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To cite this article: Xuhongyi Zheng & Changfa Zhou (2021): First detailed description of adults and nymph of *Cincticostella femorata* (Tshernova, 1972) (Ephemeroptera: Ephemerellidae), *Aquatic Insects*, DOI: [10.1080/01650424.2020.1871026](https://doi.org/10.1080/01650424.2020.1871026)

To link to this article: <https://doi.org/10.1080/01650424.2020.1871026>



Published online: 25 Jan 2021.



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# First detailed description of adults and nymph of *Cincticostella femorata* (Tshernova, 1972) (Ephemeroptera: Ephemerellidae)

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## ABSTRACT

The adults of *Cincticostella femorata* (Tshernova, 1972) (Ephemeroptera: Ephemerellidae), which were reared from mature nymphs in spring 2020, are described and photographed for the first time. Compared to the known males in the same genus, especially *C. fusca* (Kang and Yang, 1995), the male adults of *C. femorata* have simpler penis but more derived forceps and wings. Additionally, for the first time, we provided detailed nymphal description. A flat and streamline body, enlarged and serrated femora of nymphs are not only their perfect diagnostic characters but also assumed their adaptive structures to live on substrate surface.

## ARTICLE HISTORY

Received 17 July 2020  
Accepted 28 December 2020

## KEYWORDS

*Cincticostella femorata*;  
China; evolution; mayfly;  
nymphal adaptation

## Introduction

The nymphs of *Cincticostella femorata* (Tshernova, 1972) (Ephemeroptera: Ephemerellidae) were described about 50 years ago (Gose 1969; Tshernova 1972). Although this ephemerellid species was mentioned, keyed and even photographed by several later researchers from many Asian countries (Allen 1975, 1980; Kluge 2004; Jacobus, McCafferty, and Sites 2005; Jacobus and McCafferty 2008; Xie, Jia, Chen, Jacobus, and Zhou 2009; Martynov et al. 2019), its imaginal stages have remained unknown, as for the most members of the genus. So far, among 17 species, only for five male and only one female adult have been described.

Morphologically, the nymphs of *C. femorata* seem more derived than other congeners. Their flat body, enlarged genae, anterolateral projections of pronota and mesonota, and flattened and serrated femora show that they are possibly adapted to living on stony bottom or adhering to the surface of stones. However, most nymphal structures (like mouthparts, gills and claws) of this species were only drawn and shown in the original descriptions (Gose 1969; Tshernova 1972). Therefore, the exact and fine morphology of these structures also needs to be described in order to discuss the identification and phylogenetic position of the taxon. Furthermore, knowledge about

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the imaginal characters and eggs of this species will contribute to this issue significantly.

In recent years, the nymphs of *C. femorata* have been collected frequently in central to Southern China in rivers of different size. In early spring 2020, during a collecting trip at a locality in Southeastern China (Zhejiang Province), two males and one female adults were reared from nymphs. This provided an opportunity to confirm their taxonomical and phylogenetic status. Additionally, by comparing with the species with relatively plesiomorphic characters, such as *C. fusca* (Kang and Yang, 1995) (Zhang, Han, Zhang, Wang, and Zhou 2020) and some Japanese species (see Ishiwata 2003), the evolutionary trend of known species in the genus *Cincticostella* (Allen, 1971) can be revealed to a certain degree.

## Material and methods

### Material examined

#### *Cincticostella femorata* (Tshernova, 1972)

2 ♂ adults, 1 ♀ adult, 1 ♂ subimago, 2 ♀ subimagos, 20 nymphs, Tianmu Stream, Tianmu Mountain, Lin-An city, Zhejiang Province, 579 m a.s.l., 30°22'12"N, 119°25'14"E, 5.IV.2020, leg. Xu-Hong-Yi Zheng, Zhen-Xing Ma; 11 nymphs, Mengnan Village, Menghai, Banna, Yunnan Province, 1013 m, 22°8'54"N, 100°5'57"E, 23.III.2013, leg. Ning Ding; 10 nymphs, northwest of Puwen Town, Banna, Yunnan Province, 1047 m, 22°30'26"N, 101°3'26"E, 2.IV.2013, leg. Yun-Lei Zhou; 13 nymphs, Yushugou, Lantian County, Xi-An City, Shaanxi Province, 1235 m, 34°14'43"N, 109°31'55"E, V.2020, leg. Sheng Xu, Zhao Xie; 12 nymphs, Dabanacha River, Chang-An District, Xi-An City, Shaanxi Province, 1128 m, 33°55'23"N, 108°56'52"E, V.2020, leg. Sheng Xu, Zhao Xie; 10 nymphs, Liyutang Stream, Guilin, Guangxi Province, 1000 m, 24°23'54"N, 110°41'55"E, III.2008, leg. Bei-Xin Wang; 10 nymphs, Tongkuangchang, Fanjing Mountain, Guizhou Province, 900 m, 27°55'2"N, 108°41'23"E, 23.IX.2005, leg. Zhi-Jie Wang, Chun-Xia Xiao (Tshernova 1972).

#### *Cincticostella fusca* (Kang and Yang, 1995)

1 ♂ adult, 3 nymphs, unnamed creek in front of Tianmu Hotel, Lin-An City, Zhejiang Province, 579 m, 30°19'22"N, 119°26'33"E, 15.IV.2017, leg. Juan-Yan Luo, Wei Zhang, Zhen-Xing Ma, Ming Zhang; 3 nymphs, unnamed creek in front of Tianmu Hotel, Lin-An city, Zhejiang Province, 579 m, 30°19'22"N, 119°26'33"E, 3.IV.2019, leg. Wei Zhang, Xiao-Li Ying; 2 ♂ and 1 ♀, unnamed creek beside Chanyuan Temple, Lin-An City, Zhejiang Province, 7.IV.2019, leg. Zhen-Xing Ma, Yin-Fang Wang (Kang and Yang 1995).

All specimens used in this study are deposited in the mayfly collection, College of Life Sciences, Nanjing Normal University.

### Methods

Nymphs were collected from the creek by hand net. Adults were reared from mature nymphs (kept in plastic washbasin which was covered by net). All materials were stored in ethanol (about 85%).

**Table 1.** GenBank Accession Numbers of COI sequences used in this research.

Species	GenBank Accession Number
<i>C. fusca</i>	MT738744 (This research)
<i>C. tornata</i>	MT254050 (Auychinda et al. 2020a)
<i>C. femorata</i> (1)	MT738745 (This research)
<i>C. femorata</i> (2)	MT738746 (This research)
<i>C. gosei</i>	MN186574.1 (Auychinda et al. 2020b)
<i>C. levanidovae</i> (1)	MH823283.1 (Suh et al. 2019)
<i>C. levanidovae</i> (2)	MH823284.1 (Suh et al. 2019)
<i>C. orientalis</i>	MH823285.1 (Suh et al. 2019)

The specimens were photographed with a digital camera (single lens reflex) and examined under a stereomicroscope. For some small structures, like mouthparts, claws and gills, a microscope camera was used.

Eggs were dissected from female subimagos. The scanning electronic microscope samples were prepared with a standard protocol: fixed in 4% glutaraldehyde for 5–8 h, rinsed with PBS (physiological saline) 2–3 times (10–15 min each), dehydrated in concentration gradient acetone (30%, 50%, 70%, 80%, 90%, 100%, 10–15 min each), and coated with gold film in a vacuum.

### Molecular study

Total genomic DNA of three specimens was extracted from nymphal legs using Animal Genomic DNA Kit (TsingKe Biotech Co., Beijing, China). The mitochondrial genes cytochrome c oxidase subunit I were PCR-amplified using the *Premix Taq* (Takara Bio Inc., Beijing, China) with forward primer F (5'-TTC AGC CAC TTT ACC GCG-3', see Hrivniak, Sroka, Godunko, and Žurovcová 2017) and reverse primer HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3', Folmer, Black, Hoeh, Lutz, and Vrijenhoek 1994). PCR conditions included initial denaturation at 94°C for 5 min, 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 40 s, with final extension at 72°C for 10 min. COI sequences of other *Cincticostella* species were obtained from GenBank. All sequences with GenBank accession number and references are listed in Table 1. They were aligned using Muscle (Edgar 2004), and the K2P genetic distance were estimated in MEGA7 (Kumar, Stecher, and Tamura 2016).

## Results

### *Cincticostella femorata* (Tshernova, 1972)

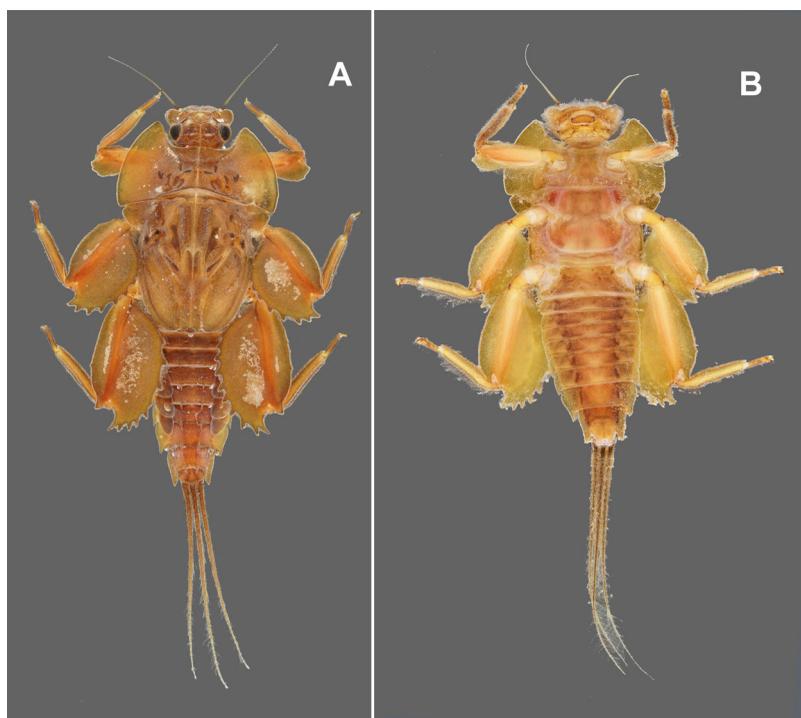
*Ephemerella* sp. TEA Gose 1969: 132, figures 23–37 (nymph, from Thailand).

*Asiatella femorata*, Tshernova 1972: 612, figure 5 (type: nymph, from Vietnam).

*Cincticostella femorata* Tshernova 1972: 614 (*Asiatella* synonymised with *Cincticostella*).

*Ephemerella* (*Cincticostella*) *boja* Allen 1975: 18 (based on the description by Gose (1969)); Jacobus et al. 2005: 733 (synonymised).

*Ephemerella* (*Cincticostella*) *femorata* (Tshernova, 1972): Allen 1975: 20 (subgeneric placement).



**Figure 1.** Nymphal habitus of *Cincticostella femorata* (Tshernova, 1972) (digital photos): (A) dorsal view and (B) ventral view.

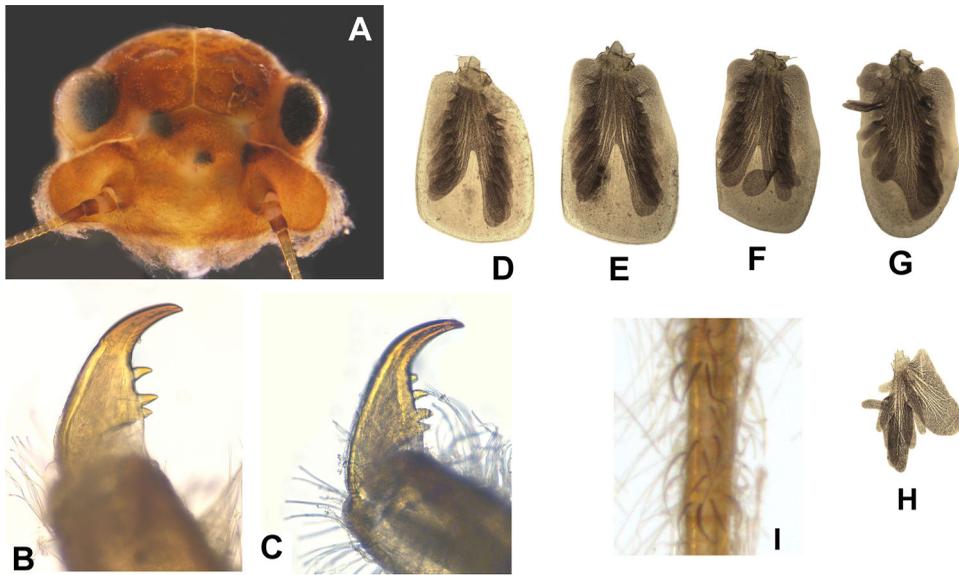
*Cincticostella* (*Rhionella*) *femorata* (Tshernova, 1972): Allen 1980: 83 (generic and subgeneric placement); Kluge 2004: 312 (review).

*Cincticostella femorata* (Tshernova, 1972): Jacobus and McCafferty 2008: 239 (*Rhionella* Allen, 1980 synonymised with *Cincticostella* Allen, 1975); Xie et al. 2009: 53 (first record from China); Martynov et al. 2019: 171 (review, Thailand).

### **Description of nymph**

**In alcohol.** Body length: male 10.0–12.0 mm, female 11.0–14.0 mm; caudal filaments length: male 7.0–9.0 mm, female 7.0–9.0 mm. Colouration yellowish to dark brown, whole body densely covered with tiny setae (Figures 1 and 2A).

**Head.** Antennae 2.0–3.0 mm, with tiny setae between segments except base; scape and pedicel subequal in length, first segment of flagellum ca. 1.5× of scape or pedicel; free margin of clypeus straight but genae extended forward and forming regularly rounded ear-like lobes, making leading margin of head much wider than posterior margin (Figures 1 and 2A); free margins of clypeus and genae with dense soft hair (Figure 2A); head and vertex surface with regular but short ridges and depressions (Figures 1A and 2A). Mouthparts: labrum: length ca. 2× width, with shallow median emargination; two-third of leading dorsal surface and free margins with relatively longer setae, whole ventral surface with yellowish bristle-like short hair, additional row of short setae near leading margin dorsally (Figure 3E). Left mandible: basal half of outer margin and dorsal surface with long and dense setae; outer incisor divided into

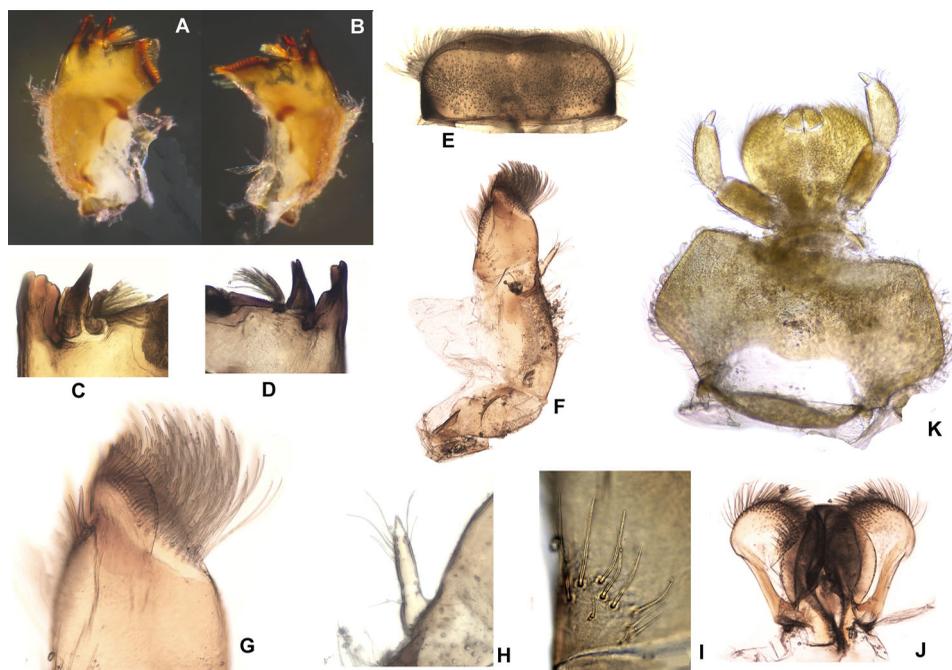


**Figure 2.** Nymphal structures of *Cincticostella femorata* (Tshernova, 1972) (digital photos): (A) head (anterior view); (B) claw of foreleg; (C) claw of foreleg (other specimen); (D) gill 1; (E) gill 2; (F) gill 3; (G) gill 4; (H) gill 5 (ventral view); and (I) terminal filament (partially enlarged, dorsal view).

three blunt denticles, inner incisor divided into two denticles; prostheca with branch of hair-like setae on common base and one broader spine (Figure 3A,C). Right mandible similar to left one except with 8–10 mesal setae near mola and prostheca with a smaller spine (Figure 3B,D). Hypopharynx: linguae with dense short setae on apical margin; superlingua oval, with very tiny setae on surface (Figure 3J). Maxillae: strong setae on apex or crown of galea-lacinia forming a brush-like tuft (Figure 3F,G); two relatively stronger dentisetae near inner-apical corner; one row of setae on apical half of mesal margin and basal half of outer margin, respectively; two rows of long setae with truncate tips and some tiny setae on ventral surface near mesal margin (Figure 3I); maxillary palp tapering, indistinctly three-segmented, with several long setae (Figure 3H); cardo with sparse setae (Figure 3F). Labium: glossae sub-triangular, slightly longer than broad, broadly rounded and slightly protruding the apices of the paraglossae; labial palp three-segmented, length of apical segment ca. 2× width; basal segment subequal to second one in length; glossae, paraglossae and labial palp of labium with dense setae on both surfaces but mentum and submentum only on ventral surfaces, some setae on submentum broader and more distinct (Figure 3K).

**Thorax.** Pronotum expanded laterally, forming obvious lateral lobes and anterolateral projections; projections surround head laterally and posteriorly, forming a semicircular structure; mesonotum with obvious square anterolateral projections (Figure 1A). Prosternum with slightly concave lateral margin; mesosternum near oval (Figure 1B).

**Legs.** All legs and body covered with tiny soft setae, those on free margins of femora, inner, dorsal and outer margins of tibiae and tarsi more longer and distinct; femora of forelegs widened with slightly serrated free margins, their length subequal to



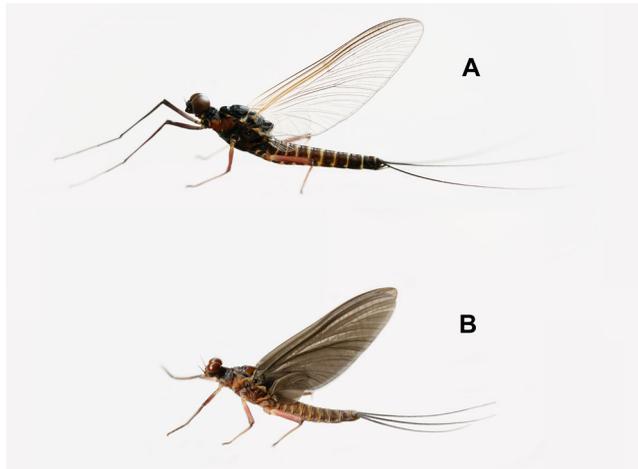
**Figure 3.** Nymphal mouthparts of *Cincticostella femorata* (Tshernova, 1972) (digital photos): (A) left mandible; (B) right mandible; (C) apex of left mandible; (D) apex of right mandible; (E) labrum (dorsal view); (F) maxilla; (G) apex of maxilla; (H) maxillary palpus enlarged; (I) setae on ventral surface of maxilla; (J) hypopharynx (ventral view); and (K) labium (ventral view).

foretibiae; femora of midlegs slightly longer than tibiae, exaggeratedly expanded into oval plates with deeply serrate outer margins; femora of hind legs obviously longer than tibiae, similar to mid-femora in shape but larger and with slightly serrated inner margins; tarsi of all legs darker than other leg segments, half-length of tibiae (Figure 1A,B); claws of all legs with 3–4 basal denticles (Figure 2B,C).

**Abdomen.** Terga III–X each with pair of median sharp spines, spines progressively larger from terga III–X, those on terga III sometimes very tiny; posterolateral corners of segments III–IX extended into sharp projections, those of segments VIII–IX more prominent (Figure 1A,B). Subanal plate of female with distinct median notch.

Gills on terga III–V similar in shape and more-or-less subequal in size, dorsal lamellae gradually slender; ventral lamellae III–V bifurcate, each parts with several leaf-like lobes (Figure 2D–F); ventral lamellae of gill VI not bifurcate but emarginate apically (Figure 2G); gill VII much smaller than others, covered by gill VI, dorsal and ventral lamellae subequal in size, ventral one with several lobes (Figure 2H).

**Caudal filaments.** Cerci length subequal to terminal filament, similar in shape and structure: with setae on whole surface, those of basal filaments denser, broader but shorter than apical setae; some setae broader than others (Figure 2I).



**Figure 4.** Male of *Cincticostella femorata* (Tshernova, 1972) (digital photos): (A) imago and (B) subimago.

#### **Description of male adult**

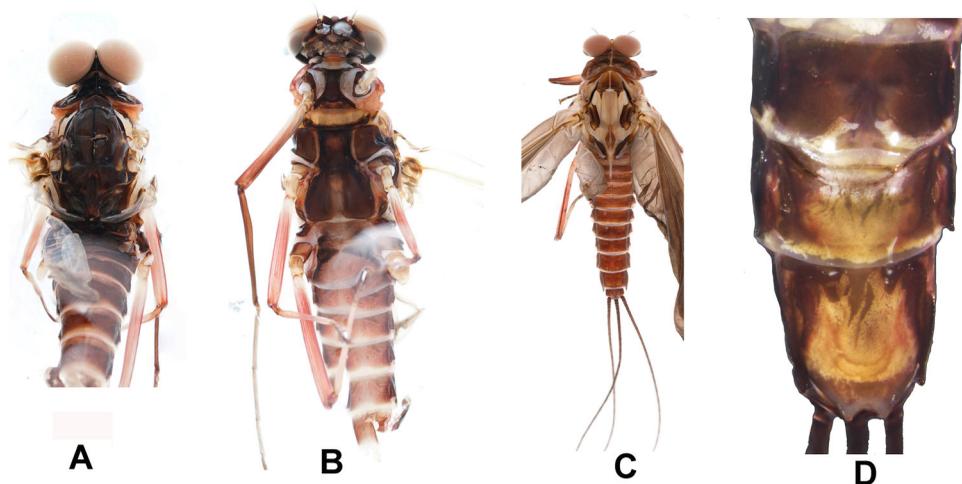
*In alcohol.* Body length 10.0–12.0 mm, caudal filaments 11.0–13.0 mm, forewing 11.0–13.0 mm, hindwing 2.0–3.0 mm. Colouration chocolate brown to dark brown, washed with irregular dark stripes and dots (Figure 4A).

**Head.** Compound eyes contiguous, upper portion dark reddish (in life) and lower portion black (Figure 5A).

**Thorax.** Dark brown dorsally, with dark sutures; Prosternum dark brown to black; basisternum of prosternum dark brown, with slightly converging anteriorly longitudinal carinae, maximum width between carinae about  $1.5\times$  minimum width; furcasterna separated by basisterum. Basisternum of mesosternum dark brown, rectangular, with parallel lateral margins and carinae; furcasternum subtriangular, median depression with parallel margins (Figure 5B).

**Legs.** Femora:tibiae:tarsi of foreleg = 1.0:1.4:1.2, tarsal segments 1–5 arranged in decreasing order = 3, 2, 4, 5, 1 (Figure 5A–B); Femora:tibiae:tarsi of midleg = 2.6:2.0:1.0, tarsal segments arranged in decreasing order = 5, 2, 3, 4, 1 (Figure 5B); Femora: tibiae: tarsi of hindleg = 1.0:3.0:3.6, tarsal segments arranged in decreasing order = 5, 4, 3, 2, 1 (Figure 5B). Legs reddish brown. Claws of all legs similar, each with one blunt and one hooked process.

**Wings.** Forewings hyaline, but costal (C) and subcostal (Sc) region semihyaline; longitudinal veins dark brown and cross veins light brown to semihyaline; cross veins in stigma region between C and Sc separated into two parts by a long vein; bullae of Sc,  $R_1$  (first radius) and  $R_5$  clear; Medius anterior (MA) forked two-third of distance from base to margin, close to the bulla of  $R_5$ ; Medius posterior (MP) forked slightly distal from fork of MA + Rs; Cubitus Posterior (CuP) recurved strongly (Figures 6A and 7A). Hindwings entirely hyaline, leading margin with costal projection; forks of



**Figure 5.** Adults of *Cincticostella femorata* (Tshernova, 1972) (digital photos): (A) male (dorsal view); (B) male (ventral view); (C) male subimago (thorax in dorsal view); and (D) terminal of female abdomen (ventral view).

MA + Rs and MP equidistant from base to margin; MA single, MP forked symmetrically (Figures 6B and 7B).

**Abdomen.** Chocolate brown, tergum IX with obvious posterolateral projections (Figure 5A,B).

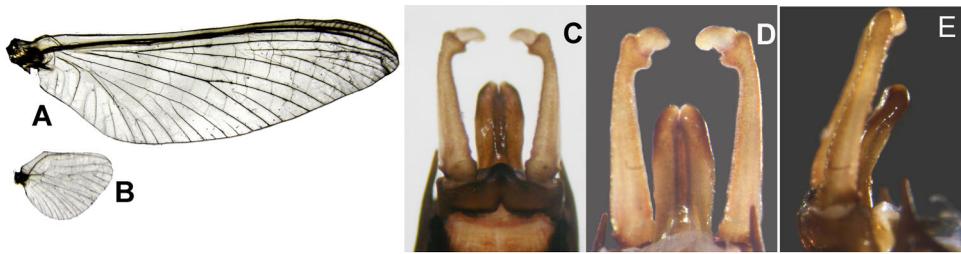
**Genitalia.** Styliger plate brown, with convex posterior lobe; forceps length about  $1.5 \times$  penis length; basal segment length ca. one-ninth of second segment length, second segment with distinct swollen apex; third segment slightly convex, length  $1.5 \times$  basal width; inner surface of forceps with tiny projections. Penis lobes fused with apicomedian cleft; lateral margins slightly convex and somewhat sinuous; dorsal and ventral surfaces flat and smooth, with indistinct median suture (Figures 6C–E and 7C).

#### **Description of female adult**

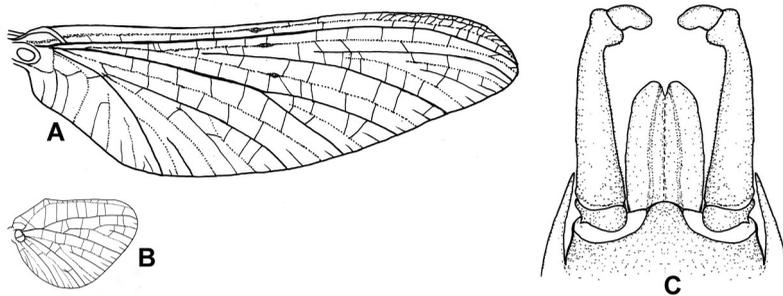
*In alcohol.* Colour pattern similar to male; body length 13.0–16.0 mm, caudal filaments 12.0–14.0 mm, forewing 14.0–16.0 mm, hindwing 2.5–3.5 mm. Length of femur: tibia: tarsus of foreleg = 2.2: 2.0: 1.0, tarsal segments 1–5 arranged in decreasing order of length = 5, 2, 4, 3, 1; femur: tibia: tarsus of midleg = 4.0: 3.0: 1.0, tarsal segments arranged in decreasing order = 5, 2, 3, 4, 1; femur: tibia: tarsus of hindleg = 5.2: 2.6: 1.0, tarsal segments arranged in decreasing order = 5, 4, 3, 2, 1. Subgenital plate produced to  $1/5$  length of sternum VIII. Posterior margin of subanal plate with semi-oval median cleft (Figure 5D).

#### **Description of male subimago**

*In alcohol.* Similar to male adult except for following characters (Figure 4B): scutellum with long and pointed posterior prolongation (Figure 5C); tarsus of foreleg



**Figure 6.** Male imago of *Cincticostella femorata* (Tshernova, 1972) (digital photos): (A) forewing; (B) hindwing; (C) genitalia (ventral view); (D) genitalia (dorsal view); and (E) genitalia (lateral view).



**Figure 7.** Male adult of *Cincticostella femorata* (Tshernova, 1972) (drawn picture): (A) forewing; (B) hindwing; and (C) genitalia (ventral view).

shorter than femur, caudal filaments shorter than body length; forewings and hindwings semi-hyaline, dark brown, with tiny setae on outer and hind margins.

#### **Description of female subimago**

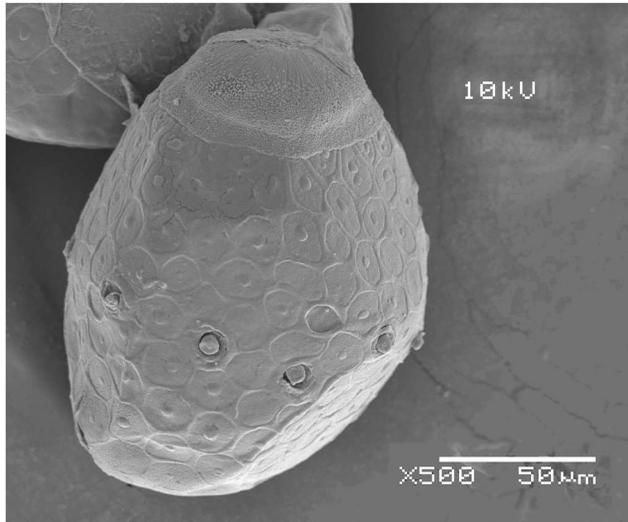
*In alcohol.* Similar to male subimago except for usual sexual differences.

#### **Description of egg**

Oval, with one polar cap, surface sculptured with hexagonal to round ridges or frames; each ridge surrounding a small tubercle; micropyle round; knob-terminated coiled threads (KCTs) at equatorial line (Figure 8).

#### **Diagnosis**

When compared with known congeners except *C. tornata* (Auychinda and Gattolliat, 2020) (Auychinda, Murányi, Li, Sartori, and Gattolliat 2020), both nymphs and male adults of *C. femorata* are easily distinguished. In nymphal stages, their flat body, especially expanded pronotum, flattened and serrated femora, enlarged genae, and quadrate head are very good recognisable characters (Figures 1 and 2A). The details added in this article, like setaceous body and mouthparts (labrum, mandibles, maxillae and labium, Figure 3), vestigial maxillary palpi, relatively smaller abdominal spines, tail having dense hairs but without spines (Figures 1 and 2), are useful additional diagnosable characters, when comparing this species to certain other congeners. The nymphal femora and pronotum of the *C. insolta*-complex species often expanded to some extent, which can be



**Figure 8.** Egg of subimago *Cincticostella femorata* (Tshernova, 1972) (digital photo).

**Table 2.** Mean values of K2P genetic distance among the DNA barcodes (COI).

Mean	<i>C. fusca</i>	<i>C. tornata</i>	<i>C. femorata</i>	<i>C. gosei</i>	<i>C. levanidovae</i>
<i>C. tornata</i>	0.226				
<i>C. femorata</i>	0.237	0.142			
<i>C. gosei</i>	0.240	0.227	0.239		
<i>C. levanidovae</i>	0.240	0.244	0.241	0.250	
<i>C. orientalis</i>	0.228	0.236	0.242	0.229	0.228

seen in *C. bifurcata* and *C. insolta*, for example, but never to the flat plate-like in *C. femorata* (see Xie et al. 2009; Martynov et al. 2019).

The male genitalia of *C. femorata* are distinctive. The almost totally fused penis without any projections or tubercles, but with slightly convex lateral margins, is a distinct feature (Figures 6C–E and 7C). The shape of the second segment of the forceps, with its swollen apex and abrupt inward curve, apex and the apical segment curved and slightly elongated, is another distinct feature (Figures 6D–E and 7C). Additionally, the unpigmented forewings, and hindwings with clear costal projections are also helpful for identification of this species (Figures 6A,B and 7A,B). Neither similar penis nor forceps have been found so far in the genus but most males of congeners are still unknown (Ishiwata 2003; Zhang et al. 2020). Only the genitalia of *C. elongatula* is even vaguely similar, but they do show fundamental differences (for instance, the shapes of their penis apex are completely different).

The major characteristics of *Cincticostella* eggs are relatively similar, regarding to their polar cap, outline, ridge shape and KCTs distribution pattern. However, the eggs of *C. femorata* show some uniqueness because of their regularly located KCTs at the equator (Figure 8). The eggs of *C. fusca* are somewhat similar, but they have fewer KCTs (Kang and Yang 1995).



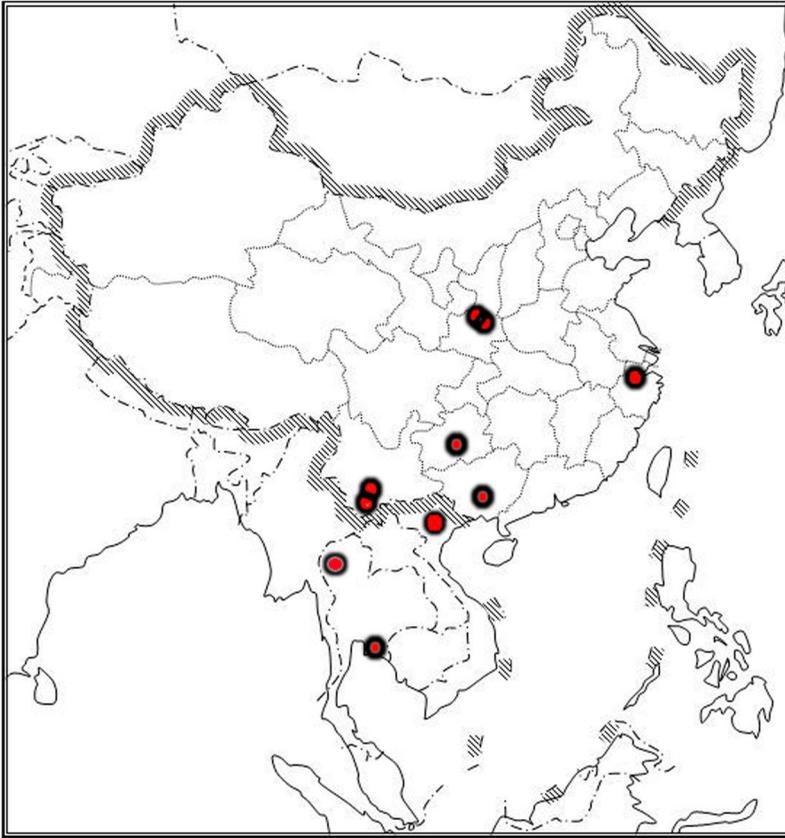
**Figure 9.** Habitat of *Cincticostella femorata* (Tshernova, 1972) in Tianmu mountain (Zhejiang Province, Southeastern China).

### ***Molecular study***

We checked all our collection of *C. femorata* from China, including some from Shaanxi Province, where the types of *C. tornata* were collected, but we found no consistent differences between the nymphs of those two species. We sequenced the COI gene of our *C. femorata* specimens and compared them to some species in the same genus. The genetic distance of two specimens of *C. femorata* is 0.002, but they differ from the sequence of *C. tornata* provided by Auychinda et al. (2020) by 0.142. The average distance of known species in the genus *Cincticostella* is 0.226–0.250 (Table 2). Ball, Hebert, Bruian, and Webb (2005) suggested the mean genetic divergence among congeneric species is greater 0.18, nevertheless, because of limited sampling locations and specimens, we treat *C. tornata* as species separate from *C. femorata* until more genetic data are available and the imaginal morphology of *C. tornata* is known.

### ***Ecology***

The creek where the *C. femorata* nymphs were collected is 2–8 m wide, with water depth 20–80 cm, and contains stones of various sizes (Figure 9). The nymphs usually hide under stones.



**Figure 10.** Distribution map of *Cincticostella femorata* (Tshernova, 1972) (the Vietnam and Thailand data from original reports on this species and Martynov et al. 2019).

The nymphs observed emerged at about 12:00 a.m. to 15:00 p.m. in April and the subimagos moulted around 1:00 a.m. to 8:00 a.m. local time after they lived for 36–40 h in rearing net.

#### ***Evolutionary and ecological trend***

Unquestionably, the nymphs of *C. femorata* are more specialised than other congeners, such as *C. fusca* and *C. nigra* (Uéno, 1928). Their extremely flat body, flattened femora, and setaceous body show they probably live on stones or other substrate surface. So we hypothesise here that the ancestor nymphs of *C. femorata*, which were somewhat similar to those of extant nymphs of *C. fusca* or *C. nigra*, evolved from hiding in plants or leaves to living on stone surface in lotic environment. To adapt to this new habitat, their body and femora flattened, and their body smoothed (with fewer and smaller spines) but covered with dense setae and streamlined to reduce water impact force.

#### ***Distribution***

China (Guangxi, Yunnan, Guizhou, Zhejiang, Shaanxi provinces), Northern Vietnam, Thailand (Figure 10).

## Acknowledgements

We thank sincerely Mr Zhenxing MA for his collection and molecular identification. Luke Jacobus (Indiana University Purdue University Columbus) improved the English and organisation of an early draft of this article. Jean-Luc Gattolliat (Museum of Zoology, Palais de Rumine, Place Riponne 6, CH-1005 Lausanne, Switzerland) read and improved our draft.

## Funding

This work was supported by the National Natural Science Foundation of China [Grant Nos. 31750002 and 32070475], funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), and supported by key projects of science-technology basic condition platform from The Ministry of Science and Technology of the People's Republic of China [Grant No. 2005DKA21402].

## Disclosure statement

No potential conflict of interest was reported by the authors.

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