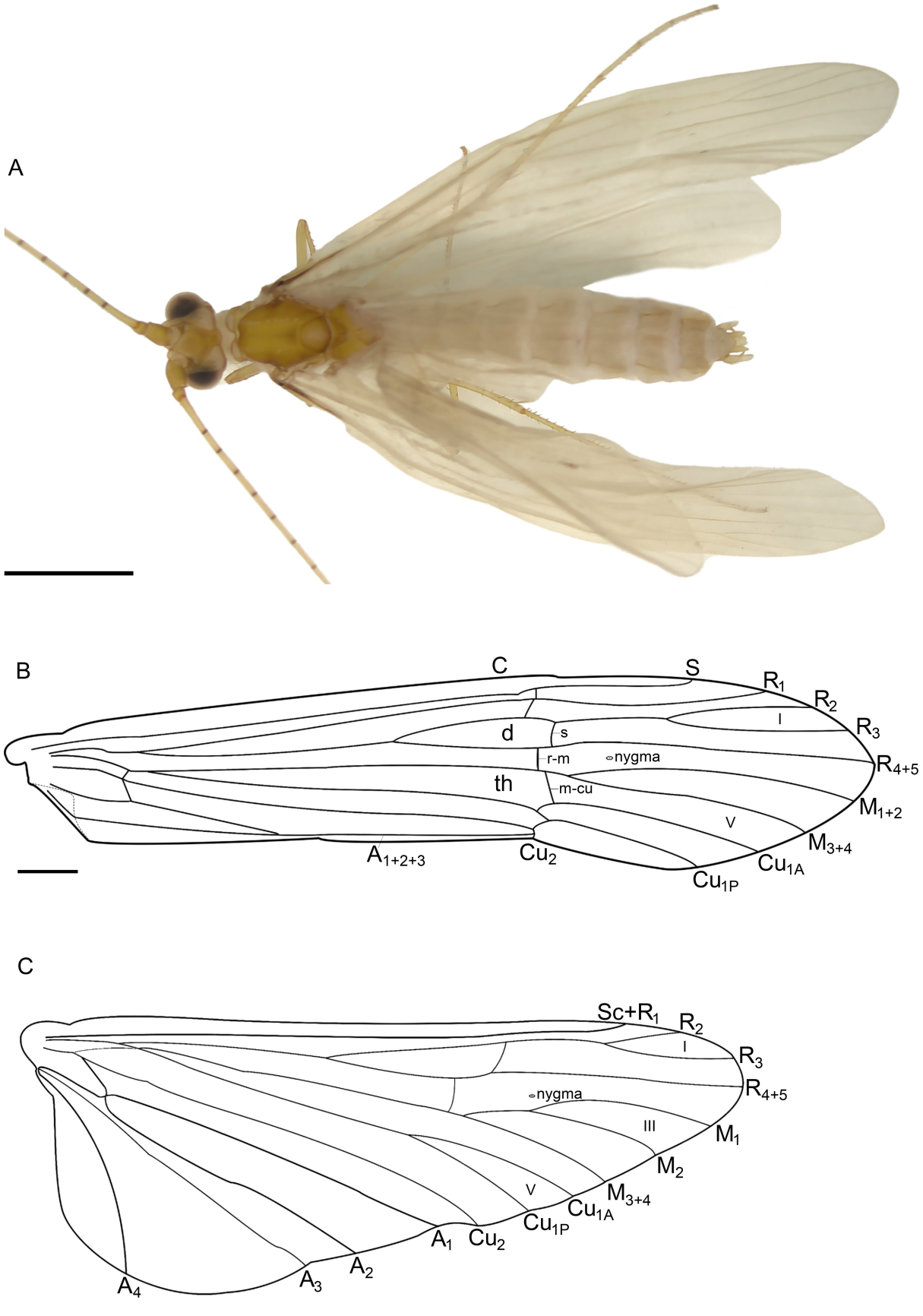

FIGURE 3. *Triplectides buengkanensis* **sp. nov.**, adult male, holotype. 3A, habitus, dorsal; 3B, venation of right forewing, dorsal; 3C, venation of right hind wing, dorsal. Scale bars: 3A = 2 mm; 3B, 3C = 1 mm.

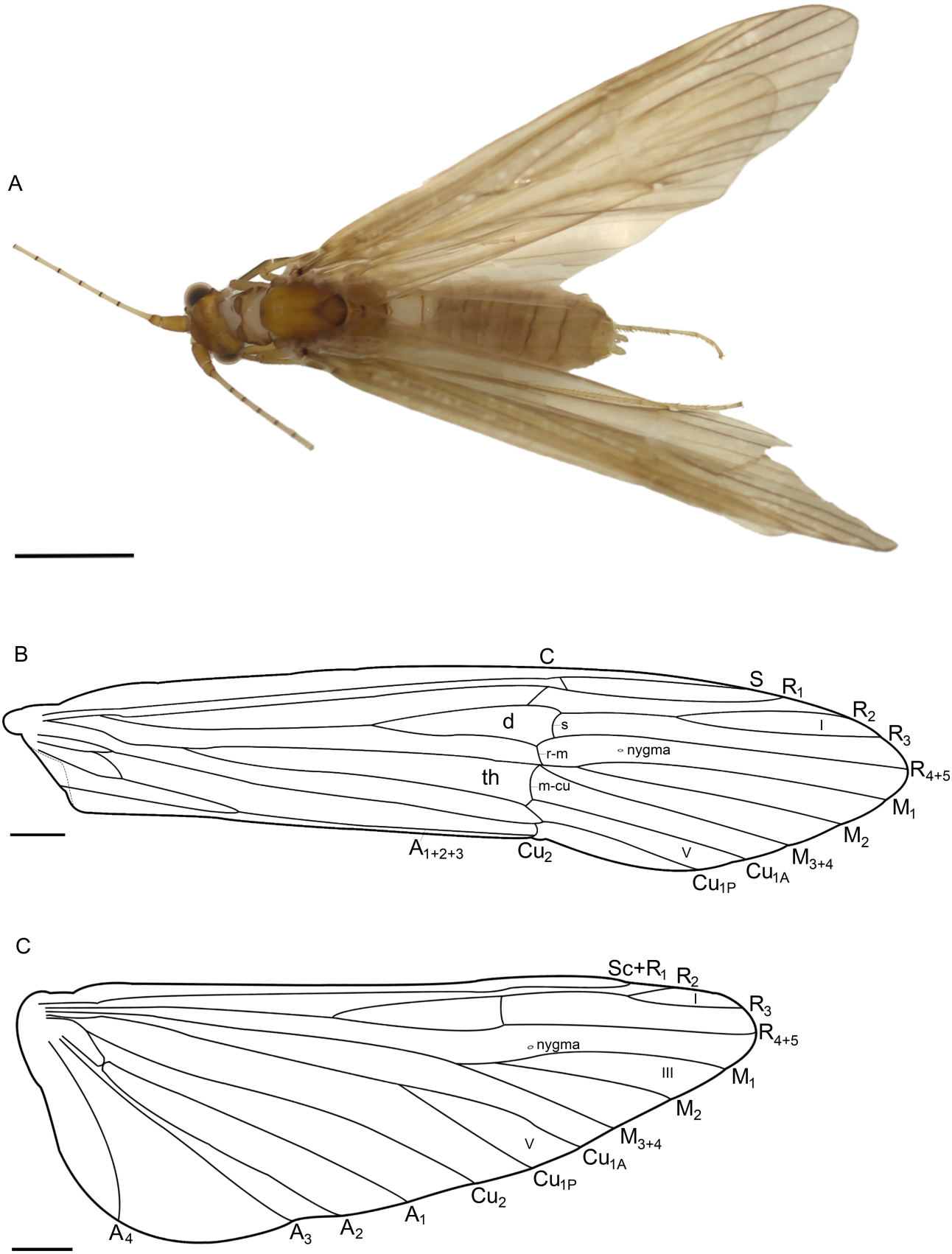

FIGURE 4. *Triplectides buengkanensis* **sp. nov.**, adult female, paratype. 4A, habitus, dorsal; 4B, venation of right forewing, dorsal; 4C, venation of right hind wing, dorsal. Scale bars: 4A = 2 mm; 4B, 4C = 1 mm.

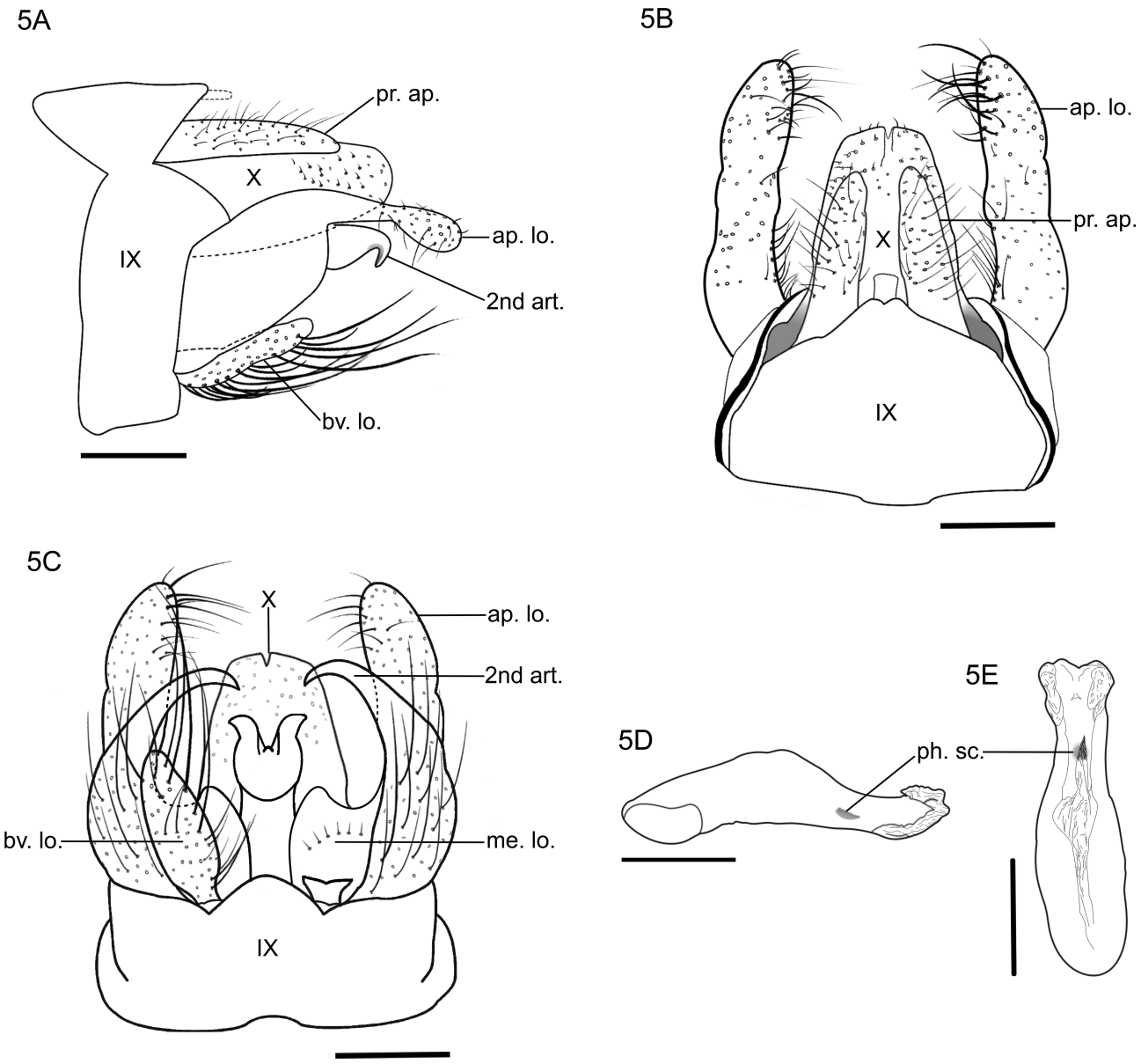

FIGURE 5. *Triplectides buengkanensis* **sp. nov.**, adult male, holotype. 5A–5C, genitalia: 5A, left lateral; 5B, dorsal; 5C, ventral. 5D, 5E, phallic apparatus: 5D, left lateral; 5E, dorsal. Abbreviations: ap. lo. = apicodorsal lobe; bv. lo. = basoventral lobe; me. lo. = basomesal lobe; ph. sc. = phallotremal sclerite; pr. ap. = preanal appendages; 2nd art. = second article. Scale bars: 0.1 mm.

However, *Triplectides buengkanensis* **sp. nov.** can be distinguished from *T. misakianus* in the female by length of the fork I petiole (shorter in *T. misakianus*) and absence of the transverse vein *r-m* in the hind wing (present in *T. misakianus*). In the male, *Triplectides buengkanensis* **sp. nov.** can be distinguished from these species by tergum X being obliquely truncate in dorsal view (suboval in *T. indicus* and *T. misakianus*) and having a shallow V-shaped apicomesal incision (deep in *T. misakianus*, *T. nessimiani*, and *T. indicus*), the absence of striae on the basomesal lobes of the inferior appendages (present in *T. nessimiani*), and the suboval phallotremal sclerite (subpentagonal in *T. nessimiani*).

Material examined. Holotype**.** 1 male. THAILAND: Bueng Kan Province, Bueng Khong Long District, Bueng [= Swamp] Khong Long; 18.02334° N, 104.01569° E; 168 m; 4.ii.2023; light trap; K. Wangwasit, P. Bunchalee & I. Thanee; Department of Biology, Faculty of Science, Mahasarakham University, Thailand.

Paratypes**.** Same data as holotype, except 11 males, 4 females; GenBank no: PQ009803–PQ009817 (COI).

Description. *Adult male* (Figs 3A–3C). Forewing length 11.0 mm–12.5 mm (Fig. 3B), body 7.5–9 mm ($n = 12$). *Adult female* (Figs 4A–4C). Forewing length 11.4–15 mm (Fig. 4B), body 8–11 mm ($n = 11$). In both sexes, body

generally appearing yellowish brown on head, thorax, forewing, abdomen, and legs in alcohol-preserved material. Tibial spur formula 2:2:2—forelegs, midlegs, and hind legs respectively—in both sexes. Wing venation similar in male and female except male forewing with R4+5 straight behind discoidal cell and apical fork III absent, female forewing with R4+5 angled posterad subapically at *r-m* crossvein behind discoidal cell as in several Australian species (Morse & Neboiss 1982) and apical fork III present.

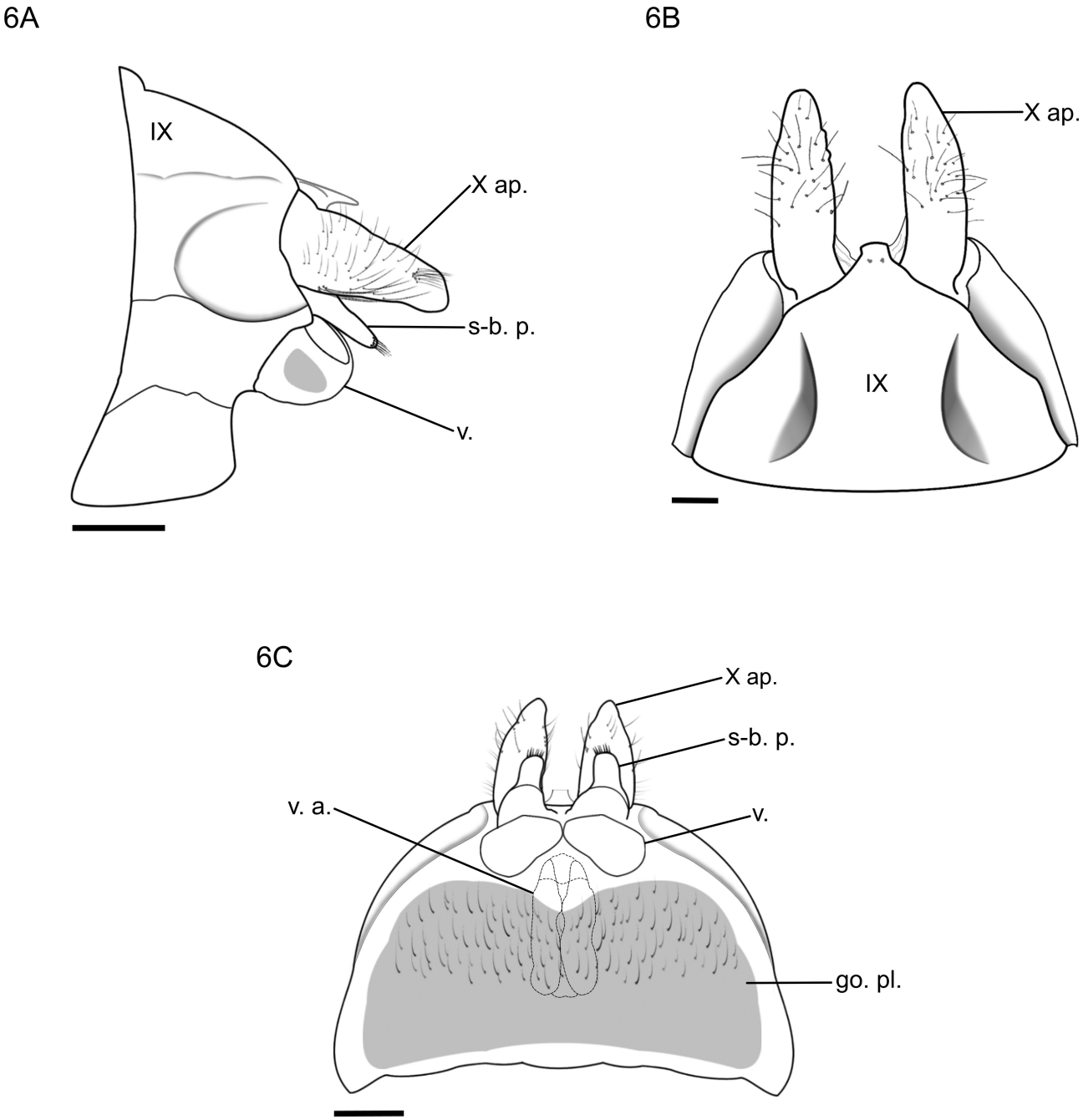

FIGURE 6. *Triplectides buengkanensis* **sp. nov.**, adult female genitalia, paratype. 6A, left lateral; 6B, dorsal; 6C, ventral. Abbreviations: X ap. = appendages of segment X; s-b. p. = sensilla-bearing processes; go. pl. = gonopod plate; v. = valves; v. a. = internal vaginal apparatus. Scale bars: 0.1 mm.

Male genitalia (Figs 5A–5E). Segment IX (IX), in lateral view, tall, longitudinally short, subrectangular, longer dorsally than ventrally, constricted at 3/4 height with acute incisions on anterior and posterior margins, posterior margins projecting and triangular at 2/3 height. In dorsal view, tergum IX subpentagonal with posterior margin almost rounded, with tiny notch and membranous digitate median processes (Fig. 5B). Preanal appendages (pr. ap.) long and slender, 3/4 as long as tergum X, blunt apically, with many setae (Figs 5A, 5B). Tergum X (X), in lateral view,

wide basally, with dorsal and ventral margins almost straight, apex round (Fig. 5A); in dorsal view, slightly tapering posterad, bearing short lateral and apical setae, apex obliquely truncate, with shallow, V-shaped incision (Fig. 5B). In lateral view (Fig. 5A), apicodorsal lobe of first article of each inferior appendage long, extending beyond hooked second article (2nd art.) and tergum X, bearing long setae; in ventral view (Fig. 5C), first article broad basally with blunt subtriangular basomesal lobe, second article crescent-shaped, wide at base, tapering apically, sharply curved inward, with pointed apex. In lateral and ventral views (Figs 5A, 5C), basoventral lobe of each inferior appendage, long, shorter than preanal appendages, apically round, with long setae. In lateral view (Fig. 5D), phallic apparatus long, tubular, thicker near middle, curved upward apically; in dorsal view, bulb-like, with small phallotremal sclerite and pair of wide apices (Fig. 5E).

Female genitalia (Figs 6A–6C). In lateral view (Fig. 6A), tergum IX subrectangular, with sclerotized concave area posterolaterally, sternum smaller; in dorsal view (Fig. 6B), subtriangular, round apically, and with pair of small papillae posteriorly. In lateral and dorsal views (Figs 6A, 6C), appendages of segment X (X ap.) long and oval, with many setae; sensilla-bearing processes almost 1/2 as long as preanal appendages, blunt apically, each with 6 or 7 short, stout setae apically. In lateral view (Fig. 6A), valves (v.) semicircular, round apically, each with short setae ventrolaterally. In ventral view (Fig. 6C), gonopod plate (go.pl.) sub-rectangular, with blunt anterolateral angles and round posteriorly. In ventral view (Fig. 6C), internal vaginal apparatus (v. a.) subrectangular, twice as long as broad.

Immature stages. Unknown

Distribution. Thailand (Buengkan Province).

Etymology. The specific epithet, *buengkanensis*, refers to the type locality of the species, Buengkan Province, in northeast Thailand.

Discussion

Traditional taxonomy of the caddisflies relies mainly on the morphological characters of adult male genitalia (Johanson 2007; Malicky 2010). Therefore, identification of the female and immature stages is difficult. DNA barcoding thus proved very useful for the association of known adult males with unknown females or larvae (Pauls *et al*. 2010; Kilian *et al*. 2022; Orfinger *et al*. 2022). In addition, DNA barcoding possibly has also uncovered cryptic genetic divergent lineages in many morphological species (Pauls *et al*. 2010; Zhou *et al*. 2016). Several of these genetically divergent lineages have been formally described (Pauls *et al*. 2010; Peng *et al*. 2023). In this study, we report DNA barcoding sequences of 13 species of caddisflies from Thailand. Among these, eight are reported for the first time. The specimens and data will be useful as references for further studies, such as the association of the different life stages.

All but one of the 13 species collected in this study exhibit low intraspecific genetic divergence, with a maximum value of 3.08% observed in *E. mammus*. These species were all monophyletic, supported by high bootstrap values (>90%). The exception was *Cheumatopsyche lucida,* which displayed exceptionally high intraspecific genetic divergence (maximum of 13.61%). This divergence was attributed to the presence of four genetically distinct lineages revealed through phylogenetic analyses, with at least 9.43% sequence divergence between them. These lineages corresponded to four Barcode Index Numbers (BINs) recorded in the BOLD system: BOLD: AAM8123, BOLD: ACC6097, BOLD: AAW6472, and BOLD: ACC6320. Specimens obtained in this study formed a clade with a sequence from Thailand (GenBank accession: KX292952; Zhou *et al*. (2016)), which belonged to BIN BOLD: AAW6472. Two additional sequences from Thailand, retrieved from GenBank, formed distinct lineages and were treated as separate species based on ASAP species delimitation analysis. The fourth lineage comprised specimens from Indonesia. While the ASAP method treated the Indonesian lineage as a distinct species, the mPTP method grouped it with two lineages from Thailand. The ASAP analysis supported a recognition of these lineages as separate species, whereas the mPTP method recognized only two species: one for the lineage including specimens from this study and another combining the Indonesian lineage with two sequences from Thailand. These divergences might be explained by misidentifications of the specimens from which the sequences were obtained or may indicate morphologically cryptic species. Further studies are required to clarify the species status of these genetically divergent lineages of *C. lucida*.

Three distinct genetic lineages are found in *T. indicus* with a minimum genetic differentiation of 4.62%. These lineages were also treated as different species according to ASAP species delimitation and placed into different BINs in the BOLD system (BOLD: AAE7972, BOLD: ABA2801, and BOLD: ACH0310) for Thailand, Indonesia, and Laos clades, respectively. Because these lineages are geographically isolated, high levels of genetic differentiation could either be intraspecific genetic structure or the presence of morphologically cryptic species (Morinière *et al*. 2017). Further studies are required to clarify the taxonomic status of these lineages.

In this study, we describe a new caddisfly species discovered in northeastern Thailand. This species was assigned to the genus *Triplectides* based on its tibial spur formula, wing venation, and male genitalia. The tibial spur formula for the new species is 2,2,2, while the genus *Triplectides* typically exhibits a range of 0,0,0 to 2,2,4. Key wing characteristics include the presence of a crossvein in the hind wings. The male genitalia are characterized by long inferior appendages with a second article and basoventral lobes. The genus *Triplectides* was first defined by Kolenati (1859) and is the most species-rich genus within Triplectidinae, comprising approximately 70 described species (Malm & Johanson 2008; Desidério *et al*. 2020). It is widely distributed across regions including India, Southeast Asia, and Japan. Prior to this study, only one species, *Triplectides indicus*, had been recorded in Thailand (Chantaramongkol *et al*. 2010).

Morphological differentiation of *T. buengkanensis* **sp. nov.** is supported genetically. The phylogenetic tree based on the COI sequences clearly resolved the new species into a monophyletic clade. Based on the phylogenetic tree, *T. buengkanensis* **sp. nov.** is closest to *T. misakianus*. Both ASAP and mPTP species delimitation methods merged *T. buengkanensis* **sp. nov.** and *T. misakianus* into the same species. These species have a minimum genetic divergence based on COI sequences of 2.18%. Although in the present study, the limited number of *T. misakianus* (n = 1) obtained from BOLD prevent a robust assessment of intraspecific genetic variation that is critical for species delimitation, this level of genetic differentiation is slightly greater than the species genetic boundary (2%) of many DNA barcoding studies of Trichoptera (Zhou *et al*. 2010; Geraci *et al*. 2011). Furthermore, low interspecific genetic divergence is not uncommon for the Trichoptera. Minimum interspecific genetic divergence among closely related species can be as low as 0.25%, and many species have a distance to the nearest neighbor of <3% based on COI sequences (Morinière *et al*. 2017). Furthermore, cryptic species even with distinct morphological and ecological differences can have a sequence divergence of as low as 1.5% such as in the genus *Hydropsyche* (Zhou *et al*. 2016). Genetically closely related between *T. buengkanensis* **sp. nov.** and *T. misakianus* is agree with morphology as both species have many similar morphological characteristics including those of the forewings, shape and length of the preanal appendages, basomesal and basoventral lobes and apically acute second articles of the inferior appendages. However, in the female, *T. buengkanensis* **sp. nov.** can be clearly distinguished from *T. misakianus* by the longer fork I petiole and the absence of a transverse vein *r-m* in each hind wing (present in *T. misakianus*). In the male, the new species can be separated from *T. misakianus* by tergum X (obliquely truncate apically in dorsal view in *T. buengkanensis* **sp. nov.** but suboval in *T. misakianus*), and its V-shaped apicomesal incision (shallow in *T. buengkanensis* **sp. nov.** but deep in *T. misakianus*).

In conclusion, we have discovered a new caddisfly species, *T. buengkanensis* **sp. nov.**, from northeastern Thailand. This species is morphologically and genetically distinct from other closely related species of the genus *Triplectides*. Additionally, we report DNA barcoding sequences for 13 species of Trichoptera from five families, nine of which are recorded in Thailand for the first time. These DNA barcoding sequences will be invaluable for further taxonomic studies, particularly in associating different life stages (e.g., larvae and adults). Furthermore, DNA barcoding uncovered cryptic genetic diversity within *C. lucida* and *T. indicus*, highlighting the need for further investigation to resolve the taxonomic status of its genetically divergent lineages. Future studies should expand molecular sampling across a broader geographic range and integrate additional taxonomic approaches (e.g., morphology, ecology, behavior, and nuclear genetic markers) to refine species boundaries.

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