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Associations of mayfly larvae with *Corbicula* clams

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Currently, the parasitic and endosymbiontic fauna of *Corbicula* clams remain poorly studied throughout their range. Here, using samples from the Mekong basin, we describe two *Symbiocloeon* species new to science: ***Symbiocloeon corbiculinus* sp. nov.** and ***Symbiocloeon laoensis* sp. nov.** Our results and a review of available published data indicate that freshwater bivalve-associated mayflies are narrow host specialists, being associated with one or a few closely related species of freshwater bivalves. The bivalve-associated mayfly larvae have several specific morphological traits compared with free-living species, which include a reduction of integument chitinization and a significant reduction of the surface structures on segments. An increase in area of the respiratory surface of larval tergalia was also recorded. The possible positive and negative effects of mayflies on the clam hosts are discussed. This study is an example of the many possible hidden associations between aquatic species that remain to be described.

ADDITIONAL KEYWORDS: adaptations – *Corbicula* – endemic species – endosymbionts – Laos – mayfly – Thailand.

INTRODUCTION

Several shortfalls can impair a comprehensive knowledge of biodiversity, including gaps in taxonomy (the Linnean shortfall) and in biotic interactions (the Eltonian shortfall), among several others (reviewed by Hortal *et al.*, 2015). These shortfalls are especially prominent in invertebrates and in aquatic ecosystems (Lopes-Lima *et al.*, 2021).

Despite the persistent lack of knowledge about biotic interactions in freshwater invertebrates, symbionts, commensals and parasites living in the mantle cavity of freshwater bivalves have begun to be studied actively (Bolotov *et al.*, 2019, 2020; Brian & Aldridge, 2019, 2021; Brian *et al.*, 2021; Chapurina *et al.*, 2021; Taskinen *et al.*, 2021). This topic attracts the full attention of researchers mainly because of a number of negative impacts of parasites on bivalve populations (Taskinen & Valtonen, 1995; Taskinen, 1998; Jokela *et al.*, 2005; Walker, 2017; Müller *et al.*, 2015). In addition, bivalves can play fundamental ecological roles in aquatic ecosystems, but these roles (nutrient cycling, habitat for other species, and transfer of

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energy from the water column to the benthos; Vaughn & Hakenkamp, 2001; Zieritz *et al.*, 2022) are density dependent, hence the possible effects of endosymbionts (or predators, competitors, etc.) on bivalve population density and structure or even on the performance of a given function (or services) should not be ignored (e.g. see Brian *et al.*, 2022). Therefore, understanding the interactions between bivalves and their endosymbionts is one of the high-priority areas of research for assessing population trends and species health (Brian & Aldridge, 2019; Ferreira-Rodríguez *et al.*, 2019).

The native range of the genus *Corbicula* Megerle von Mühlfeld, 1811 extends into the Middle East, India, Southeast Asia, East Asia, the Indonesian Archipelago, Australia and Africa (Haponski & Ó'Foighil, 2019). Asian *Corbicula* clams (Bivalvia: Cyrenidae) are one of the most successful invaders in American and European aquatic ecosystems (Pigneur *et al.*, 2014; Crespo *et al.*, 2015; Bespalaya *et al.*, 2018, 2021). Despite decades of intense studies on *Corbicula* clams, reliable data on their parasitic fauna and symbionts are relatively sparse (Taskinen *et al.*, 2021; Brian *et al.*, 2022). Currently, three genera of parasitic trematodes have been reported in the native range of *Corbicula* clams in Asia (*Phyllodistomum*, *Echinostoma* and *Aspidogaster*; Tang, 1992; Chung *et al.*, 2001; Keeler & Huffman, 2009; Alves *et al.*, 2015). Moreover, there are two parasitic flatworms (aspidogastrid and echinostomatid trematodes) associated with invasive populations of Asian clams from North America (Danford & Joy, 1984; Karatayev *et al.*, 2012). It was also described that *Corbicula fluminea* (Müller, 1774) is infested by *Unionicola* mites (Abdel-Gaber *et al.*, 2018) and oligochaete (Liquin *et al.*, 2021). Finally, it was discovered that mayflies, chironomids and gudgeon fish can use *Corbicula* clams as hosts for their larval development in the native range (Bespalaya *et al.*, 2022). However, recent studies did not find parasites in *Corbicula* spp. in invaded European waterbodies (Taskinen *et al.*, 2021). Even less information is available on relationships of *Corbicula* clams and their possible endosymbionts.

The family Baetidae is one of the most diverse and widespread groups of mayflies (Sartori & Brittain, 2015; Tungpairojwong & Bae, 2015; Suttinun *et al.*, 2020). This family comprises nearly 1070 species in 110 genera worldwide (Barber-James *et al.*, 2008; Sartori & Brittain, 2015; Suttinun *et al.*, 2020). However, the state of our knowledge of this family varies considerably depending on the geographical region; some areas of North America and Europe are well known, whereas others, such as Southeast Asia, remain insufficiently studied (Sartori & Brittain, 2015). According to the literature, 150 species of mayflies have been described from the Oriental Region (Barber-James *et al.*, 2008; Sartori & Brittain, 2015). However, the number of species known from this area certainly remains much lower than the real diversity (Sartori & Brittain, 2015; Suttinun *et al.*, 2020), because large areas are still *terra incognita* (Barber-James *et al.*, 2008). Associations of the mayfly genus *Symbiocloeon* Müller-Liebenau, 1979 with freshwater mussels (Order Unionida) were previously recorded in tropical Asia (Thailand) (Müller-Liebenau & Heard, 1979) and India (Subramanian & Sivaramakrishnan, 2009), and those of the genus *Mutelocloeon* Gillies & Elouard, 1990 (Ephemeroptera: Baetidae) in Sub-Saharan Africa (Mali and Guinea) (Gillies & Elouard, 1990). Mayfly larvae were also discovered on the gills of *C. fluminea* from Thailand and Laos (undescribed species *Symbiocloeon* sp. 1 and *Symbiocloeon* sp. 2, respectively; Bespalaya *et al.*, 2022). It was established that the mayfly larvae in both genera have some adaptations for a hidden lifestyle inside the mantle cavity of freshwater bivalves (Müller-Liebenau & Heard, 1979; Gillies & Elouard, 1990). This type of biotic association was considered to be commensalism (Müller-Liebenau & Heard, 1979) or even inquilinism (a variety of commensal relationship) (Gillies & Elouard, 1990). However, it is still unclear whether this relationship should be classified, given the lack of ecological studies assessing these biotic relationships and the benefits and/or costs for both species involved.

Given all the above-mentioned taxonomic gaps, the main goals of the present study were as follows: (1) to introduce two new *Symbiocloeon* species based on molecular genetic and morphological data; (2) to describe morphological adaptations of these larvae to a specific lifestyle; and (3) to discuss the relationships of mayflies with their clam hosts within a broader evolutionary and ecological context.

MATERIAL AND METHODS

Data collection and DNA analyses

The Mun River and its tributary, the Lam Chae Stream, in Thailand and the Sein Kaphoe River in Laos were surveyed in March 2018 and March 2020, respectively (Fig. 1). Both sites are located within the Mekong Basin. Samples of *Corbicula* clams were collected by snorkelling and wading. The samples were fixed in 96% ethanol immediately after collection. A total of 211 clam specimens were collected. The mayfly larvae were collected from the mantle cavity of the clams using forceps. The body lengths of 25 mayfly larvae were measured by using a Leica M165C research microscope (Supporting Information, Table S1).

We also measured a total of 36 shells of *Corbicula* from Sein Kaphoe River in Laos. The shell length (SL), height (SH) and width (SW) were measured to the nearest 0.1 mm using dial callipers (Supporting Information, Table S2).

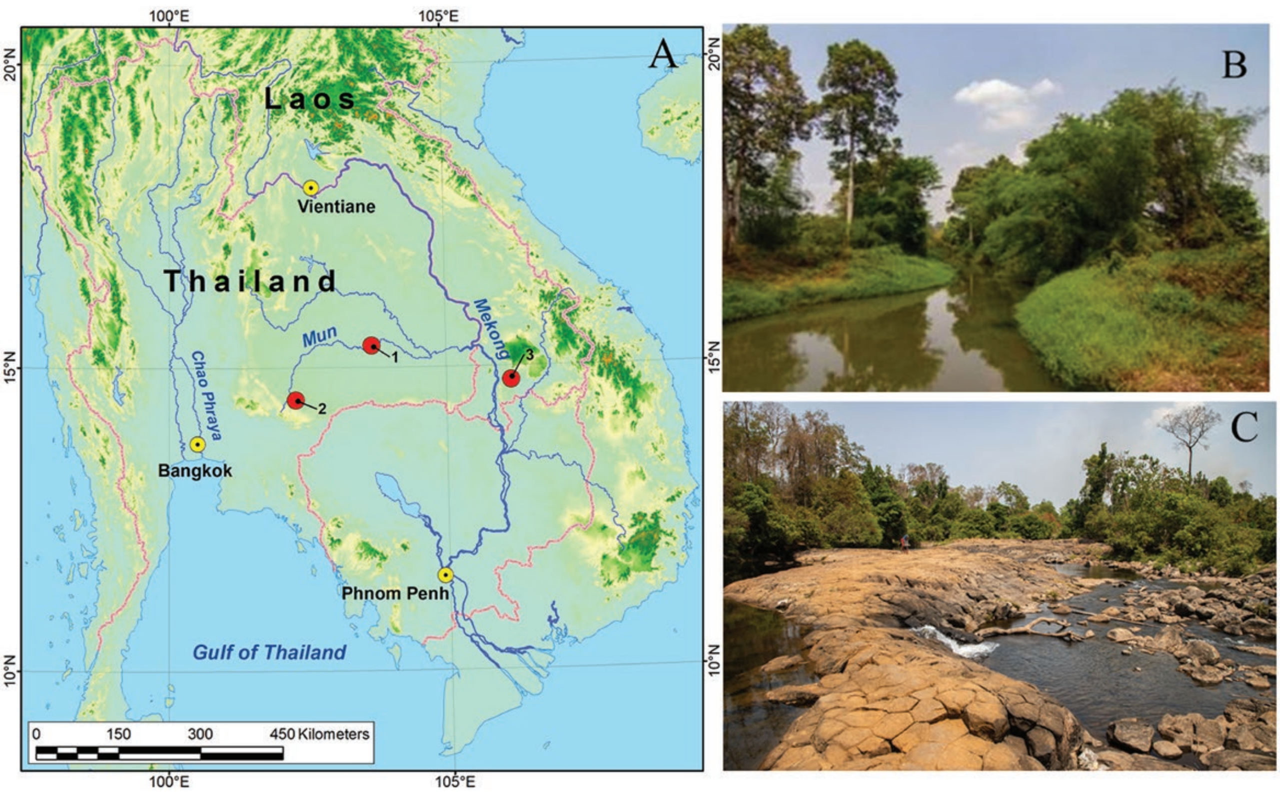

Figure 1. Localities of the new bivalve-associated mayfly species. A, map of the localities (red circles): ***Symbiocloeon corbiculinus*** (1 and 2) and ***Symbiocloeon laoensis*** (3). B, type locality and habitat of ***S. corbiculinus***. C, type locality and habitat of ***S. laoensis***. Photographs: Ilya V. Vikhrev.

The materials are stored in the Russian Museum of the Biodiversity Hotspots (RMBH) of N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Arkhangelsk, Russia). The holotypes of *Symbiocloeon* species (Ephemeroptera: Baetidae) are deposited in the collection of the Zoological Museum of Moscow State University, Moscow, Russia (ZMMU).

DNA ISOLATION, POLYMERASE CHAIN REACTION AND SEQUENCING

Total DNA was extracted from 96% ethanol-preserved *Corbicula* foot tissues and from whole *Symbiocleon* larvae using the NucleoSpin Tissue Kit (Macherey-Nagel, Germany), following the manufacturer's protocol (Supporting Information, Table S3). As the primer pairs for *Corbicula* clams, we used LCO 1490 and HCO2198 (Folmer *et al.*, 1994), and for mayflies we used the primer pairs LoboF and LoboR for the cytochrome *c* oxidase subunit I (*COI*) gene fragment (Lobo *et al.*, 2013). The PCR mix contained ~100 ng of total cell DNA, 10 pmol of each primer, 200 µmol of each dNTP, 2.5 µL of PCR buffer (with 20 mmol $MgCl_2$) and 0.8 units Taq DNA polymerase (SibEnzyme, Russia), with H_2O added, for a final volume of 25 µL. Temperature cycling for *Corbicula* clams was as follows: 95 °C for 4 min; 36 cycles of 95 °C for 50 s, 52 °C for 50 s and 72 °C for 50 s; and a final extension at 72 °C for 5 min. Temperature cycling for mayflies was as follows: 95 °C for 4 min; 28 cycles of 95 °C for 50 s, 50 °C for 50 s and 72 °C for 50 s; and a final extension at 72 °C for 5 min. Forward and reverse sequencing was performed on an automatic sequencer (ABI PRISM 3730; Applied Biosystems) using the ABI PRISM BigDye Terminator v.3.1 reagent kit. The resulting sequences were checked manually using the sequence alignment editor BIOEDIT v.7.2.5 (Hall, 1999).

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

The sequences were aligned using the MUSCLE algorithm of MEGA7 (Kumar *et al.*, 2016).

The phylogeographical analyses for *C. fluminea* were performed based on a median-joining network approach using NETWORK v.4.6.1.3 software with default settings (Bandelt *et al.*, 1999). In addition, 71 *COI* sequences of *Corbicula* clams were obtained from NCBI GenBank (Supporting Information, Table S4).

The *Symbiocleon* sequence alignment was collapsed to unique haplotypes using an online FASTA sequence toolbox FaBox v.1.61 (Villesen, 2007). The maximum likelihood (ML) phylogenetic reconstruction was performed using IQ-TREE v.1.6.12 through an online server (http://iqtree.cibiv.univie.ac.at), with an automatic identification of the most appropriate evolutionary model (Nguyen *et al.*, 2015; Trifinopoulos *et al.*, 2016). Node support values were calculated using an ultrafast bootstrapping algorithm (Hoang *et al.*, 2018). In addition, six *COI* sequences of mayfly species (Baetidae) were obtained from NCBI GenBank and BOLD System v.4 (Supporting Information, Table S5).

MORPHOLOGICAL STUDIES

Macrophotographs of the type specimens of two mayfly species (larval stage) studied here were taken through Leica M205 C and Leica Z16 APO stereomicroscopes equipped with a Leica DFC450 digital camera using Leica Application Suite v.3.1.8, and taken using a Canon EOS 7D camera with a Sigma AF 24–70 mm, f/2.8 IF EX DG Aspherical HSM Canon EF lens (Canon, Tokyo, Japan). Photograph stacks were processed with Helicon Focus Pro v.6.4.1 to obtain combined photographs with extended depth of field and, subsequently, enhanced with Adobe Photoshop CS3.

Permanent slides were prepared with Faure–Berlese's mounting medium. Morphological features were photographed using a digital camera (ToupCam 9.0 MP; Hangzhou ToupTek Photonics, Hangzhou, China) attached to a light microscope (Olympus CX21; Olympus, Tokyo, Japan).

Scanning electron microscopy was used for investigation and visualization of larval structures (mouthparts and the surface of cuticula) of the two new species described here. Dissected body parts were dehydrated through a stepwise immersion in ethanol, dried by critical point drying, and mounted on scanning electron microscope stubs. The larval material was analysed using Vega3 Tescan in the Palaeontological Museum of the Palaeontological Institute, Russian Academy of Sciences, Moscow, Russia [PIN]. All the photographs were subsequently sharpened and adjusted for contrast and tonality in Adobe Photoshop CS6.

TERMINOLOGY AND NOMENCLATURAL ACTS

The terminology and respective acronyms proposed by Gaino & Rebora (1996, 2003) and Godunko *et al.* (2015) were used to describe body setation of larvae: *B* (for sensillum basiconicum), *FT* (for designation of flat-tipped sensillum), *Hr* (for hair-like setae), *STS* (for stout setae), *SC* (for scales) and *SCS* (for scales sockets), followed by alphabetical subscripts. The subscripts refer to the size of setae (symbols *e*, *m* and *s*), and setae/scales and the shapes their sockets (for details, see Godunko *et al.*, 2015: table 1 and fig. 41).

Morphological terms are given according to Müller-Liebenau (1969), Gaino & Rebora (1996, 2003), Kluge (2004), Godunko *et al.* (2017) and Bauernfeind & Soldán (2012).

RESULTS

RECORD OF MAYFLY LARVAE FROM THE GENUS *SYMBIOCLOEON* IN MANTLE CAVITY OF *CORBICULA*

The Asian clams in the Lam Chae Stream were collected in sandy sediments, with depths ≤ 1.5 m. *Corbicula* specimens in the Sein Kaphoe River were collected in sandy and rocky sediments, with depths ≤ 1 m.

The mayfly larvae were discovered in the mantle cavity of a *Corbicula* species from the Lam Chae Stream (Mun River basin) in Thailand, and from the Sein Kaphoe River in Laos. In total, 175 *Corbicula* individuals from Thailand were examined, with 47 clams being infested by 48 larvae. Among 36 *Corbicula* clams examined from Laos, eight were infested by eight larvae.

The average shell measurements (±SD; $N = 36$) from the Sein Kaphoe River are as follows: SL = 11.7 ± 2.6 mm (min–max 8.0–17.3 mm), SH = 9.4 ± 2.2 mm (min–max 6.0–15.3 mm) and SW = 5.7 ± 1.5 mm (min–max 3.9–9.0 mm) (Supporting Information, Table S2). Unfortunately, we did not measure specimens from Lam Chae Stream.

The highest percentage of infested *Corbicula* clams (52%) was found in Site 2 of the Lam Chae Stream (Thailand). In the Sein Kaphoe River (Laos), 22% of *Corbicula* clams were infested. Usually, each infested individual of *Corbicula* housed a single mayfly larva. In one individual of *Corbicula* from Thailand, we found two larvae in the mantle cavity. Larvae were mainly attached to the gills and, to a lesser degree, to the base of the foot (Supporting Information, Table S6).

The body length of larvae of *Symbiocloeon corbiculinus* Palatov sp. nov. varied from 4.3 to 9.3 mm and in *Symbiocloeon laoensis* Palatov sp. nov. from 5.5 to 8.2 mm (Supporting Information, Table S1).

We did not find significant differences between the size of the shells of infested and non-infested individuals of *Corbicula* clams from the Sein Kaphoe River (Mann–Whitney *U*-test: $P > 0.05$).

Three freshwater mussel species were found living in sympatry with *Corbicula* clams, namely *Pilsbryoconcha compressa* (Martens, 1860) ($N = 10$)

from Lam Chae Creek, and *Bineurus anodontinum* (Rochebrune, 1882) (*N* = 18) and *Bineurus exilis* (Morelet, 1866) (*N* = 22) from the Sein Kaphoe River Stream. In general, 50 individual freshwater mussels were examined for the presence of parasites. However, the mayfly larvae were found only in *Corbicula*.

Mitochondrial DNA sequences

A total of seven new *COI* sequences from mayfly specimens from the Lam Chae Stream and Sein Kaphoe River were obtained during this study. Our molecular genetic (Fig. 2) and morphological analyses (Figs 3–12) indicated that the mayfly larvae belonged to two species from the genus *Symbiocloeon* (Ephemeroptera: Baetidae). The distance between our two species was 2% (this was 13 substitutions for a length of 658 bp of *COI*).

In addition, six new *COI* sequences from the *Corbicula* sample were retrieved. The *COI* dataset contained the single FW5 haplotype. The *COI* haplotype network revealed that this haplotype belonged to the widespread (in the native and non-native ranges) species *C. fluminea* (Supporting Information, Fig. S1).

TAXONOMY

Given that DNA-based studies on the type species of the genus *Symbiocloeon* have not been carried out, the generic affiliation of the studied mayfly larvae was determined solely on the basis of their morphological structure. Principally, this was the structure of the mouthparts: a characteristic shape of the labium, especially the labial palps with fused segments I and II and narrow dorsal and wide ventral lobes; the structure of the maxillae, especially the maxillary palps that are noticeably expanded and widely rounded at the top and consist of fused segments; and the structure of the mandibles, particularly the area of their canines. The legs, especially the distal part of the femora, also match the genus characteristics, and the chitinization (significantly reduced) and surface structures on segments of the abdomen (developed to varying degrees in different species) agree with the generic diagnosis.

Here, we describe two new species of *Symbiocloeon* (Ephemeroptera: Baetidae) based on diagnostic morphological (Figs 3–12) and molecular characters.

Order Ephemeroptera Hyatt & Arms, 1891

Family Baetidae Leach, 1815

Genus *Symbiocloeon* Müller-Liebenau, 1979

Type species: Symbiocloeon heardi Müller-Liebenau, 1979.

Imago and subimago: Unknown.

Species composition: Symbiocloeon heardi Müller-Liebenau in Müller-Liebenau & Heard, 1979, *Symbiocloeon madhyasthai* Subramanian & Sivaramakrishnan, 2009, *Symbiocloeon corbiculinus* Palatov sp. nov. and *Symbiocloeon laoensis* Palatov sp. nov.

Distribution: The Oriental Region: India, Thailand and Laos.

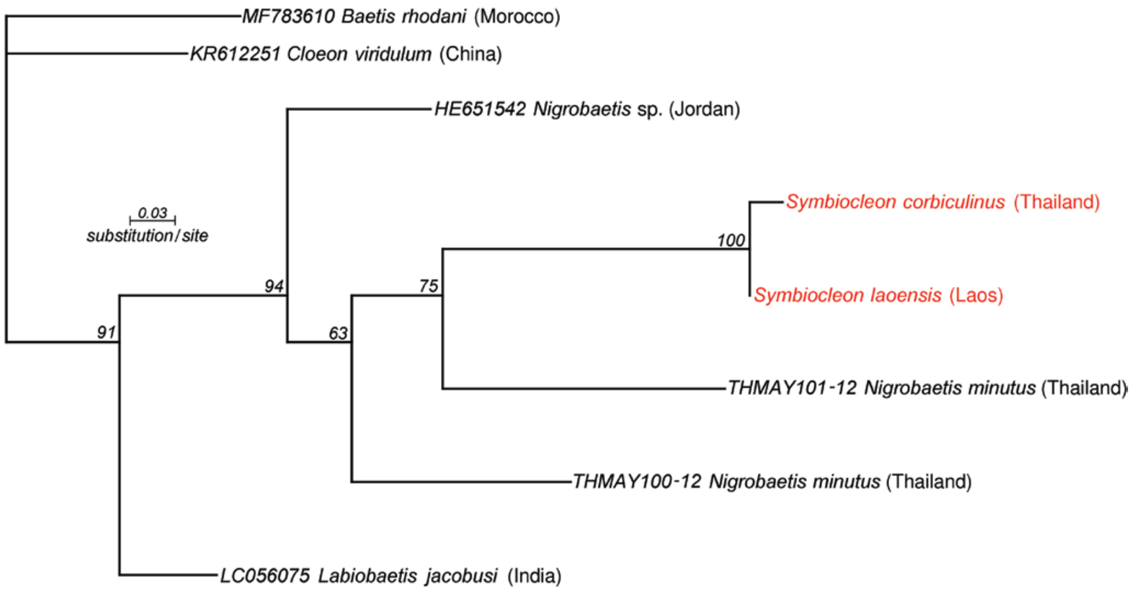

Figure 2. Maximum likelihood phylogeny of *Symbiocloeon* spp. based on the *COI* dataset.

Figure 3. Larvae of ***Symbiocloeon corbiculinus***, total view. A, B, male larvae. C–E, female larvae. Scale bars: 1 mm. Photographs: Dmitry M. Palatov.

SYMBIOCLOEON CORBICULINUS PALATOV SP. NOV.

(FIGS 1, 3–8)

Type material

Holotype: Voucher number *ZMMU Eph-0002*. Thailand: Lam Chae Stream, 14.4712°N, 102.2828°E, from the mantle cavity of *C. fluminea*, 13 March 2018, I. N. Bolotov, I. V. Vikhrev, S. Tumpeesuwan, K. Tanmuangpak, B. Nahok, U. Chanlabut leg.

Paratypes: RMBH N-12. Thailand: Lam Chae Stream, type locality, same host species, date and collector (five

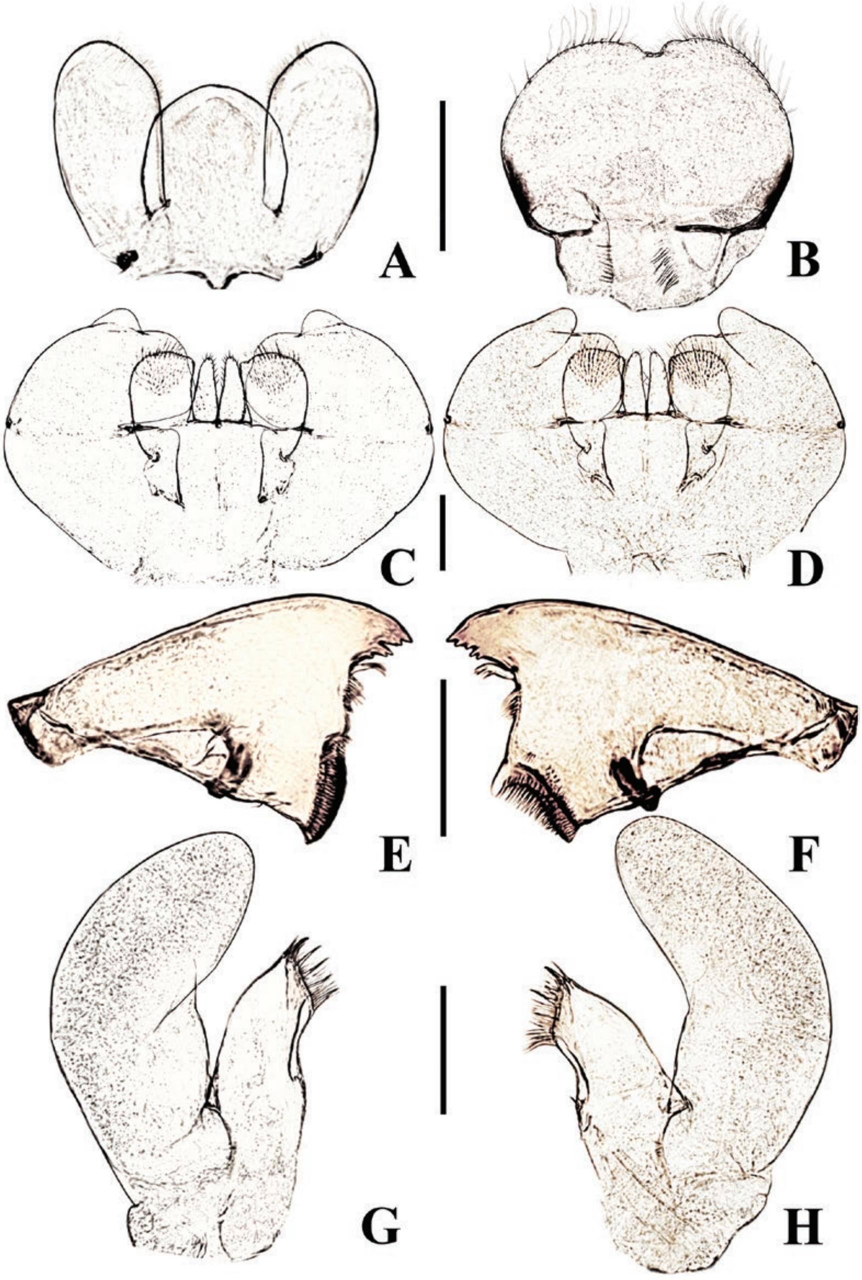

Figure 4. Larva of ***Symbiocloeon corbiculinus*** (holotype). A, hypopharynx. B, labrum. C, labium, ventral view. D, labium, dorsal view. E, right mandible. F, left mandible. G, left maxilla. H, right maxilla. Scale bars: 0.2 mm. Photographs: Dmitry M. Palatov.

specimens). Remaining material is deposited in the RMBH, Arkhangelsk, Russia (37 larvae).

Diagnosis

[Based on larvae.]

Larvae of *S. corbiculinus* differ from all other representatives of genus *Symbiocloeon* by the following combination of larval characters (see Supporting Information, Table S6): (1) tarsal claws with eight to ten stout teeth increasing in length, arranged into two rows, distributed close to claw apex; (2) surface of terga covered only with regular *B*- and *Hr*- setae and a few cells; (3) posterior margin of all terga nearly straight, without any marginal teeth and spines; (4) maxillary palp wider and longer than galealacinia; (5) paraglossae nearly square

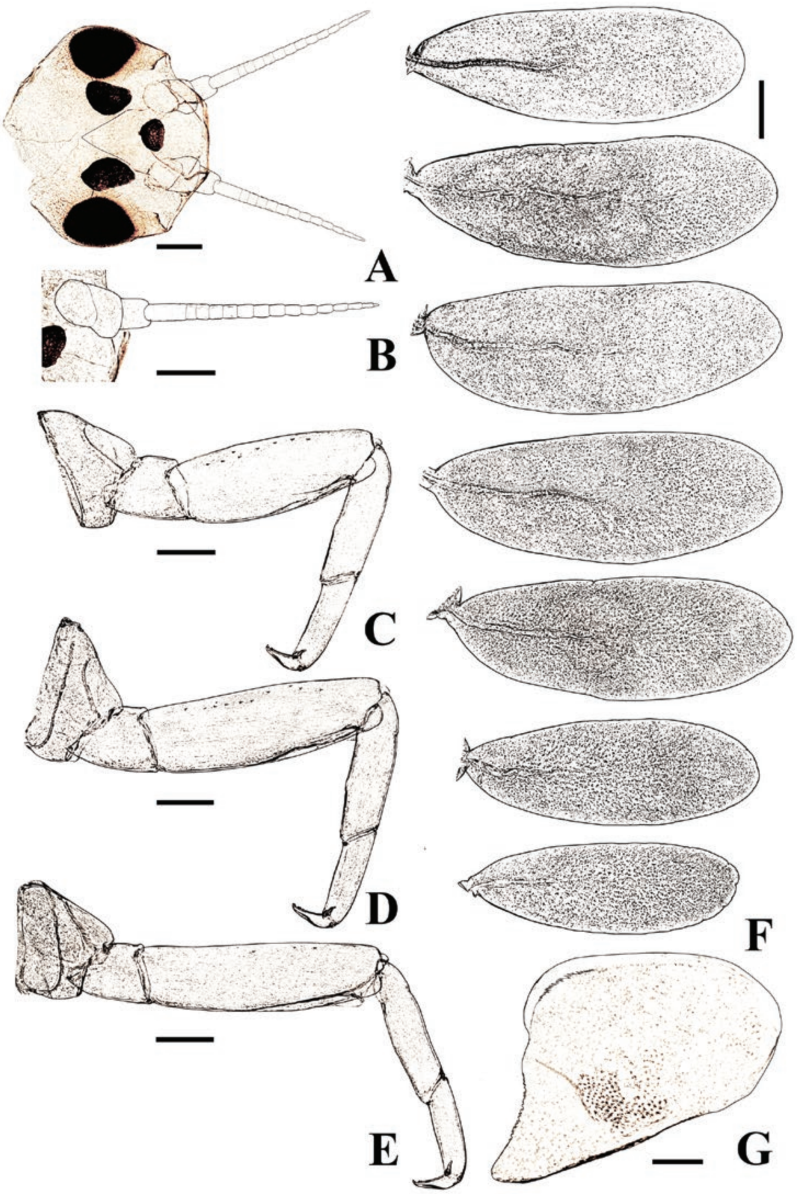

Figure 5. Larva of ***Symbiocloeon corbiculinus*** (holotype). A, head. B, antenna. C, foreleg. D, middle leg. E, hind leg. F, abdominal gills I–VII. G, paraproct. Scale bars: 0.2 mm. Photographs: Dmitry M. Palatov.

in shape, with rounded outer apical margin; (6) lingua of hypopharynx distinctly shorter than superlinguae; and (7) tarsi of forelegs not elongated.

Etymology

The species was named in reference to the freshwater bivalve genus *Corbicula*, in which it dwells.

Description

Larva: Body length 6.5–8.2 mm (males), 6.0–8.5 mm (females); length of cerci 1.6–1.8 mm; length of paracercus 1.4–1.6 mm. Body long and slender (Fig. 3).

General body coloration distinctly pale, whitish to light brown and dirty yellow; mesonotum darkest, with brownish maculae posteriorly and along base of forewing pads; lateral and ventral sides of meso- and metathorax darker than abdominal segments, whitish to light brown or dirty yellow. Abdominal segments paler than thorax, except occasionally brown terga V–IX; a pair of small pale dots and oblique strokes on terga III–IX centrally and anteriorly. Legs lightest, whitish, with slightly darker tarsi and claws. Body cuticle distinctly thin, delicate; surface of cuticle flattened and smooth, without protruded corrugation (Fig. 3).

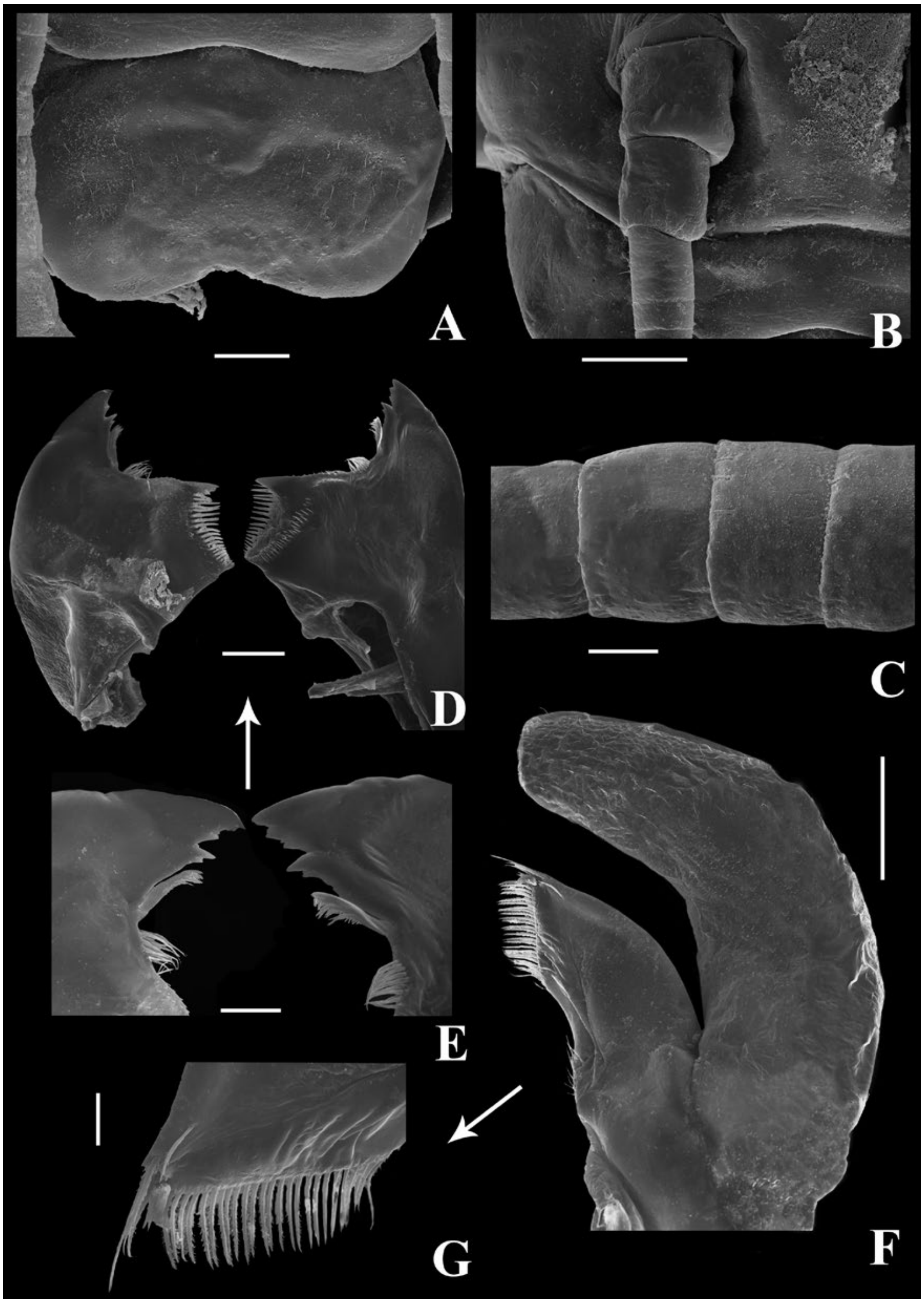

Figure 6. Larva of ***Symbiocloeon corbiculinus***. A, labrum. B, scapus, pedicellus and basal annuli of flagellum of antenna. C, flagellar segments of antenna. D, E, mandibulae. F, maxilla. G, apical part of maxilla. Scale bars: 100 µm in B, G; 50 µm in A, C; 20 µm in D–F. Photographs: Dmitry M. Palatov. The arrows show enlarged sections of the same structures and objects: E - mandibular canines; G - galelacinia, anterio-medial part.

Head: Colour whitish to yellowish and dirty yellow; light yellow area between ocelli and diffused light greyish maculation between eyes; clypeus and genae slightly darker than frons and vertex. Faceted surface of larval turbinate eyes unicolorous, intensely brown (Fig. 5A). Head cuticle smooth; surface of frons and clypeus sparsely covered with occasional small *B* [6.0–8.7 µm in length; sensillum basiconicum as defined by Gaino & Rebora (1996, 1999a, b, 2003), with modifications discussed by Godunko *et al.* (2015, 2018)], and a few even smaller *Hr* (4.2–5.5 µm in length; thin, hair-like setae, distributed over whole

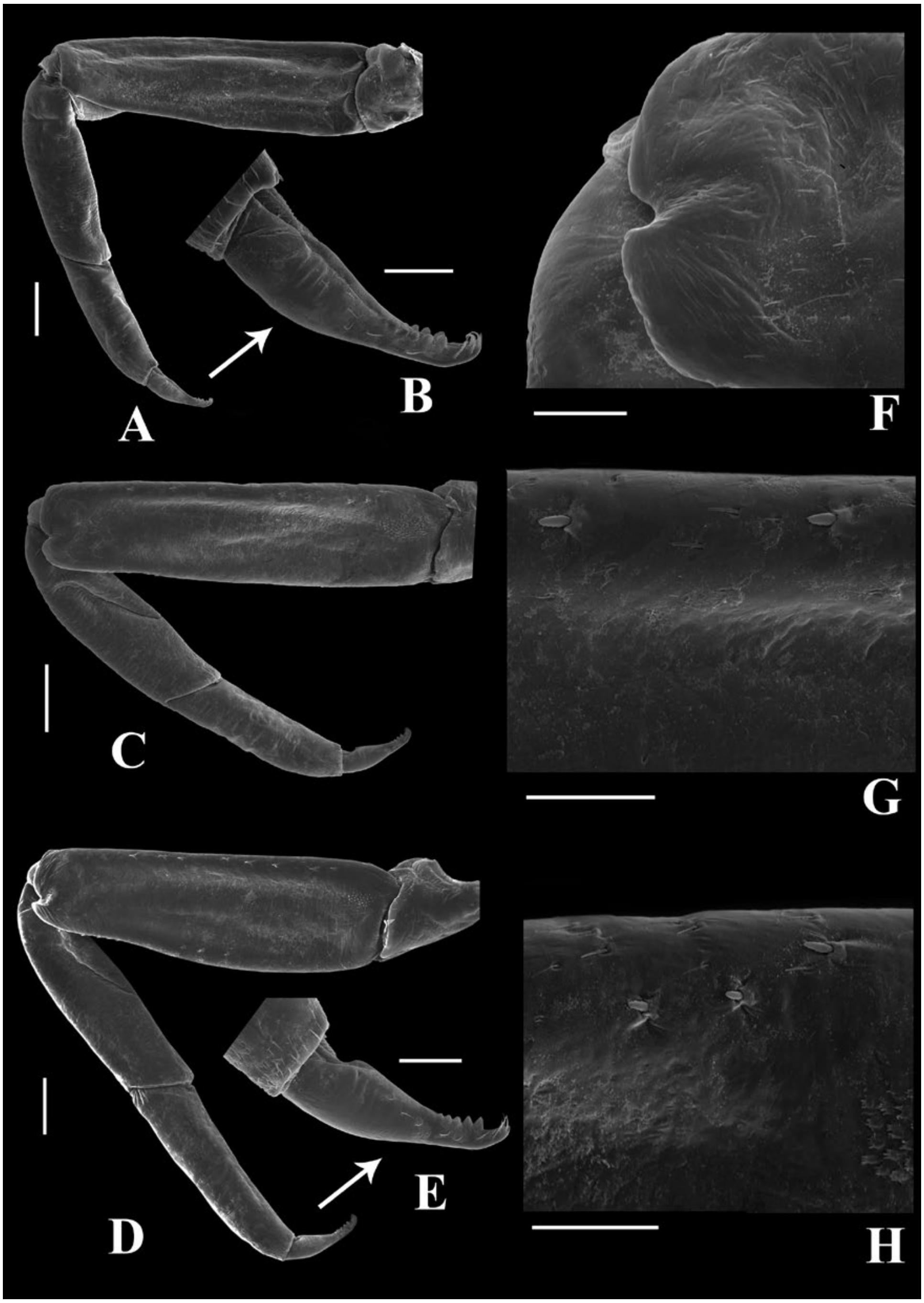

Figure 7. Larva of ***Symbiocloeon corbiculinus***. A, foreleg. B, fore tarsal claw. C, middle leg. D, hind leg. E, hind tarsal claw. F, distal part of middle femora. G, outer margin of middle femora, central part. H, outer margin of middle femora, proximal part. Scale bars: 100 μm in A, C, D; 30 μm in B, E–H. Photographs: Dmitry M. Palatov. The arrows show enlarged sections of the same objects.

body surface). Antennae unicolorous, whitish to light yellow, nearly as long as head length; scape slightly longer than pedicle; flagellum consists of 12–20 segments; base of antennae close together (Fig. 5B). Surface of scape and pedicel with regular small *B* and *Hr*, more densely scattered on first antennal segment; occasionally, surface of scape and pedicel with a few *B* only; lack of setae on surface of flagellar segments; posterior margin of flagellar segments nearly flat, without spines and setae, with sparse row of six to eight submarginal *B* (5.7–7.2 μm in length) (Figs 3, 5A, 6B, C).

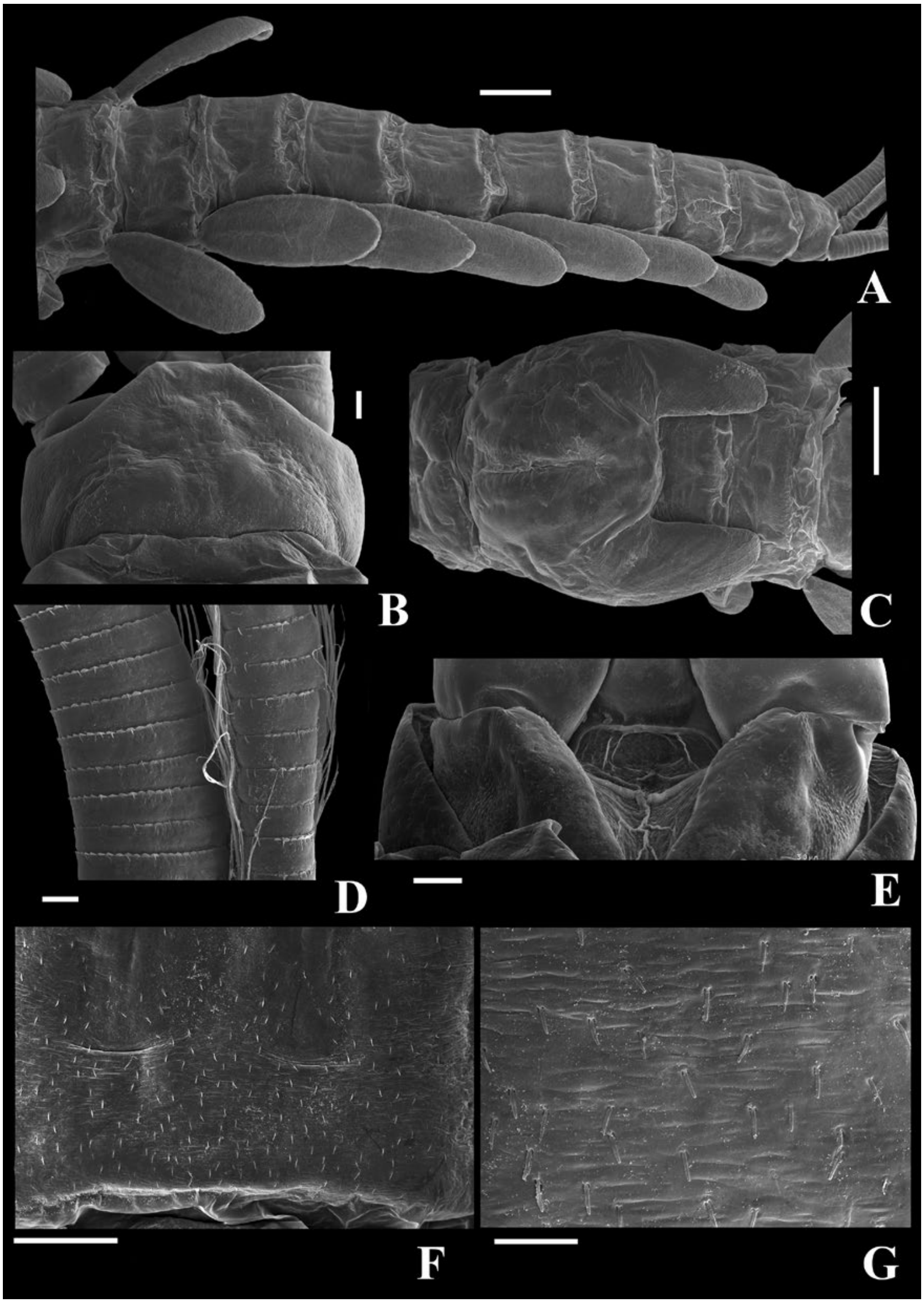

Figure 8. Larva of ***Symbiocloeon corbiculinus***. A, abdomen, lateral view. B, abdominal tergum X. C, thorax, dorsal view. D, segments of caudal filaments. E, paraprocts. F, posterior margin of abdominal tergum V. G, surface of abdominal tergum VI. Scale bars: 300 μm in A, C; 30 μm in B, D–F. Photographs: Dmitry M. Palatov.

Mouthparts: Labrum expanded laterally; broadest part near to middle of its length; lateral margins moderately curved; anteriolateral margins symmetrically rounded, slightly obtuse. Labrum ~1.45–1.60 times broader than long. Median notch not flattened, U-shaped, distinctly narrow, moderately deep. Dorsal surface of labrum covered with tiny, sparsely scattered *B* and *Hr*, fairly uniform in size; lacking dorsal submarginal bristles along outer margin; ventral surface with row of submarginal short setae (Figs 4A, 6A).

Superlinguae of hypopharynx slightly asymmetric, oval in shape, narrow, elongated, widely rounded

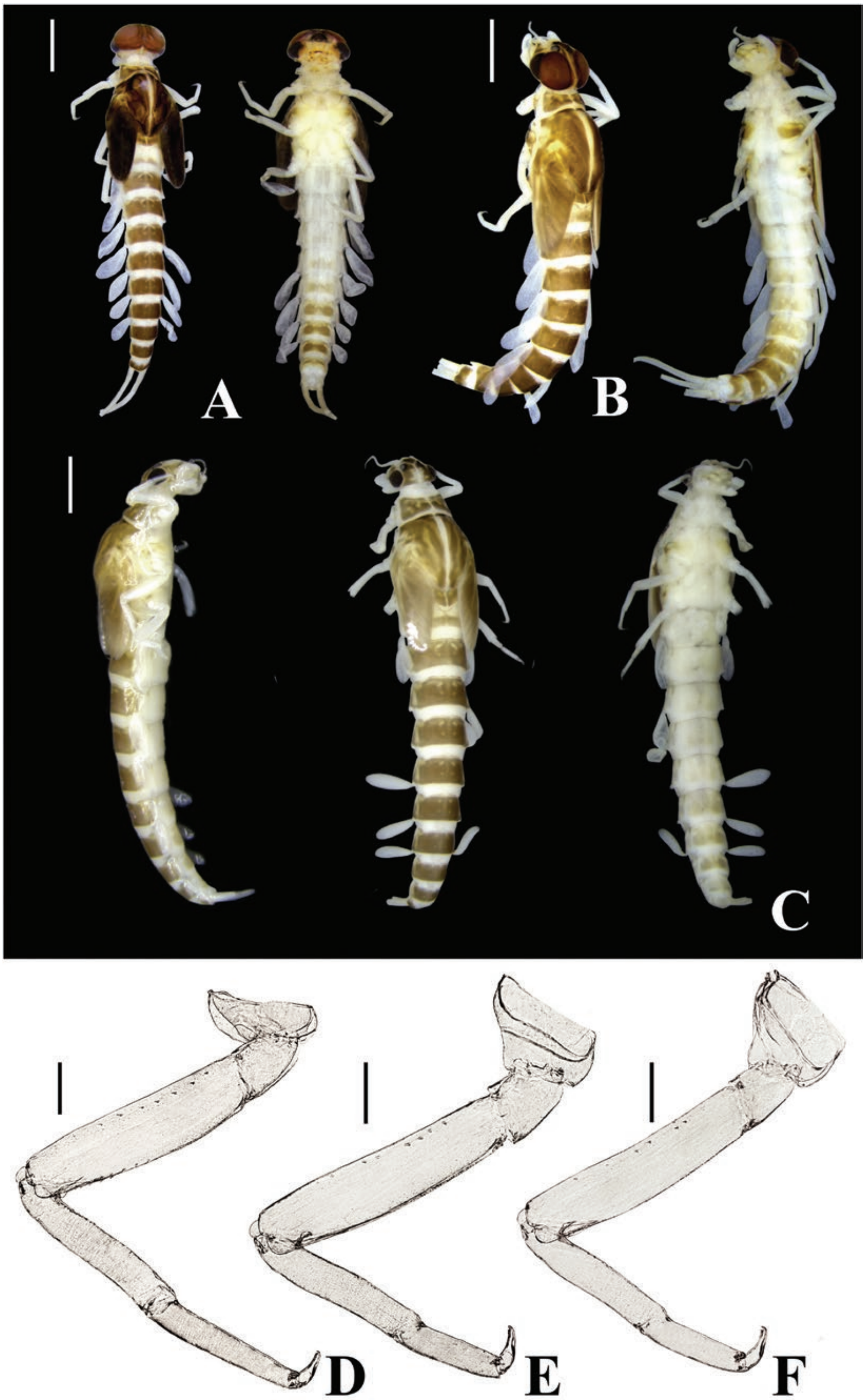

Figure 9. Larvae of ***Symbiocloeon laoensis***. A, B, male larvae, total view. C, female larvae, total view. D, foreleg. E, middle leg. F, hind leg. Scale bars: 1 mm in A–C; 0.2 mm in D–F. Photographs: Dmitry M. Palatov.

apically, without any projections; lingua widely rounded apically, distinctly shorter than superlinguae (Fig. 4A).

Mandibles stout; canines of both mandibles fused; outer margin flat medially, without distinct vault, and smoothly curved apically near base of canines. Surface of mandibles with a few small *B* and *Hr* (Figs 4E, F, 6D, E).

Maxillary palp consists of two almost fused segments, large, stout, bent inwards, widely rounded apically;

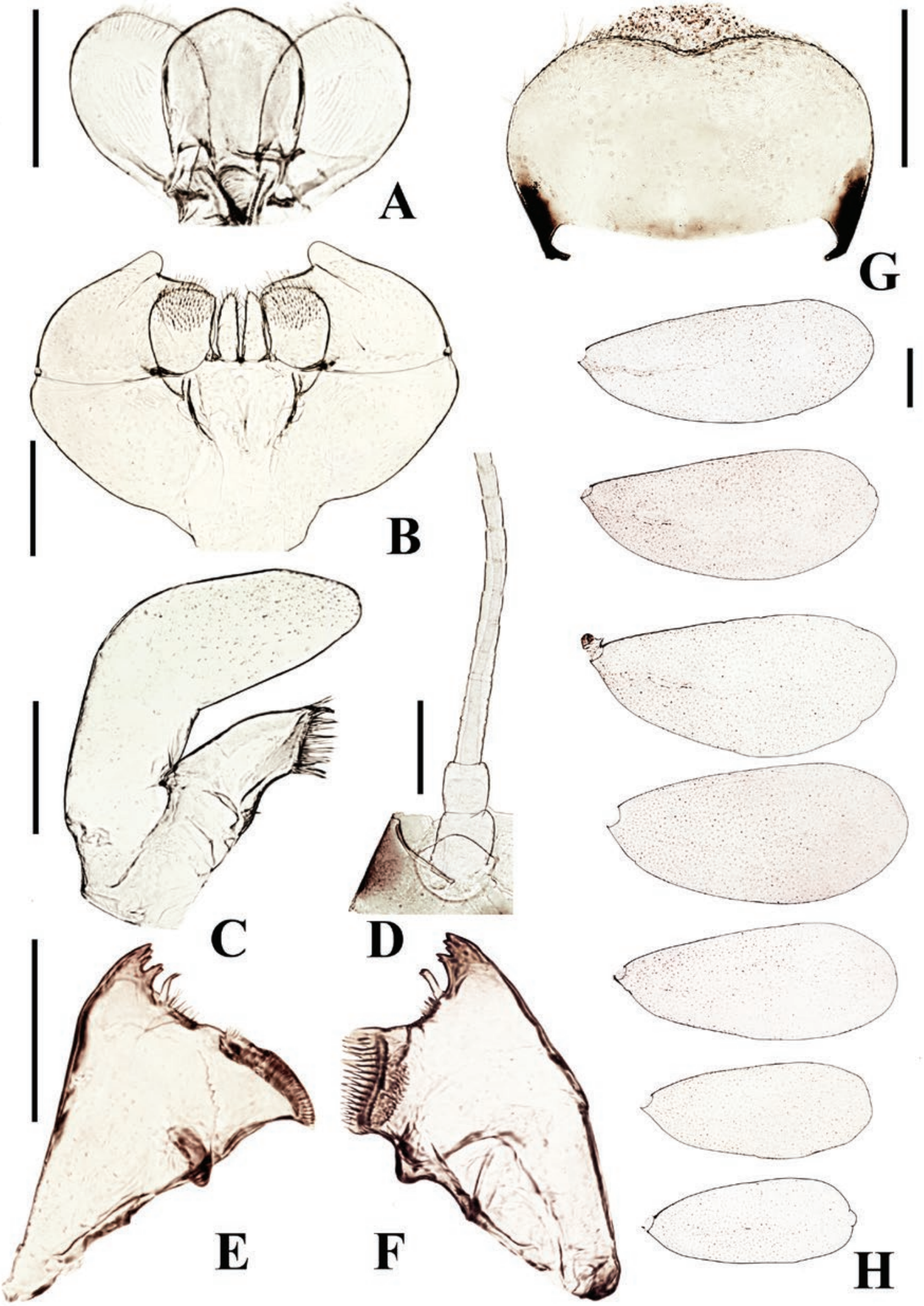

Figure 10. Larva of ***Symbiocloeon laoensis*** (holotype). A, hypopharynx. B, labium, dorsal view. C, maxilla. D, antenna. E, right mandible. F, left mandible. G, labrum. H, abdominal gills I–VII. Scale bars: 0.2 mm. Photographs: Dmitry M. Palatov.

maxillary palp wider and longer than galealacinia; surface of palp almost without setae and bristles; a few tiny *B* grouped near the tip of palp; four to six stout bristles along outer apical angle of galea on ventral side, alternating with numerous long, slender, feather-like bristles; five to eight tiny setae on inner surface of galea basally; surface of galealacinia with several *Hr* and one or two small feather-like setae (Figs 4G, H, 6F, G).

Labial palp three-segmented; segment I expanded apically, with proximal margin nearly straight; segments II and III almost fused to form a large, stout structure, bilobed apically; dorsal lobe [corresponding to second segment of Baetidae according to

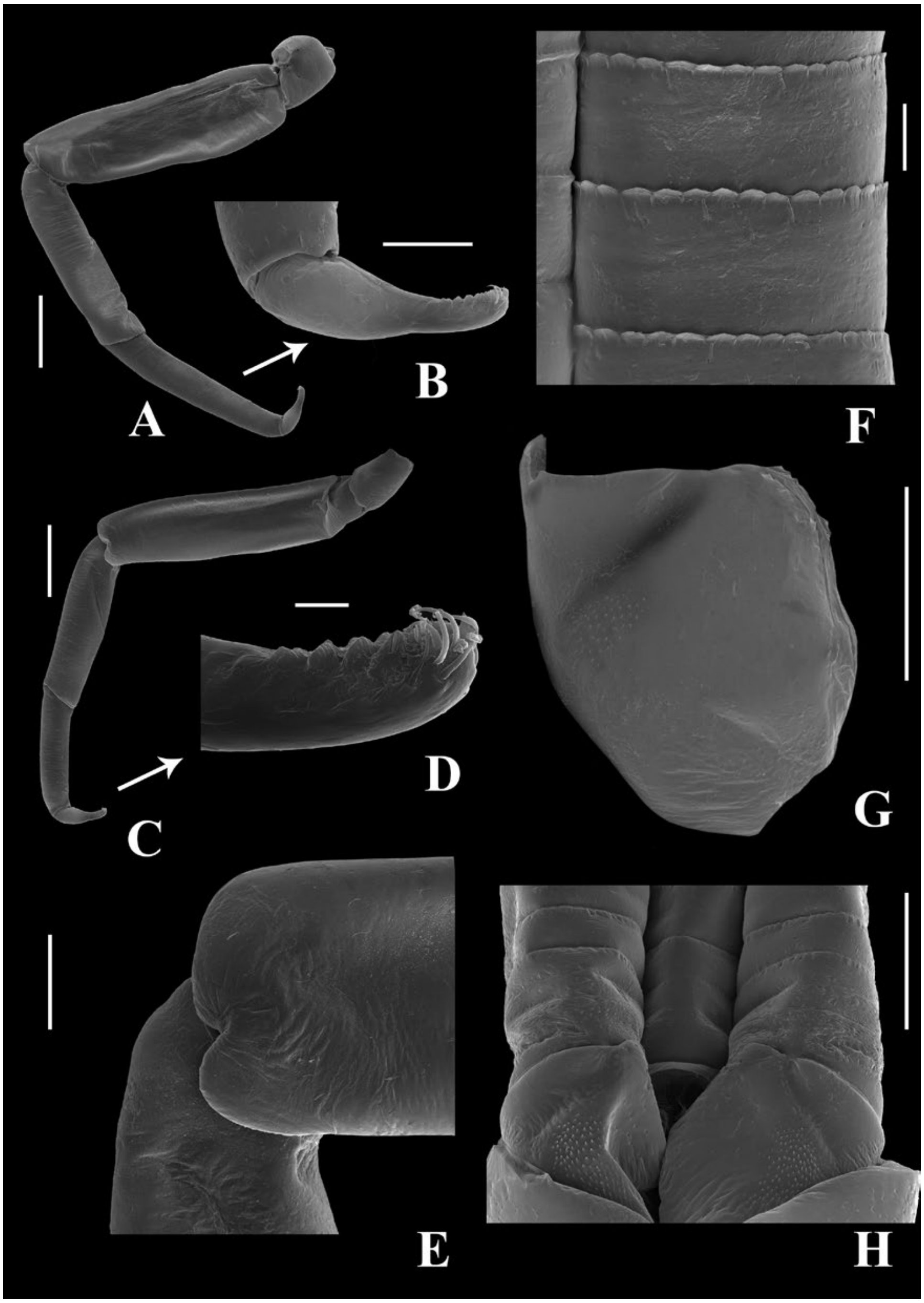

Figure 11. Larva of ***Symbiocloeon laoensis***. A, foreleg. B, fore tarsal claw. C, hind leg. D, hind tarsal claw. E, distal part of hind femora. F, segments of caudal filaments. G, paraproct. H, paraprocts and proximal part of caudal filaments. Scale bars: 200 µm in A, C; 100 µm in G, H; 50 µm in B, E; 20 µm in F; 10 µm in D. The arrows show enlarged sections of the same objects. Photographs: Dmitry M. Palatov.

Müller-Liebenau & Heard (1979)] rises above ventral lobe (corresponding to third segment), finger-like and narrow distally, rounded at tip; ventral lobe large, with nearly rectangular and moderately oblique inner margin; lacking stout setation on surface of dorsal and ventral lobes (Fig 4C, D).

Glossae and paraglossae of different shape; glossae nearly triangular in shape, narrow, rounded apically; several short, stout setae along inner margin and tip; surface of glossae covered with tiny *Hr*; paraglossae as long as glossae, nearly square in shape, with rounded outer apical margin; paraglossae densely

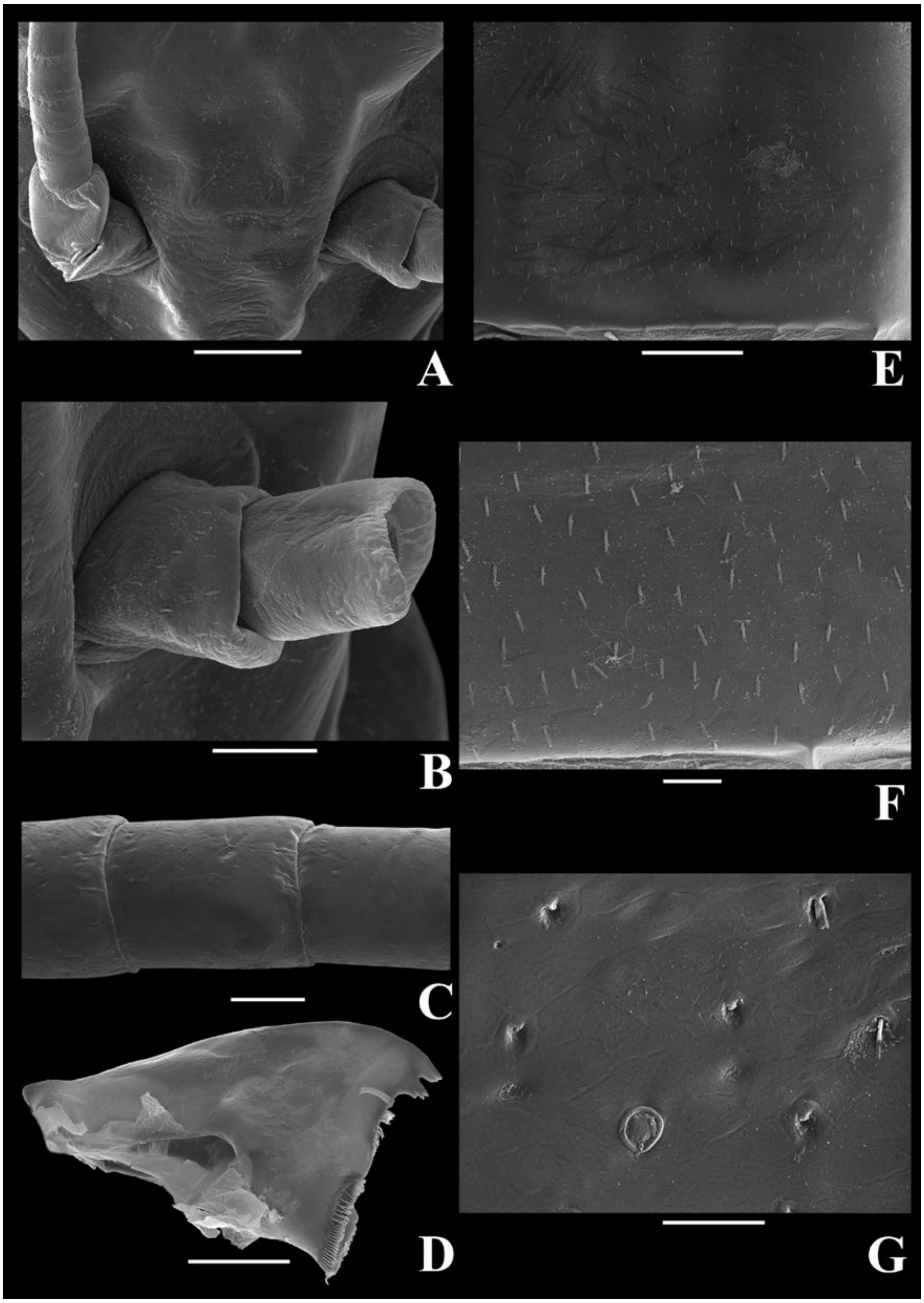

Figure 12. Larva of ***Symbiocloeon laoensis***. A, frons. B, scapus and pedicellus. C, flagellar segments of antenna. D, right mandible. E, posterior margin of abdominal tergum V. F, posterior margin of abdominal tergum II. G, surface of abdominal tergum VII. Scale bars: 100 µm in A, C, E; 50 µm in B; 20 µm in C, F; 10 µm in G. Photographs: Dmitry M. Palatov.

covered with numerous stout bristles prevailing on dorsal side (Fig 4C, D).

Thorax: Pronotum narrow, ~2.8–3.7 times longer than wide. Pronotum with diffuse whitish-yellow to light brown maculae along posterior margin. Thoracic pleura pale, and sterna the same colour as the dorsal side of the thorax. Hind wing pads absent. Surface of thoracic cuticle flattened and smooth, without protruding corrugation; almost no setae and strong spines on surface of thorax; sparse small *Hr* on mesonotum centrally (Fig. 8C).

Legs pale, whitish to yellow and dirty yellow; all pairs relatively slender and short. Middle femora parallel sided; fore- and hind femora with slightly convex inner margin proximally. Patellar–tibial suture is developed on all legs. Surface of legs without protruded corrugation. Dorsal surface of forefemora almost without stout setae centrally; up to eight bluntly pointed setae [10–12.5 µm in length; *STS-bp* type of setae according to Godunko *et al.* (2015)] irregularly scattered close to outer margin; *B* and *Hr* sparsely scattered on surface; foretibiae and foretarsi evenly covered with dominant *B* and a few *Hr* densely scattered distally. Dorsal surface of middle and hind legs with the same type of setation; up to ten blunt and bluntly pointed setae [8.0–9.5 µm in length (middle legs); 9.0–11.0 µm in length (hind legs)] situated submarginally along outer margin, and four to six small *STS-bp* (5.5–8.0 µm in length) close to inner margin; tibiae and tarsi with relatively dense *B- and Hr-*. Length ratio of femur:tibia:tarsus: foreleg, 1:0.65:0.50; middle leg, 1:0.65:0.48; and hind leg, 1:0.72:0.64 (Figs 5C–E, 7A–H).

Tarsal claw as long as 0.34–0.38 of tarsus length, one-third of its distal end moderately narrow; tip distinctly hooked; eight to ten stout teeth increasing in length, arranged in two rows, distributed close to claw apex; a group of five to ten stout bristles (10.0–15.0 µm in length) at the tip of claw (Fig 7B, E).

Abdomen: Terga pale, uniformly coloured whitish, occasionally yellow to light brown with paler tergum X. Sterna the same colour as terga, occasionally slightly paler. Surface of terga with flattened and smooth cuticle on terga I–III(IV); terga IV(V)–X with not protruded, undulating corrugation of cuticle. Surface of terga covered with regular *B* (7.5–12.0 µm in length), several small *Hr* and a few cells (2.5–3.2 µm in diameter); posterior margin of all terga nearly straight, without any marginal teeth and spines. Cuticle of sterna flattened and smooth, covered with rare *Hr* and *B*; posterior margin of sterna nearly straight (Fig 6B, F, G).

Gills elongated, each about two to three times longer than respective segment, slightly asymmetrical, narrow, broadly rounded apically; gills II and III largest; gill VII smallest; tracheization poorly visible, whitish. Both margins of gills straight; no setae and hairs on surface and margins of gills (Figs 5F, 8A).

Paraproct plate with 25–30 small, pointed teeth along inner margin; distal margin smooth; single row of eight to 12 very small unequal spines along inner margin of cercotractor; ventral surface of paraproct plate covered with occasional *B* and *Hr* (Figs 5G, 8E).

Cerci pale, whitish, approximately as long as 0.22–0.25 of body length (Fig. 3).

Posterior margin of caudal filament segments with small triangular-shaped teeth, blunt and bluntly pointed apically, and a sparse row of short submarginal *B* setae; surface of these segments without setae and hairs (Fig. 8D).

Biology

A mayfly species that seems to use a specific clam as a larval host (Table 1; Supporting Information, Table S7). The clams with mayfly larvae were found in the Lam Chae Stream, at depths ≤ 1.5 m, with sandy sediments.

Distribution

Thailand, Lam Chae Stream in Nakhon Ratchasima Province, a tributary of the Mun River, Mekong Basin.

SYMBIOCLOEON LAOENSIS PALATOV SP. NOV.

(FIGS 9–12)

Type material

Holotype: Voucher number *ZMMU Eph-0003*. Laos: Sein Kaphoe River, 14.7710°N, 106.1704°E, from the mantle cavity of *C. fluminea*, 3 March 2020, I. N. Bolotov, I. V. Vikhrev, E. S. Konopleva and Y. E. Chapurina leg.

Paratypes: RMBH N-68, Laos: Sein Kaphoe River: type locality, same host, date and collectors (three specimens).

Remaining material is deposited in the RMBH, Arkhangelsk, Russia (two specimens).

Diagnosis

[Based on larvae.]

Larvae of *S. laoensis* differ from all other representatives of genus *Symbiocloeon* by the following combination of larval characters (see Supporting Information, Table S6): (1) tarsi of forelegs noticeably elongated; (2) tarsal claws with eight to ten stout teeth increasing in length, arranged in two rows, distributed close to claw apex and with a group of stout bristles at the tip; (3) surface of terga covered with regular *B-* and *Hr-* setae and a few cells; (4) posterior margin of all terga nearly straight, without any marginal teeth and spines; (5) paraproct plate with ten to 15 small, pointed teeth along inner margin; (6) maxillary palp wider and longer than galealacinia; and (7) lingua of hypopharynx nearly equal to length of superlinguae.

Table 1. Taxonomic overview of freshwater bivalve-associated mayfly species (Ephemeroptera: Baetidae)

Species	Type locality	Distribution	Hosts*	Localization	Reference
Symbiocloeon corbiculinus	Lam Chae Stream, tributary of Mun River, Mekong Basin, Nakhon Ratchasima Province, Thailand	Not known beyond its type locality	*Corbicula fluminea* (Müller, 1774)	Gills	This study
Symbiocloeon laoensis	Sein Kaphoe River, Mekong Basin, Laos	Not known beyond its type locality	*Corbicula fluminea* (Müller, 1774)	Gills	This study
Symbiocloeon heardi Müller-Liebenau, 1979	Mae Nam Khwae Yai River at Bang Nong Bua, Kanchanaburi Province, Thailand	Thailand, Mae Klong River basin	*Hyriopsis myersiana* (Lea, 1856)	Gills	Muller & Heard (1979)
Symbiocloeon madhyasthai Subramanian & Sivaramakrishnan, 2009	Karnataka, Shimoga district, Theerthahalli taluk, Mandagadde, Tunga River, India	Not known beyond its type locality	*Pseudomulleria dalyi* (Smith, 1898)	Gills	Subramanian & Sivaramakrishnan (2009)
Muteloclοeon bihoumi Gillies & Elouard, 1990	Niandan River, Sassambaya, Guinea	Rivers in Mali and Guinea, West Africa	*Aspartharia* spp.; *Chambardia wissmanni* (Martens, 1883); *Mutela dubia* (Gmelin, 1791)	Gills	Gillies & Elouard (1990)
Muteloclοeon corbeti (Kimmins, 1956)	Jinja on the shores of Lake Victoria at the outlet of the Nile River	Lake Victoria, Uganda, East Africa	The larval stage and host are unknown	Unknown	Gillies & Elouard (1990)

*The names of freshwater mussel hosts are given based on the most recent checklist of the order Unionida (Graf & Cummings, 2021).

Etymology

The specific name *laoensis* indicates that this species was found in Laos (Lao People's Democratic Republic).

Description

Larva: Body length 6.7–8.2 mm (males), 3.4–5.5 mm (females); length of cerci 1.1–1.5 mm; length of paracercus 1.0–1.1 mm. Body long and slender (Fig. 9A–C).

General body coloration dark, whitish-yellow to intensely brown and black, darker in mature larvae and nymphs. Thorax darkest, with brownish-black maculae on pronotum and mesonotum; metanotum paler, light brown. Ventral side of thorax markedly paler than dorsal side; with dirty yellow sterna, occasionally with unclear light brown smudges laterally; pleura whitish-yellow to brown. Abdominal segments dark, the same colour or slightly paler than thorax; tergum I yellowish anteriorly, brown to dirty brown in posterior half; terga II–IX(X) uniformly dark, with a pair of small pale dots and oblique strokes on each tergum centrally and anteriorly; sterna paler than terga; sterna I–III lightest, dirty whitish to yellowish, occasionally with darker macula near posterior margin; sterna IV and V with the same colour pattern as previous sterna, but dark macula posteriorly intensely coloured, dirty yellow to light brown; sterna VI–IX with broad light brown to intensely brown smudge centrally and paler area laterally; occasionally sternum IX with brownish spot anteriorly and two paler rounded spots posteriorly; a pair of small pale dots and two short oblique strokes mainly near anterior margin of sterna III–IX. Legs lightest, whitish to light yellow; tarsi and claws dirty yellow, with slightly darker tarsi and claws. Body cuticle flattened and smooth on thorax and abdominal segments I and II or shaped by moderately protruded undulating corrugation on dorsal surface of head and abdominal segments III–X, and more protruded on legs (Fig. 9A–C).

Head: Colour relatively dark, dirty yellow to brown; whitish to yellow maculation between eyes. Faceted surface of larval turbinate eyes liver-brown to brown, surrounded by thin blackish ring; shaft of turbinate eyes of the same colour or slightly paler. Frontal suture widely V-shaped. Head cuticle shaped by moderate corrugation on frons and genae, and almost flat on vertex. Surface of head covered with sparse small *B* (5.0–6.5 µm in length) and a few long *Hr* ≤ 20 µm in length. Antennae unicolorous, as long as 0.85–1.0 of head length; flagellum consists of ten to 15 segments; base of antennae close together. Surface of scape and pedicel covered with sparse small *B* and *Hr*, less numerous on second antennal segment; lack of setae on surface of flagellar segments proximally and centrally; posterior margin of flagellar segments nearly flat, without spines and setae; sparse row of five to eight submarginal *B* along posterior margin (4.5–5.5 µm in length) (Figs 9A–C, 10D, 12A–C).

Mouthparts: Labrum expanded laterally; broadest part near to middle of its length; lateral margins widely rounded; anteriolateral margins symmetrically rounded, not markedly obtuse. Labrum distinctly broader than long (width-to-length ratio ~1.65–1.75). Median notch flattened, widely U-shaped, not deep. Dorsal surface of labrum covered with tiny, sparsely scattered *B* and *Hr*; lacking dorsal submarginal bristles along outer margin; ventral surface with short setae (Fig. 10G).

Superlinguae of hypopharynx slightly asymmetrical, leaf-shaped, wide, shortened, without any projections; lingua vaulted apically, with triangular-shaped tip; its length nearly equal to the length of superlinguae (Fig. 10A).

Mandibles stout; outer margin nearly flat medially, smoothly curved apically near base of canines, occasionally with indistinct vault medially; canines of both mandibles partly fused, divided into two groups apically; left mandibular incisor groups terminated by 3 + 2 stout denticles; left prostheca brush-shaped, terminated by a group of three stout bristles; right mandibular incisor groups terminated by 3 + 3 stout denticles; right prostheca thin, stick-shaped; no setae and hairs on surface of mandibles (Figs 10E, F, 12D).

Maxillary palp one-segmented, large, stout, bent inwards, widely rounded apically; maxillary palp wider and longer than galealacinia; surface of palp covered with tiny *Hr* and sparse *B*; setation denser distally; a few sparse hairs near base of palp; six to eight stout bristles along outer apical angle of galea on ventral side, alternating with numerous slender feather-like bristles; five to seven tiny setae on inner surface of galea basally; surface of galealacinia with several *Hr* (Fig. 10C).

Labial palp three-segmented; segment I expanded apically, with proximal margin nearly straight or concave; segments II and III almost fused to form a large, stout structure, bilobed apically; dorsal lobe rises above ventral lobe, finger-like and narrow distally, rounded at the tip; ventral lobe large, with moderately rounded inner margin; a few strong setae on dorsal surface; numerous *Hr* dorsally and ventrally on both lobes (Fig. 10B).

Glossae and paraglossae of different shape; glossae nearly triangular in shape, narrow, rounded apically; several short stout setae along inner margin and tip; surface of glossae covered with tiny *Hr*; paraglossae as long as glossae, nearly square in shape, with rounded apical inner margin; paraglossae densely covered with numerous stout bristles prevailing on dorsal side (Fig. 10B).

Thorax: Pronotum narrow, ~2.8–3.7 times longer than wide, with diffuse light brown maculae along posterior margin. Thoracic pleura pale, and sterna same colour as dorsal side of thorax. Hind wing pads absent. Surface of thorax cuticle flattened and smooth, without protruded corrugation; almost no setae and strong spines on surface of thorax; sparse small *Hr* on mesonotum centrally (Fig. 9A–C).

Legs pale, whitish to yellow and dirty yellow; all pairs relatively slender and short. Forefemora with slightly convex inner margin proximally; middle and hind femora parallel-sided. Patellar–tibial suture is developed on all legs. Surface of legs without protruded corrugation. Dorsal surface of forefemora almost without stout setae centrally; up to seven bluntly pointed apically setae [6–7 µm in length; *STS-bp* type of setae according to Godunko *et al.* (2015)] irregularly scattered close to outer margin and ten or 11 small *STS-bp* (4.5–6.0 µm in length) close to inner margin; *B* and *Hr* sparsely scattered on surface; foretibiae and foretarsi evenly covered with dominant *B* and a few *Hr* densely scattered distally. Dorsal surface of middle and hind legs with the same type of setation; up to eight blunt and bluntly pointed setae (5.0–6.5 µm in length) situated submarginally along outer margin, and three to five small *STS-bp* (4.5–6.0 µm in length) close to inner margin; tibiae and tarsi with relatively dense *B* and *Hr*. Tarsi of forelegs noticeably elongated. Length ratio of femur:tibia:tarsus: foreleg, 1:0.79:0.68; middle leg, 1:0.67:0.40; and hind leg, 1:0.65:0.42 (Figs 9D–F, 12A, C).

Tarsal claw as long as 0.23–0.26 times length of tarsus of foreleg and 0.39–0.42 times length of tarsus of middle and hind legs, one-third of its distal end moderately narrow; tip distinctly hooked; eight to ten stout teeth increasing in length, arranged in two rows, distributed close to claw apex; a group of five to ten stout bristles (10.0–15.0 µm in length) at the tip of the claw (Fig. 11B, D).

Abdomen: Terga intensely brown, uniformly coloured, with a pair of small pale dots and oblique strokes on each tergum centrally and anteriorly. Sterna I–III lightest, dirty whitish to yellowish, occasionally with darker macula near posterior margin; sterna IV and V with the same colour pattern as previous sterna, but dark macula posteriorly intensely coloured, dirty yellow to light brown; sterna VI–IX with broad, light brown to intensely brown smudge centrally and paler area laterally. Surface of terga with flattened cuticle having a polygonal rhombic sculpture and weakly pronounced undulating corrugation. Surface of terga covered with regular *B* (7.0–12.0 µm in length), several small *Hr* and a few cells and micropores (2.5–4.5 µm in diameter); posterior margin of all terga nearly straight, without any marginal teeth and spines. Cuticle of sterna flattened and smooth, covered with rare *Hr* and *B*; posterior margin of sterna nearly straight (Fig. 12E–G).

Gills elongated oval, relatively wide, each approximately two times longer than respective segment, slightly asymmetrical, broadly rounded apically; gills III and IV largest; gill VII smallest; tracheization poorly visible, whitish. No setae and hairs on surface and margins of gills (Fig. 10H).

Paraproct plate with ten to 15 small, pointed teeth along inner margin; distal margin smooth; inner margin of cercotractor smooth; ventral surface of paraproct plate covered with occasional *B* and *Hr* (Fig. 11G, H).

Cerci pale, whitish, approximately as long as 0.22–0.25 of body length (Fig. 7A–C).

Posterior margin of caudal filament segments with small triangular-shaped teeth, blunt and bluntly pointed apically, and sparse row of short submarginal *B* setae; surface of these segments without setae and hairs (Fig. 11F).

Biology

A mayfly species that seems to use a specific clam as a larval host (Table 1; Supporting Information, Table S7)

Distribution

Laos, Sein Kaphoe River, Mekong Basin.

DISCUSSION

DNA SEQUENCES AND PHYLOGENETIC ANALYSES

Molecular genetic studies of species in the genus *Symbiocloeon* have not been performed before. In this study, we provide, to the best of our knowledge, the first molecular data on *Symbiocloeon* species from Thailand and Laos. The genus *Symbiocloeon*, with *S. corbiculinus* and *S. laoensis*, was recovered as a well-supported monophyletic group (Fig. 2). These species represent divergent phylogenetic lineages, which are distant from each other by 2% (Fig. 2). The mean uncorrected *COI* percentage distance between the new species *S. laoensis* and its nearest neighbour, *Nigrobaetis minutes* Müller-Liebenau, 1984 , is 18.7% (Fig. 2; Supporting Information, Table S4). However, the genus *Symbiocloeon* includes many more undescribed species that need further research.

DIVERSITY AND DISTRIBUTION OF BIVALVE-ASSOCIATED MAYFLIES

Here we show, for the first time, that mayflies belonging to the genus *Symbiocloeon* can use *Corbicula* clams as a host for larval development. We describe two *Symbiocloeon* species new to science from Thailand and Laos, increasing the number of bivalve-associated mayfly taxa globally. Our results and a review of available published data indicate that bivalve-associated mayflies are narrow host specialists, being associated with one (*S. corbiculinus*, *S. laoensis*, *S. heardi* and *S. madhyasthai*) or a few (*Muteloclоeon bihoumi* Gillies & Elouard, 1990) species of freshwater bivalves (Table 1). It is likely that the stage of larval development in the mantle cavity of a freshwater bivalve is mandatory in the life cycle of the bivalve-associated mayfly species. It was shown that most species of freshwater mussel-associated mites (Acari: Unionicolidae) and leeches (Hirudinea: Glossiphoniidae) reveal the same narrow host preference, being obligate parasites (Bolotov *et al.*, 2019; Chapurina *et al.*, 2021).

Only large larvae of *Symbiocloeon* spp. were found in the mantle cavity of a *Corbicula* species. Similar results were obtained in previous studies in Thailand, African countries and India (Müller-Liebenau & Heard, 1979; Gillies & Elouard, 1990; Subramanian & Sivaramakrishnan, 2009). However, despite this, Müller-Liebenau & Heard (1979) assumed that the young larvae also live in the mantle cavity of freshwater mussels. According to our unpublished data, larvae might penetrate the mantle cavity of clams at a very early developmental stage. Thus, the larvae of an undescribed species of *Symbiocloeon* from Myanmar have been found in freshwater mussels at different stages of development from 2 mm (first instar larva) to 11 mm (adult larva). We therefore believe that the *Symbiocloeon* larvae might develop inside the mantle cavity of their host from the first instar to adult stage.

Until the present study, associations of mayflies with freshwater bivalves were known to occur in

Southeast Asia, India and Sub-Saharan Africa (Müller-Liebenau & Heard, 1979; Gillies & Elouard, 1990; Subramanian & Sivaramakrishnan, 2009; Table 1). The African genus *Mutelocloeon* contains two species: *Mutelocloeon bihoumi* from West Africa (Mali and Guinea) and *Mutelocloeon corbeti* (Kimmins, 1956) from East Africa (Uganda); the larval stage of the latter species is unknown (Gillies & Elouard, 1990). It has been established that *Mutelocloeon bihoumi* inhabits the mantle cavity of several freshwater mussel species from the family Iridinidae: *Chambardia wissmanni* (Martens, 1883), *Aspartharia chaiziana* (Rang, 1835), *Aspartharia dahomeyensis* (Lea, 1859), *Aspartharia rochebrunei* (Jousseaume, 1886) and *Mutela dubia* (Gmelin, 1791) (Gillies & Elouard, 1990). The overall percentage of mussel hosts was low. In addition, the unionid genus *Coelatura* and etheriid genus *Etheria* were examined, but none of the mayfly larvae was detected in these mussels (Gillies & Elouard, 1990).

The Oriental genus *Symbiocloeon* was established based on *S. heardi* from the Khwae Yai River, a medium-sized freshwater basin in western Thailand (Müller-Liebenau & Heard, 1979). This species is associated with the freshwater mussel *Hyriopsis myersiana* (Lea, 1856), Unionidae (Müller-Liebenau & Heard, 1979). In addition, mussel species from the Mekong and Chao Praya basins were examined, namely *Lens contradens* (Lea, 1838), *Indonaia caerulea* (Lea, 1831) and *Physunio superbus* (Lea, 1843). Mayfly nymphs were not found in these taxa (Müller-Liebenau & Heard, 1979).

Later, one more species in this genus, *S. madhyasthai*, was discovered from the freshwater 'oyster' *Pseudomulleria dalyi* (Smith, 1898), a peculiar cemented bivalve from the Western Ghats of India (Subramanian & Sivaramakrishnan, 2009). This bivalve species has clear Gondwanan affinities and is most likely to belong to the family Unionidae (Parreysiinae) (Bogan & Hoeh, 2000; Bolotov *et al.*, 2022), although it is currently placed in the Etheriidae (Graf & Cummings, 2021).

Overall, bivalve-associated mayflies might represent a species-rich group, the taxonomic richness of which appears to be greatly underestimated in tropical areas.

MORPHOLOGICAL ADAPTATIONS OF THE BIVALVE-ASSOCIATED MAYFLY LARVAE

The bivalve-associated mayfly larvae have several specific morphological traits compared with free-living species, which appear to be adaptations to a hidden lifestyle in the mantle cavity of freshwater clams and mussels. These features include a reduction of integument chitinization (primarily at the abdomen and limbs), a reduction in the size of the antennae and legs, and a distinctive shape of the claws. In addition, a significant reduction of breast and abdomen segments (disappearance of stiff bristles, teeth and spines) was discovered (Fig. 13). The spines and sharp segments of the integument could irritate the sensitive surface of the gills of the host. It is likely that selection in this case is aimed to decrease the damage to the gills of the clam host (Gillies & Elouard, 1990). Similar modifications have been identified in other commensals of freshwater bivalves, for example in chironomids inhabiting the mantle cavity of the freshwater mussel species *Echyridella menziesii* (Gray, 1843) (Hyriidae) in New Zealand (Forsyth & McCallum, 1978).

An increase in area of the respiratory surface of larval tergalia was also recorded. This trait expands the oxygen supply of mayfly larvae via the water current through the shell of the host. Furthermore, the larvae also have specific modifications of the mouthparts, such as an extension of the labium (Fig. 4). This adaptation is probably necessary for collecting food particles from the gill surface of the host.

Usually, a clam host houses one mayfly larva, except for a single case in which two larvae were recorded in one *Corbicula* individual. Our new data align with previous observations on a limited number of mayfly larvae in freshwater mussels (Müller-Liebenau & Heard, 1979; Gillies & Elouard, 1990; Ricciardi, 1994). It is possible that there is some regulation of the number of larvae, perhaps by competition between mayflies (Gillis & Elouard, 1990). Earlier, it was established that competition between non-biting midge larvae (*Xenochironomus canterburyensis* (Freeman, 1959) ; Chironomidae) in the mantle cavity of their mussel host can cause severe larval mortality (Forsyth & McCallum, 1978).

RELATIONSHIP OF BIVALVE-ASSOCIATED MAYFLIES WITH THEIR HOSTS

The association between mayflies and freshwater bivalves was previously considered to be a type of commensalism, which is beneficial to the insect and neutral for the bivalve (Müller-Liebenau & Heard, 1979). The impact of larvae on the bivalve host might be limited by possible discomfort and competition for food resources, as the cited authors suggested. In contrast, Gillies & Elouard (1990) assumed that the relationships between mayfly larvae and their hosts might be treated as inquilinism.

The benefit for the mayfly from this type of association is obvious. First, the survival rate of larvae in the mantle cavity of the bivalve host undoubtedly increases. In this case, the clam and its shell will limit the risk of predation. Second, the larvae are provided

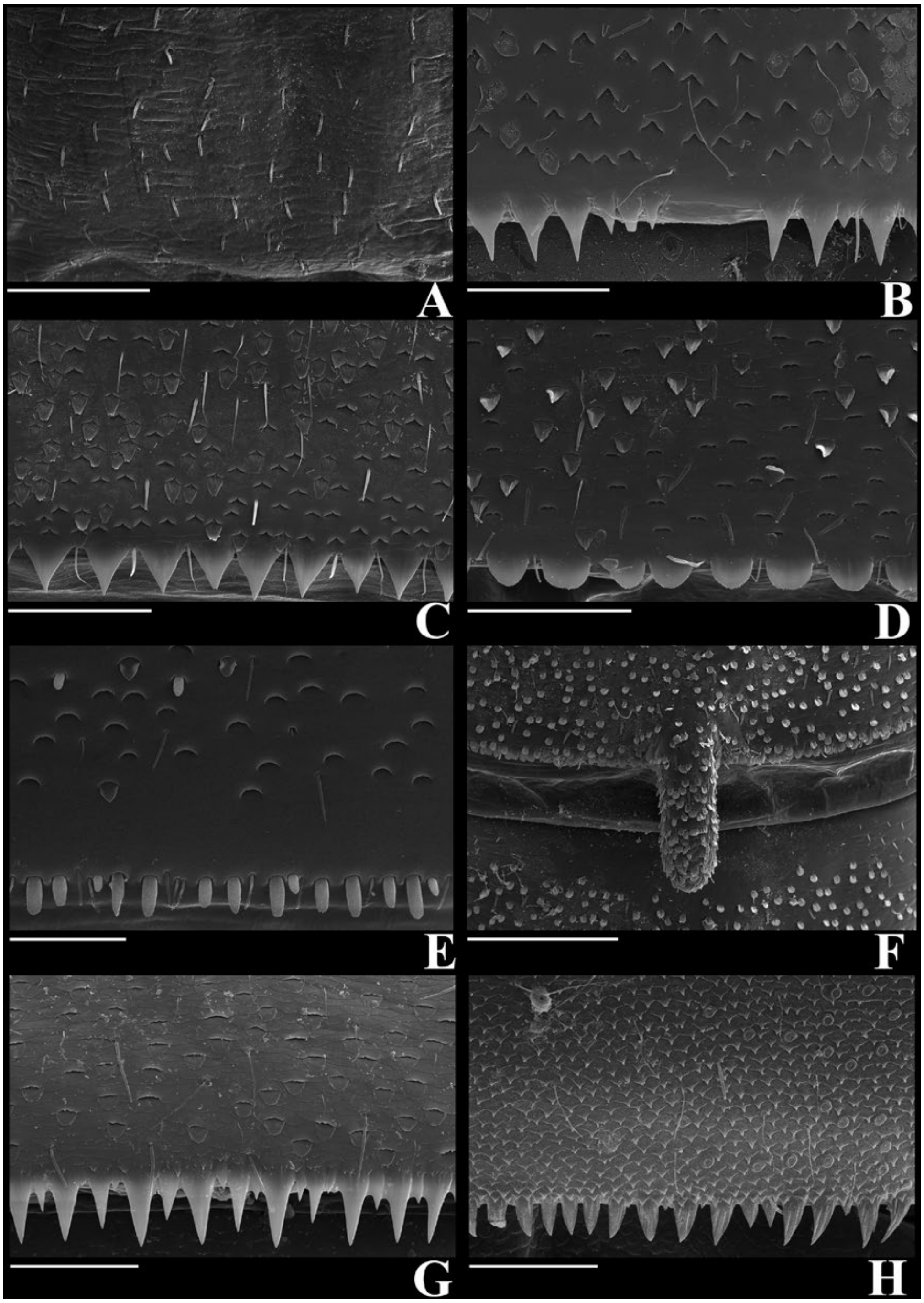

Figure 13. Posterior margin of abdominal tergum VIII in different groups of Baetidae. A, ***Symbiocloeon corbiculinus***. B, *Nigrobaetis* sp. (Baetinae). C, *Baetis* (*Labiobaetis*) sp. (Baetinae). D, *Baetis* (*Baetis*) sp. (Baetinae). E, *Baetis* (*Rhodobaetis*) sp. (Baetinae). F, *Gratia* sp. (Baetinae). G, *Cloeon* sp. (Cloeoninae). H, *Centroptilum luteolum* (Müller, 1776) (Cloeoninae). Scale bars: 100 µm in F; 50 µm in A–E, G, H. Photographs: Dmitry M. Palatov. Comparative material: B–D, F, G, India, Uttarakhand, left tributary of Kosi River, 29.4732°N, 79.1640°E; E, Tajikistan, Gorno-Badakhshan Autonomous Region, Rushanskiy District, spring in front of the mouth of the Bartang River, 37.9249°N, 71.6031°E; H, Russia, Moscow Region, Stupino City-district, small tributary of Bunchikha River, 54.9787°N, 38.2042°E.

with suspended food particles and a continuous supply of fresh, oxygenated water from the inhalant current created by their host during its filtration activity. Rocky and sandy substrates are inhospitable to small insects, and the evolutionary strategies available to mayflies for using this environment are limited. It is possible that mayflies avoid the risks of living on sandbanks by adapting to life in the body cavity of mussels (Gillies & Elouard, 1990).

The negative influence of larvae on bivalve hosts is probably attributable to competition for food resources. The larvae may also negatively affect the respiratory function of the mussels. We did not detect any tissue damage in the examined *Corbicula* hosts (Supporting Information, Fig. S2). Perhaps, the association between the *Corbicula* clam and the mayfly is a form of inquiline commensalism. Similar associations classified as inquiline commensalism have been found between chironomid larvae and dreissenid clams (Forsyth & McCallum, 1978; Riccardi, 1994). Presumably, the larvae also have some positive influence on their clam hosts. For example, mayfly larvae can consume small parasites, such as infusorians and mite nymphs. Here, we can assume that they might be useful as cleaners, being an example of cleaning symbiosis (Bronstein, 1994; Thomas *et al.*, 2016). Regarding the possible benefits and/or costs of this association for the clam, this subject is still speculative, and only further ecological studies can clarify some of the hypotheses raised above.

To conclude, the results of the present study provide new data on associations of mayfly larvae with *Corbicula* clams from the native range. Future studies should focus on studying the nature of the relationships between clams and mayflies, the possible positive and negative effects of these associations, the life cycles of mayflies associated with clams and the role of abiotic factors in the infestation process.

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DATA AVAILABILITY

The data underlying this article are available in GenBank Nucleotide Database (accession numbers OL964337 - OL964343, ON093196 - ON093199).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article on the publisher's website.

Figure S1. Median-joining network of *Corbicula fluminea* based on the *COI* sequences. Circles represent different haplotypes, with the size reflecting their frequency (smallest = 1). Numbers near branches are numbers of nucleotide substitutions per site.
Figure S2. Gill of *Corbicula fluminea* (Lam Chae Stream, Mekong Basin, Thailand).

Table S1. Body length of larvae of ***Symbiocloeon corbiculinus*** from Lam Chae Stream, Mun River basin, Thailand and ***Symbiocloeon laoensis*** from Sein Kaphoe River, Mekong drainage, Laos.
Table S2. Shell measurements of *Corbicula fluminea*, non-infested and infested by larvae of ***Symbiocloeon laoensis*** from Sein Kaphoe River, Mekong Basin, Laos.
Table S3. Information on newly sequenced specimens of *Symbiocloeon* spp. from Thailand and Laos. Materials are from the collection of the Russian Museum of Biodiversity Hotspots (RMBH), N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Arkhangelsk, Russia).
Table S4. Information on additional *COI* sequences of *Corbicula* clams obtained from NCBI GenBank, including taxon, gene, accession number, specimen code, locality information and data source.
Table S5. Information on additional *COI* sequences of Baetidae obtained from NCBI GenBank and BOLD IDS, including taxon, gene, accession number, specimen code, locality information and data source.
Table S6. Comparison of morphological characters of *Symbiocloeon* species.
Table S7. *Symbiocloeon* species associated with *Corbicula* clams.