Tropical Natural History, Supplement 8 (2025): 227–237
https://doi.org/10.58837/tnh.25.8.266890
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Ephoron debaratana sp. nov., A New Species of Burrowing Mayfly (Ephemeroptera: Polymitarcyidae) from Thailand

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Received: 27 March 2025; Accepted: 10 July 2025; Date of Publication: 14 October 2025
https://zoobank.org/urn:lsid:zoobank.org:pub: 720D2BA0-91BF-4FB9-94FF-87A70CD493DA

ABSTRACT.– A new species of burrowing mayfly, *Ephoron debaratana* sp. nov., is described based on the larval stage. The morphological characteristics distinguished the new species from other known *Ephoron* species are the followings: 1) pigmented lateral tracheal branches on the abdominal gills, 2) number of tubercles on the tusk, and 3) triangular frontal process. The morphological evidence presented in this study is strongly supported by cytochrome c oxidase subunit I (*COI*) gene data. This new species was found in a headwater stream in Kanchanaburi Province, Thailand. The habitat information and a comparison to the known species of Thai larvae are also presented.

KEYWORDS: *COI*, Oriental region, headwater stream, new species

INTRODUCTION

The genus *Ephoron* Williamson, 1802 is a member of the family Polymitarcyidae (pale burrowers), belonging to the Order Ephemeroptera, and is classified as burrowing mayflies within a group called Ephemeroidea (Miller et al., 2018). This genus is widely distributed on continents around the world in the Holarctic, Oriental, Nearctic and Afrotropical regions (Ishiwata, 1996; Kluge, 2004). The nymphs of the genus *Ephoron* can be distinguished from other polymitarcyid mayflies by the presence of long mandibular tusks with numerous tubercles (Ishiwata, 1996). Their habitat consists of streams and rivers, where they construct U-shaped burrows in clay, clay-rock, or gravel-cobble substrates and employ a filter-feeding strategy (Edmunds et al., 1976; Bae and McCafferty, 1995; Edmunds and McCafferty, 1996). Approximately 15 species are known around the world (Techakijvej et al., 2021). In the Oriental region, the following six species were previously reported: *E. indicus* (Pictet, 1843); *E. annandalei* (Chopra, 1927); *E. birmanus* (Navás, 1933); *E. nanchangi* (Hsu, 1937); *E. punensis* (Dubey, 1970); and *E. ookaewae* Techakijvej & Phalaraksh, 2021. At present, only two species from the Oriental region are known at the larval stage: *Ephoron ookaewae* and *Ephoron nanchangi* (Techakijvej et al., 2021; Pang et al., 2024). In Thailand, two species of this genus have been reported: *Ephoron indicus* from only one imago (Chiang Mai Province) and *E. ookaewae* from larvae, a male imago, and a female imago (Nakhon Sawan Province) (Uéno, 1961; Techakijvej et al., 2021). In the present study, we describe a new species of the genus *Ephoron* based on larval specimens collected from a headwater stream in Kanchanaburi Province. In addition, cytochrome c oxidase subunit I (*COI*) gene data for the *Ephoron* genus from Thailand are provided.

MATERIALS AND METHODS

The larvae of *Ephoron* were collected from a headwater stream in Kanchanaburi Province, Thailand. Specimens were preserved in absolute ethanol. Measurements (in mm) and photographs were taken using a Nikon SMZ800 and ZEISS Stemi 305 stereoscopic microscope. For scanning electron microscopy (SEM), mandibles were dried in a critical point dryer and coated with gold. The specimens were then observed and photographed using a Philips XL30 SEM instrument. The final plates were prepared with Adobe Photoshop CC 2022. The material is deposited in the collection of the Zoological Museum at Kasetsart University (ZMKU) in Bangkok, Thailand.

Species identification was performed based on external morphological characteristics under a stereomicroscope. Key diagnostic characters such as the shape of frontal process, shape and number of tubercles on tusk, and spine between teeth. Morphological traits were compared with published descriptions of known *Ephoron* species.

Selected specimens were dissected for DNA extraction. Total DNA was extracted using a genomic DNA purification kit (NucleoSpin, Macherey-Nagel, Germany), following the manufacturer's protocol. The *COI* gene amplification was performed using LCO

1490 and HCO2198 (Folmer et al., 1994). The polymerase chain reaction (PCR) conditions and procedure were as described by Kwanboon et al. (2021). The PCR products were sent for purified sequencing by U2Bio Co., Ltd. (Korea). The genetic distances between the species were determined using Kimura-2-parameter (K2P) distances (Kimura, 1980), calculated using the MEGA11 program (Tamura et al., 2021). Sequence alignment and editing were performed using ClustalW in MEGA11. A phylogenetic tree was analysed using the maximum likelihood (ML) method, and the most appropriate evolutionary model was calculated using the Find Best DNA/Protein Models (ML) option test provided with MEGA11. The general time-reversible (GTR) nucleotide substitution model with a gamma distribution for rate variation across sites (+G) and a proportion of invariable sites (+I) was prepared using MEGA11 and the likelihood-ratchet method with 1000 bootstrap replicates. The nucleotide sequences obtained in this study were deposited in GenBank.

RESULTS

Taxonomy

Family Polymitarcyidae

Genus *Ephoron* Williamson, 1802

Type species.– *Ephoron leucon* Williamson, 1802, by monotypy

Ephoron debaratana sp. nov.

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(Figs 1–6)

Material examined.– Holotype larva (ZMKU-Ephe-046), THAILAND, Kanchanaburi Province, Thong Pha Phum District, Pat Sadu Klang, 349 m a.s.l., 14°33' 10.8"N, 98°33'34.3"E, 01.IX.2019, leg. B. Boonsoong. Paratypes 13 larvae (ZMKUEphe-047), same data as holotype.

Diagnosis.– The larva of *Ephoron debaratana* sp. nov. has a reddish-brown body and can be separated from *E. ookaewae* by the following characters: (1) 17–21 tubercles on mandibular tusk dorsally, (2) mandible with relatively large triangular shaped spine between outer and inner incisors, (3) frontal process triangular, and (4) abdominal gill with lateral tracheal branches pigmented.

Etymology.– The specific epithet is named in honour of Her Royal Highness Princess Maha Chakri Sirindhorn, who has a deep passion for natural history and Thailand's biodiversity. The name is derived from Debaratana Rajasuda, one of Her Royal Highness's royal titles.

Description of Holotype.– Immature larva (in alcohol, Fig. 1). Body length 12 mm without caudal filament and cerci; body milky white with reddish-brown markings.

Head: Pale yellowish, reddish brown between ocelli, anterior margin of head with a triangular frontal process medially, frons with dense fine setae (Fig. 2B). Compound eyes on dorsolateral margin; ocelli white, inner margin of ocelli black. Head width and pronotum width equal (Fig. 2A). Labrum rounded, overall covered densely with setae, with emargination medially (Fig. 5A). Mandibular tusks yellowish to golden in colour, shorter than pronotal width, curving downwards, straight as viewed laterally, with numerous brownish blunt tubercles (17–21 tubercles) (Figs 2B–D, 3A, B), with short and dense setae on upper surface near bases of tusk, with long setae on lateral surface; right mandible with strong, large, acute spine between outer and inner incisors (Figs 4A, B). Labium with numerous setae, paraglossae drop shaped covered with numerous setae above glossae, labial palpi two-segmented, second segments quite longer than others, held beneath and at right angles to glossae (Fig. 5D). Maxillae stout, with nearly trapezoidal galea-lacinia, crown with numerous setae; maxillary palpi two segmented, second segment twice as long as first segment, with densely shorter setae on inner margin and with longer setae on outer margin (Fig. 5B). Hypopharynx, lingua and superlinguae nearly rounded with setae on dorsal surface of both sides, densely on apical margin (Fig. 5C).

Thorax: Brown markings on dorsal surface. Legs whitish, each covered with long fine yellowish setae on dorsal and ventral surfaces. Forelegs strongest, stout and fossorial with several distinct rows of long simple setae; ventral margin of femora with a row of tubercles basally; tibiae flattened, inner margin with two rows of tubercles; tarsal claws hooked without denticles (Fig. 6A), length ratio of femur: tibia: tarsus ca. 1.0: 1.4: 0.5. Midleg shortest, similar in structure to foreleg. Hindleg similar in structure to foreleg.

Abdomen: Colour white with a black dot marking at base of each gill; abdominal terga with reddish-brown banding dorsolaterally, darker and wider towards the posterior end (Fig. 6C); abdominal sterna uniformly milky white. Gills on abdominal segments 1–7; gills 1 uniramous, leaf-shaped, light yellow in colour (Fig.

FIGURE 1. *Ephoron debaratana* sp. nov. Habitus of larva, dorsal view.

FIGURE 2. *Ephoron debaratana* sp. nov. **A.** head and prothorax, dorsal view. **B.** mandibular tusks and frontal process, dorsal view. **C.** head, lateral view. **D.** mandibular tusk, ventral view (upper) and dorsal view (lower).

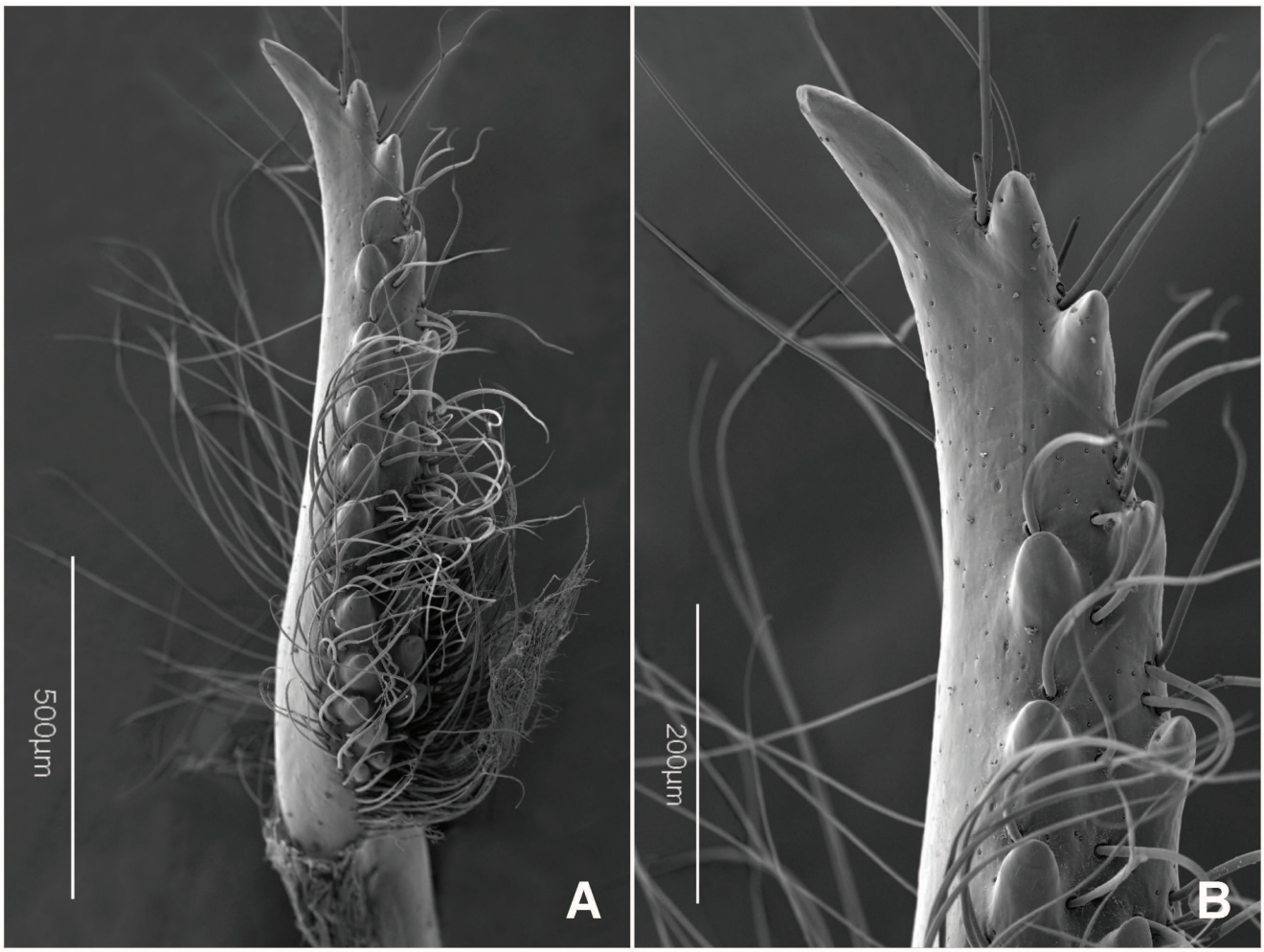

FIGURE 3. *Ephoron debaratana* sp. nov. **A.** right mandibular tusk, lateral view. **B.** apex and tubercles of right mandibular tusk, lateral view.

6B); gills 2–7 similar in shape, biramous; each lamella of gills 2–7 with lateral margin fringed, with lateral tracheal branches pigmented (Fig. 6C), dorsal lamella longer than ventral lamella and slightly curved, ventral lamella approximately 3/4 the length of dorsal lamella. Three caudal filaments with setae, ca. 1/3 body length.

DNA barcodes.– DNA materials were extracted from two paratypes. Other analysed *Ephoron* sequences were obtained from GenBank, with accession numbers given in Table 1. *Protobehningia merga* (Behningiidae), retrieved from GenBank (MW292224), was used as an outgroup.

Distribution and habitat.– The new species is known only from the type locality of Kanchanaburi Province, Thailand (Fig. 7A). The larvae of *E. debaratana* sp. nov. were collected from a sandy bottom with cobble and pebble substrates in a shallow headwater stream (Fig. 7B).

Molecular analysis

The partial sequences of the mitochondrial *COI* gene (658 bp) of the two species (unpublished sequence data for *Ephoron* sp. A) found in Thailand were obtained from specimens of each locality. These sequences were compared with *COI* sequences of five valid species retrieved from database: *E. ookaewae* (Thailand), *E. nanchangi* (China) and *E. shigae*, *E. limnobium E. eophilum* (Japan) (Table 1) to investigate the species delineation. The K2P analysis revealed mean intraspecific genetic distances of 2.76% for *E. debaratana* sp. nov. and 4.07% for *Ephoron* sp. A. The mean interspecific distances between Thai *Ephoron* are high, ranging from 15.77–17.05% (Table 2). The sequences from two specimens of *E. debaratana* and three sequences from *Ephoron* sp. A present a mean K2P interspecific distance of 17.05%, while the mean interspecific distance between *E. debaratana* and *E. ookaewae* is 16.17% and the interspecific distance between *E. ookaewae* and *Ephoron* sp. A is 15.77%

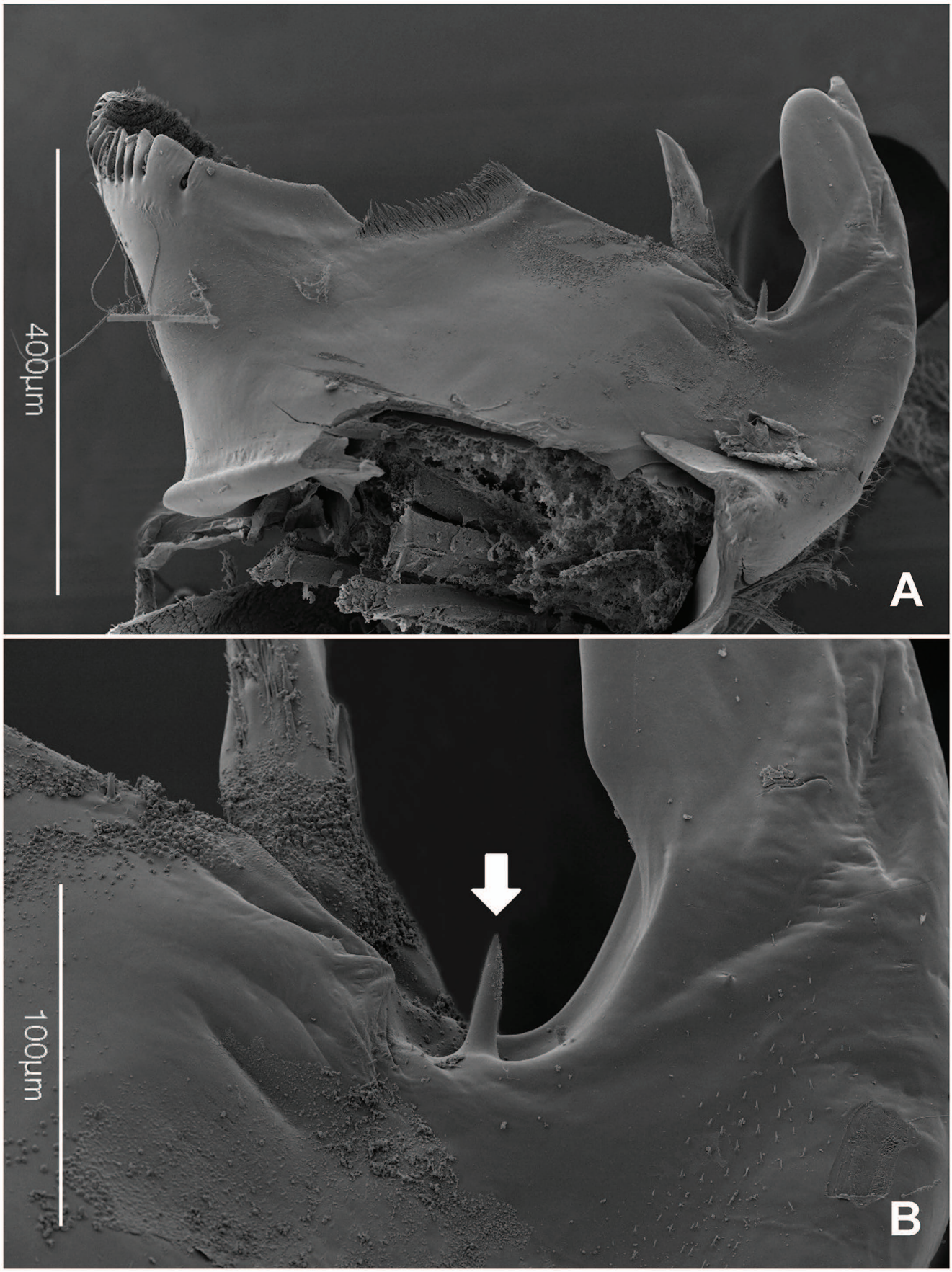

FIGURE 4. *Ephoron debaratana* sp. nov. **A.** right mandible, anteroventral view. **B.** spine (arrow) between outer and inner incisors of right mandible.

TABLE 1. Sequenced specimens of the genus *Ephoron* in this study.

Species	Locality	GenBank accession no. / Code
E. debaratana sp. nov.	Kanchanaburi, Thailand	PV920441
	Kanchanaburi, Thailand	PV920440
Ephoron sp. A	Chiang Rai, Thailand	EP05CR
	Chiang Rai, Thailand	EP06CR
	Chiang Mai, Thailand	EP07CM
E. ookaewae	Nakhon Sawan, Thailand	MW168821
E. nanchangi	China	OQ439818
E. shigae	Japan	LC635033
	Japan	LC635042
E. limnobium	Japan	AB711781
	Japan	AB711785
E. eophilum	Japan	AB711807
	Japan	AB711808

TABLE 2. Genetic distances (COI) between sequenced specimens using the Kimura 2-parameter.

	Species	1	2	3	4	5	6	7
1	*E. debaratana* sp. nov. (Thailand)	2.76						
2	*E. ookaewae* (Thailand)	16.17	-					
3	*Ephoron* sp. A (Thailand)	17.05	15.77	4.07				
4	*E. nanchangi* (China)	16.63	17.78	17.34	-			
5	*E. shigae* (Japan)	14.91	17.45	17.58	16.19	1.26		
6	*E. limnobium* (Japan)	15.33	17.38	17.16	16.11	4.12	1.63	
7	*E. eophilum* (Japan)	13.38	16.91	15.82	17.45	12.93	13.48	0.18

(Table 2). The mean interspecific distances between the Thai species and the East Palaearctic region species vary from 13.38–17.78%. The lowest value is observed for *E. debaratana* and *E. eophilum* from Japan, at 13.38%, and the highest value is observed for *E. ookaewae* and *E. nanchangi* from China, at 17.78%.

The maximum likelihood (ML) analysis of the *COI* gene data showed that *E. debaratana* sp. nov. forms a monophyletic lineage that is included within the Palaearctic species clade but is separated from the other Thai species. *E. nanchangi* displays a monophyly among the others, and two species from Northern Thailand, *E. ookaewae* and *Ephoron* sp. A, are included in the same clade (Fig. 8).

DISCUSSION

In this study, the morphology of *E. debaratana* sp. nov. reveals that it is closely related to *E. shigae* from Japan, as both share key characteristics, such as a triangular frontal process, an acute spine between the outer and inner incisors on the right mandible and gills 2–7 with fringed lateral margins and pigmented lateral tracheal branches. However, both species differ in some characters, including the number of tubercles on the mandibular tusk and body colouration; these two species also inhabit different geographic areas, as *E. shigae* has been reported in the East Palaearctic region (Ishiwata, 1996).

In 2021, *Ephoron ookaewae* Techakijvej & Phalaraksh, 2021 was reported from Thailand. It is the only species with nymphs recorded in the country. *E. ookaewae* is characterised by a short mandibular tusk that is not curved inwards as much as in other species and by the presence of a few tubercles on their tusks (Techakijvej et al., 2021). Surprisingly, the new species, *E. debaratana* sp. nov., differs from *E. ookaewae* in both its tusk length and number of tubercles, highlighting its distinct morphological identity. A comparison of the larval characteristics of Asian *Ephoron* species is shown in Table 3.

The first *COI* data for the genus *Ephoron* in the Oriental region was documented by Techakitvej et al. (2021) with a single sequence of *E. ookaewae*. Our study represents the first comprehensive molecular analysis of this genus from the Oriental region, combined with selected sequences from the East Palaearctic region. There is no doubt that *E. debaratana* sp. nov. is a new species, based on the molecular data. This new species forms a monophyletic lineage, which is included within the East Palaearctic species clade but is distinct from other Thai species, as indicated by the high mean K2P genetic distance of 16.17–17.05%. This was surprising, as we had expected the molecular data to show that other new

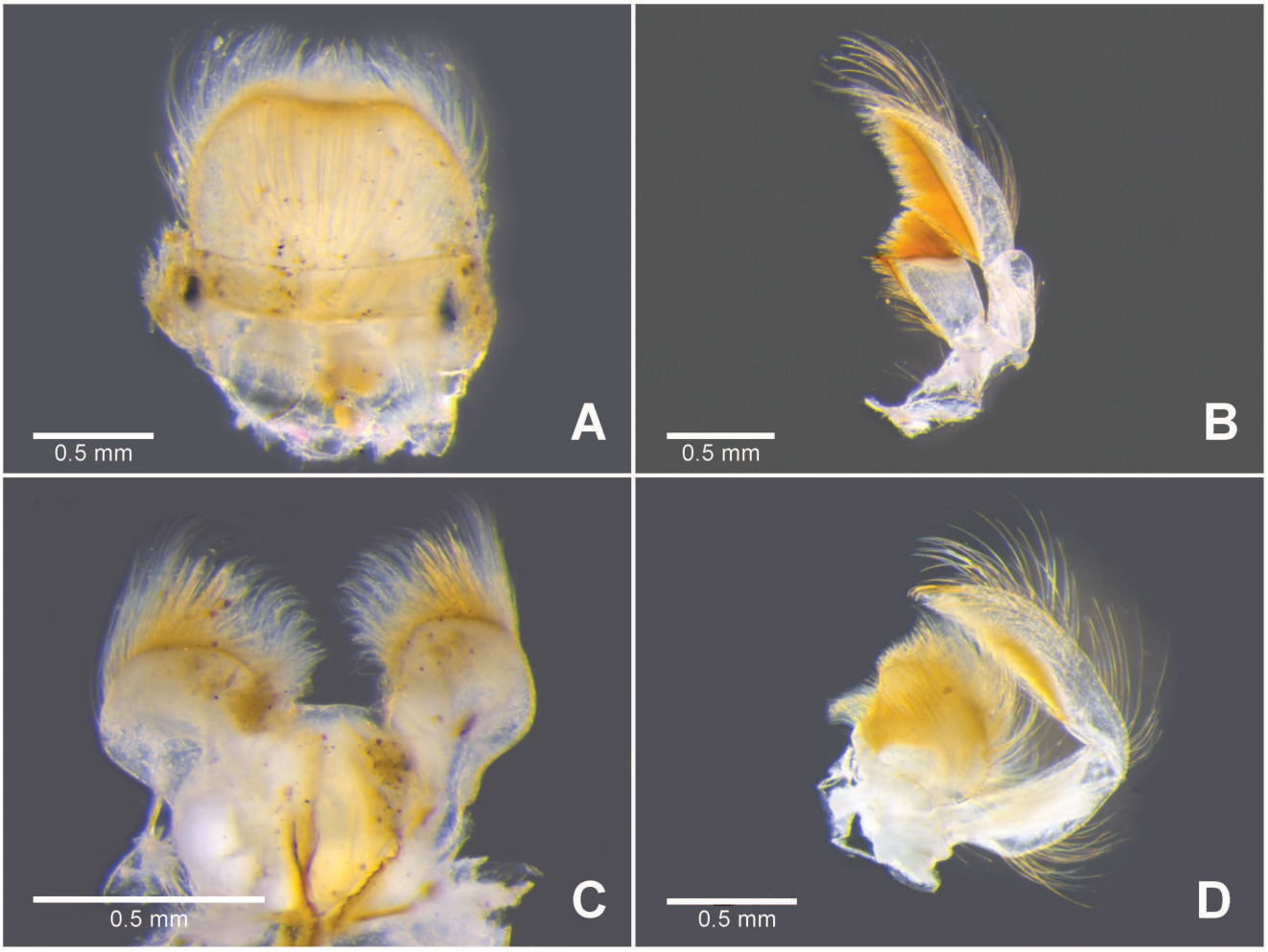

FIGURE 5. *Ephoron debaratana* sp. nov. **A.** labrum, dorsal view. **B.** left maxilla, ventral view. **C.** hypopharynx, dorsal view. **D.** labium (left side), ventral view.

TABLE 3. Comparison of larval characteristics of Asian *Ephoron* species.

	***E. debaratana* sp. nov.**	***E. ookaewae***	***E. limnobium***	***E. shigae***	***E. eophilum***	***E. nanchangi***
Marking on abdominal terga	reddish-brown	dark brown	light brown	brown	dark brown	light brown
Shape of frontal process	triangular	3-4 point median	triangular	triangular	semicircular	triangular
Spine between outer and inner incisors on right mandible	acute spine	acute spine, small	acute spine	acute spine	blunt spine	blunt spine
Lateral branch tracheal of gill II-VII	with pigment	no pigment	no pigment	with pigment	no pigment	no pigment
Number of tubercles on tusk	17-21	5-7	16-27	17-29	12-25	21-25
Habitat	headwater stream	river	lake	river	river	river
Distribution	Thailand	Thailand	Japan	Japan	Japan	China
References	This study	Techakijvej et al. (2021)	Ishiwata (1996)	Ishiwata (1996)	Ishiwata (1996)	Pang et al. (2024)

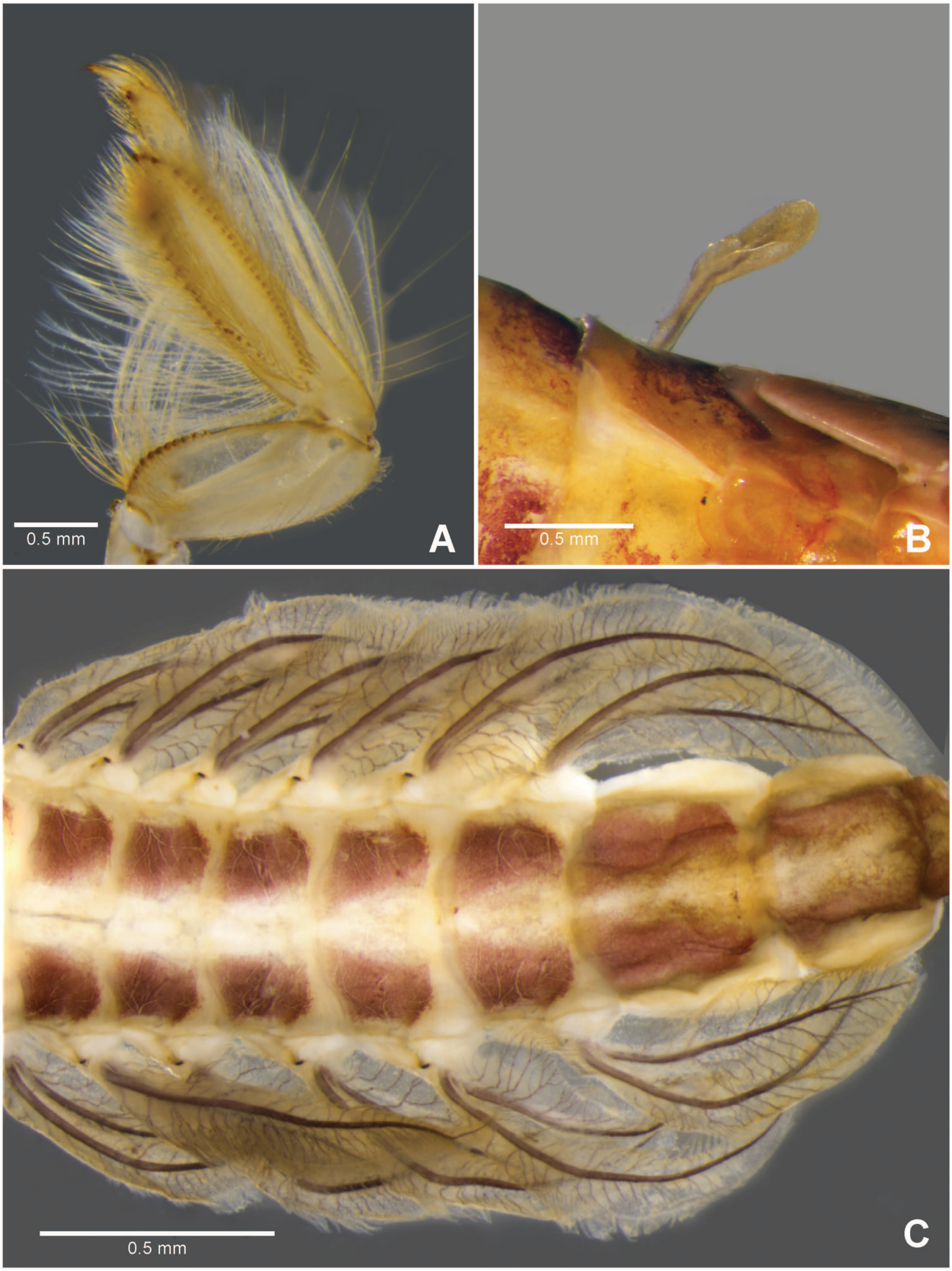

FIGURE 6. *Ephoron debaratana* sp. nov. **A.** foreleg (ventral view). **B.** gill 1 (ventral view). **C.** abdomen (dorsal view).

FIGURE 7. Habitat of *Ephoron debaratana* sp. nov. **A.** type locality. **B.** microhabitat.

species of this genus from northern Thailand were more closely related to *E. ookaewae*. Additional material and morphological investigations will be necessary to confirm their status in the future.

The ecological factors also differ between the habitats of *E. debaratana* sp. nov. and those of other Asian species. The larvae of *E. debaratana* sp. nov. were collected from a headwater stream (Fig. 7), whereas most Asian species were collected from rivers and lakes (Chopra, 1927; Ishiwata, 1996; Techakijvej et al., 2021, Pang et al., 2024). Among the Thai *Ephoron* species, the distribution of the two previously reported species (*E. indicus* and *E. ookaewae*) has been recorded in northern Thailand, in Chiang Mai Province and Nakhon Sawan Province, respectively (Uéno, 1961; Techakijvej et al., 2021). Although the adult stage of *E. debaratana* could not be collected at this time, the species is proposed based on its distinct larval morphology, molecular characteristics, and ecological isolation. Habitat specialization has been recognized as an important supporting factor in species delimitation, particularly among burrowing mayflies, where micro-habitats often correlate with genetic and morphological divergence. In this context, ecological niche of *E. debaratana* suggests adaptation to headwater streams, as opposed to other *Ephoron* species in the Oriental region.

Two Oriental *Ephoron* species (*E. indicus* and *E. birmanus*) are limited status and distribution in Southeast Asia. The adult of *E. birmanus* was shortly described by Navás (1933) based on material from Myanmar. The adult record of *E. indicus* in Thailand is limited (Uéno, 1961) and other localities of this species collected near rivers (Chopra, 1927). In Thailand, the adults of *E. ookaewae* and *Ephoron* sp. A were found only the river habitat of northern Thailand. Taylor et al. (2020) found that mayfly species with specialised habitat preferences have significant genetic hetero-geneity across catchments, indicating adaptability to varied environmental conditions. Similarly, Okamoto et al. (2021) observed that closely related mayfly species in Japan occupy different microhabitats along river gradients, resulting in niche segregation and genetic divergence.

Herein, we report a new species of *Ephoron* from western Thailand, Kanchanaburi Province, based on a combination of morphological, molecular evidence and ecological isolation. The finding of a distinct habitat preference compared to other known Oriental region species also supports the validation of this new species. This study also extends the distribution of this poorly studied genus in Thailand, as we gain a more in-depth understanding of the distribution patterns of this genus in the Oriental region.

ACKNOWLEDGEMENTS

We are most grateful to our colleagues for their assistance during field trips. We thank the MZL team (Muséum Cantonal des Sciences Naturelles (Natu-réum), Lausanne) and the Department of Zoology, the Faculty of Science and the Faculty of Engineering at Kasetsart University in Bangkok for their assistance and use of their facilities. This research was approved by the Institutional Animal Care and Use Committee, Faculty of Science, Kasetsart University, Thailand, under Project number ACKU61-SCI-028. This study was financially supported by the Centre of Excellence on Biodiversity (BDC) Office of Higher Education Commission (BDC-PG2-161004). We are grateful to all the reviewers for their helpful comments and suggestions that contributed to improving the manu-script. This research was funded by Kasetsart University through the Graduate School Fellowship Program.

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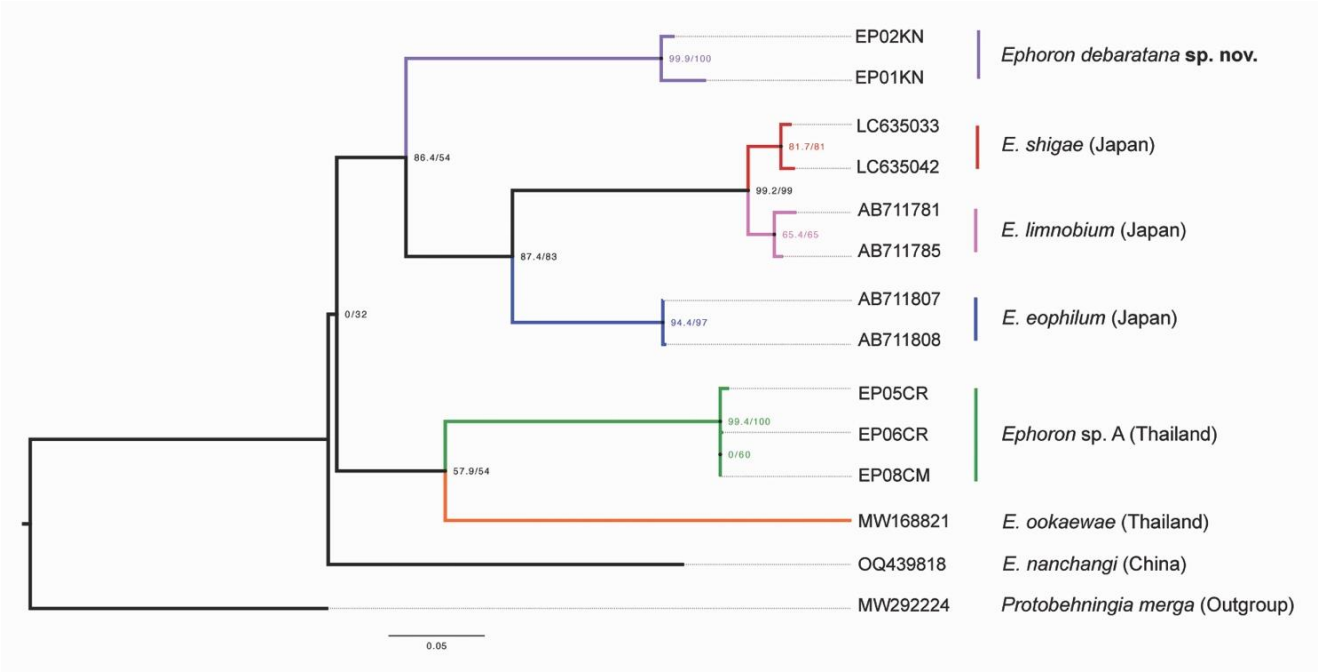

FIGURE 8. Consensus phylogenetic tree based on the maximum likelihood (ML) analysis of seven Asian *Ephoron* species. *Protobehningia merga* from GenBank was used as the outgroup. There were a total of 658 positions in the final dataset. Abbreviations are the same as those found in Table 1.

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