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# The Imaginal Characters of *Cincticostella gosei* (Allen, 1975) linking the genus *Cincticostella* Allen, 1971 to *Ephacerella* Paclt, 1994 (Ephemeroptera: Ephemerellidae)

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#### Abstract

The imagos of *Cincticostella gosei* (Allen, 1975), which were reared from mature nymphs in Spring 2021 in China, are described in detail for the first time, along with the eggs. Junior synonyms *Ephemerella nigromaculata* Xu *et al.*, 1980 and *Ephemerella tianmushanensis* Xu *et al.*, 1980 are confirmed upon new materials and types. Compared to congeners, the males of *C. gosei* have dark tibiae and gently curved forceps. In addition, the nymphs of this species are redescribed and photographed in detail. Morphologically, the nymph can be identified by pairs of smaller tergal spines on the abdomen and lack of maxillary palp. The smaller body size is also helpful to its identification among Chinese *Cincticostella* species. The characters of *C. gosei* seem like those of *Ephacerella* Paclt, 1994 in smoothly bent forceps and narrowed penes from base to apex.

Key words: mayfly, morphology, taxonomy, evolution, China

## Introduction

The nymphs of the ephemerellid species *Cincticostella gosei* (Allen, 1975) were first reported from Thailand by Gose in 1969 and formally named by Allen in 1975. After that, this species has been mentioned by several researchers working on Asian Ephemerellidae and the genus *Cincticostella* Allen, 1971 (Edmunds & Murvosh, 1995; Kluge, 2004; Jacobus & McCafferty, 2008; Xie *et al.*, 2009; Martynov *et al.*, 2019; Zheng & Zhou, 2021) and its nymphs were found recently in India (Martynov *et al.*, 2021), but its imago has never been reported before.

In China, the nymphal stages of this species were first collected and reported by Zhou *et al.* (1997). At that time, they also found several subimagos of *C. gosei*, which led them to hypothesize that *Ephemerella* (*Serratella*) *nigromaculata* and *E.* (*Serratella*) *tianmushanensis* named by Xu *et al.* (1980) are actually the imaginal stages of *C. gosei*, as reflected in the mayfly species lists of Zhou (2013) and Zhou *et al.* (2015). However, because of lacking imago to compare, these two synonyms have not been confirmed until now.

In April 2021, encouraged by the success in rearing imagos of *C. fusca* (Kang & Yang, 1995) and *C. femorata* (Tshernova, 1972) (Zhang *et al.*, 2020; Zheng & Zhou, 2021), the authors of this paper spent two weeks at Tianmu mountain (Zhejiang province, eastern China), where the nymphs of *C. gosei* were found several times before and types of *Ephemerella* (*Serratella*) *nigromaculata* and *E.* (*Serratella*) *tianmushanensis* were collected, to rear imagos. Fortunately, more than twenty male and female imagos were gathered and their molting processes from last nymphal instar to adults were observed. As a result, not only imagos are now known for *C. gosei* but also the former synonyms were confirmed. In order to add more details to the descriptions of Allen (1975) and Xu *et al.* (1980), and to provide more pictures of the species *C. gosei*, we describe all stages of its life cycle below. The renewed interest in the morphology of this species will also deepen our understanding about phylogeny and biology of the genus *Cincticostella*.

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# Materials

*Cincticostella gosei* (Allen, 1975): 32 larvae, Zhou-Jia-Tan (580 m a.s.l.,  $30^{\circ}22'12"N$ ,  $119^{\circ}25'14"E$ ), Tianmu Mt., Lin-An city, Zhejiang Province, leg. Xiao-Li YING, Min ZHANG, Wen-Juan LI, 18.iv.2021 (333699 subimagos emerged at night of 20.iv, imagos molted on 21-22.iv); 4331099 (reared) and 30 larvae, Lao-Tian-Mu, same as the former, 19-21.iv.2021 (additional 5331099 subimagos emerged from those larvae at night of 23-26.iv, imagos molted on 24-28.iv).

Holotype (male imago) of *Ephemerella (Serratella) nigromaculata* Xu *et al.* (1980) and holotype (male imago) of *Ephemerella (Serratella) tianmushanensis* Xu *et al.* (1980), Lao-Tian-Mu, Tianmu Mt. (119°24'11"E, 30°18'30"N, 620 m), Lin-An city, Zhejiang Province, leg. by Jia-Zhu XU, V.1979.

Others: 1 larva, Geng-Yu-Gou, Zhou-Zhi County, Xi-An City, Shaanxi Province, leg. Na HAN, Min ZHANG, 04.viii.2019; 9L, Wu-Dong Village, Lei-Gong County, Guizhou Province, leg. Xu-Hong-Yi Zheng, Zhen-Xing Ma, 2019-VIII-02-06; 8 larvae, Fu-Tian Village, Er-Yuan County, Yunnan Province, leg. Hui XIE, Yan-Yan JIA, Ping CHEN, 08.vii.-2008; 2 larvae, Shang-Dan-Bao, Wen County, Gansu Province, leg. Qiang XIE, Chang-Fa ZHOU, 31.vii.2000; 4 larvae, Zhai-Xi Village, Huang-Shan District, Huang-Shan City, Anhui Province, leg. Dan ZHOU, Jun-Zhi SUN, Yi-Ke HAN, 29.v.2014; 20 larvae, Wo-Qi-Wo Stream, Da-Feng National Nature Reserve, Mei-Gu County, Sichuan Province, leg. Chang-Fa ZHOU, Chang-Hai SUN, 06-VI-2005; 30L, Tong-Mu-He, Wuyi Mountain Nature Reserve Protection, Station downstream, Jiangxi Province, leg. Chang-Fa ZHOU, 03.vi.2005; 80 larvae, Tian-Chi Mountain, Song County, Luo-Yang city, Henan Province, leg. Chang-Fa ZHOU, Peng LI, 13.vii.2004; 20 larvae, Ming-Ling Mountain, Yi-Xing city, Jiangsu Province, leg. Chao-Dong ZHU, Guo-Yuan WU, v.1995.

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

# Methods

The nymphs were collected from streams by hand net. Most adults were reared from mature nymphs in white plastic trays, a few females were attracted by collecting light. All materials were stored in ethanol (more than 75%).

All specimens were examined under a stereomicroscope and photographed with a digital camera (Single Lens Reflex). Some small structures, like mouthparts, legs and gills were observed and photographed with a microscope camera.

Eggs were dissected from female subimagos. Before being placed on the stage of the SEM (scanning electron microscope) for photographs, they were prepared with a standard protocol: fixed in 4% glutaraldehyde for 4–8 hours, rinsed with PBS (phosphate buffer saline) 2–3 times (10–15 minutes each), dehydrated in concentration gradient acetone (30%, 50%, 70%, 80%, 90%, 100%, 10–15 minutes each), and coated with gold film in a vacuum.

TABLE 1. GenBank accession numbers of Cincticostella COI se	quences used in this research
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	1
C. fusca	MT738744
C. tornata	MT254050
C. levanidovae	LC481984
C. orientalis	LC481983
C. nigra	MK774402
C. elongatula	LC481973
C. femorata	MT738746
C. gosei (Thailand)	MN186574
C. gosei (China)	MZ712088 (this research)

COI sequence: total genomic DNA of *C. gosei* (collected from Tianmu Mt. in 2020) 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 *et al.*, 2017) and reverse primer HCO2198 (5'-TAA ACT

TCA GGG TGA CCA AAA AAT CA-3', Folmer *et al.*, 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 (see their accession number in Table 1). They were aligned using Muscle (Edgar, 2004), and the K2P genetic distance were estimated in MEGA7 (Kumar *et al.*, 2016).

Abbreviations used in this study are as follows: L (larvae), C (Costa), Sc (Subcosta), Rs (Radial sector), R<sub>1</sub> (Radius), MA (Medius anterior), MP (Medius posterior).

# Results

# Description

## Cincticostella gosei Allen, 1975

urn:lsid:zoobank.org;act:9190CC31-1200-4BDD-A463-6FE7D8E7411D

*Ephemerella* TEB Gose, 1969: 135, figs. 38–55 (nymph). Types: nymph, from Thailand. Renamed as *Ephemerella* (*Cincticostella*) *gosei* by Allen, 1975: 20.

*Cincticostella gosei*: Allen 1980: 82; Zhou *et al.*, 1997: 222 (first record from China). Kluge, 2004: 307; Jacobus & McCafferty, 2008: 239; Xie *et al.*, 2009: 53; Martynov *et al.*, 2019: 148; Zheng & Zhou, 2021: 3; Martynov *et al.*, 2021: 145 (nymph photographed, India).

Serratella thailandensis Allen, 1980: 76. Types: nymph, from Thailand. Synonymized by Edmunds & Murvosh, 1995: 157.

*Ephemerella (Serratella) nigromaculata* Xu *et al.*, 1980: 5, figs. 1–7 (male). Type: male, from China (Zhejiang province); Gui, 1985: 89; You & Gui, 1995: 131, fig. 140 (male). Synonymized by Zhou, 2013: 177.

Notacanthella nigromaculata: Jacobus & McCafferty, 2008: 236.

*Ephemerella (Serratella) tianmushanensis* Xu *et al.*, 1980: 5, figs. 8–14 (male). Type: male, from China (Zhejiang province); Gui, 1985: 89; You & Gui, 1995: 132, fig. 141 (male). Synonymized by Zhou, 2013: 177.

Notacanthella tianmushanensis: Jacobus & McCafferty, 2008: 236.

Nymph (in alcohol): body length 5.5–7.0 mm, caudal filaments 3.0–4.5 mm, antennae 1.0–1.5 mm. Body reddish brown to dark brown (Fig. 1A), whole body covered with very tiny pale hair-like setae. Head: dark brown dorsally but area around ocelli pale, forming three obvious dots; antennal length ca. 1.2X of head width, yellowish to brown, with tiny hair-like setae between segments except base; genae slightly expanded to form clear elbow; free margins of clypeus with pale cilia (Fig. 1B). Mouthparts: labrum subquadrate, leading margin with shallow emargination; submedian margin with two rows of long hair-like setae; dorsal surface of labrum with numerous hair-like setae while ventral surface with same kind of setae beside median groove only (Fig. 1C). Left mandible: basal half of outer margin with long hair-like setae; outer incisor divided into 4 denticles but inner one with 3 denticles only; prostheca strong (Fig. 1F). Right mandible: both outer and inner incisor with 3 apical denticles, with hair-like setae on outer margin and mesoapical margin as in Fig. 1E. Maxillae: hair-like setae on basal half outer margin and apical mesal half inner margin; crown with dense hair-like and spine-like setae, two dentisetae stronger than others and more conspicuous; cardo with sparse hair-like setae on outer margin; maxillary palp absent (Fig. 1G). Hypopharynx: lingua round, with tiny cilia on apex; superlinguae with almost straight outer margins, with hair-like setae on apical and inner margins (Fig. 1D). Labium: all parts with hair-like setae or cilia, those on glossae, paraglossae and labial palpi longer; glossae sub-round in shape, surrounded by sub-oval paraglossae; basal segment of labial palpi subequal to second one in length but slightly broader, with hair-like setae on outer margin; both inner and outer margins of second segment with same setae; apical segment length ca. 1/3 length of segment two, with very tiny setae on surface; apex slightly narrower than base; its length ca. 3X width (Fig. 1H). Thorax: Anterolateral angle of pronotum expanded forwards into acute projections, pronotum extended laterally into same level of anterolateral projections of mesothorax, lateral lines of pronotum almost straight and parallel. Foreleg brown except base and apex of femora; length ratio of femora: tibiae: tarsi=2.0: 1.6: 1.0; femora with row of transverse spine-like setae subapically, outer margin with spine-like and tiny hair-like setae (Fig. 2D); tibiae darker than other segments, with spines and setae on outer, inner and dorsal margins; tarsi also with setae and spines on surface, those on apical area denser (Fig. 2A). Midleg: slightly longer than foreleg, femora: tibiae: tarsi=2.4: 1.8: 1.0, femora expanded progressively from base to apex (Fig. 2E); other structures similar to foreleg (Fig. 2B). Hindleg: longer and flatter than midleg, femora: tibiae:

tarsi=2.9: 2.5: 1.0, others similar to midleg (Fig. 2C). Femora of midleg and hindleg with clear longitudinal ridge in middle, posterior margins slightly serrated (Fig. 2E-F). Claws of all legs similar in shape and structure, with several apical setae and seven denticles along inner margins but basal one much smaller (Fig. 2L). Abdomen: terga II–IX with pair of median ridges which expand into distinct tubercles on posterior margins; posterolateral angles of terga extended into small but sharp spines (Fig. 1A). Gills on terga III–V similar, subquadrate to oval, ventral lamellae with numerous leaf-like lobes further divided into two clusters (Fig. 2G–I); gill on terga VI slightly more slender than anterior gills, ventral lamellae consisting of several lobes but without bifurcation (Fig. 2J); gill on terga VII much smaller than others, completely covered by gills of previous segment, both dorsal and ventral lobe membranous with irregular shape (Fig. 2K). Caudal filaments with brown base, but progressively paler from base to apex, each articulation with rings of short spines and setae (Fig. 1A).



**FIGURE 1.** Nymph and mouthparts of *Cincticostella gosei* (digital photos): A. Habitus in dorsal view; B. Head capsule; C. Labrum (dorsal view); D. Hypopharynx (ventral view); E. Right mandible; F. Left mandible; G. Maxilla; H. Labium (ventral view).

Male imago: body length 7.0-8.0 mm, caudal filaments 7.0-9.0 mm, forewing 7.0-8.0 mm, hindwing 1.5-2.0 mm. Body generally brown to black (Fig. 3A). Antennae brown except apex, with setae ring between segments. Upper portion of compound eyes reddish to pink while basal portion dark; distance between two eyes shorter than half width of median ocellus. Ocelli with dark base but pale apex. Prosternum dark brown, with slightly converging anteriorly longitudinal carinae, maximum width between carinae ca. 1.5X minimum width. Basisternum of mesosternum dark brown, with parallel furcasternum (Fig. 4C). Forewing: transparent except around stigma area; a long vein between C and Sc of stigma divided crossveins into two portions. Rs leaves MA at very base, MA forked at 2/3 point from base to outer margin; MP forked more basal than fork of Rs; marginal intercalaries almost meet nearby crossveins (Figs. 4A, 5A). Hindwing: costal projection small, rounded, located at 1/3 point from base to apex; MP forked between forks of R<sub>1</sub>+MA and MA (Figs. 4B, 5B). Legs: femora: tibiae: tarsi of foreleg=1.4: 2.2: 1.7, tarsal segments 1-5 arranged in decreasing order= 2, 3, 4, 5, 1; femora brown, tibiae dark, tarsi pale, first segment of tarsi much shorter than others (Fig. 3A). Femora: tibiae: tarsi of midleg=1.0: 0.7: 0.4, tarsal segments arranged in decreasing order=5, 1, 2, 3, 4; color pattern similar to foreleg except basal tarsi brown. Femora: tibiae: tarsi of hindleg=1.3: 1.0: 0.5, tarsal segments arranged in decreasing order=5, 1, 2, 3, 4, color pattern similar to midleg (Fig. 3A). Claws of all legs similar: one blunt and one hooked. Genitalia: styliger plate with median projected lobe. Basal segment of forceps broader than others but much shorter than second one; second segment slightly constricted at point in apical 1/3, bending inwards smoothly. Length of segment III ca. 2X width, slightly tapered from base to apex. Penes length 2/3 length of forceps, with irregular dark stripes or streaks, slightly narrowed from base to apex, with shallow median emargination (Fig. 4E–G). Caudal filaments dark in basal 1/3, other portion pale (Fig. 3A).



**FIGURE 2.** Nymphal characters of *Cincticostella gosei* (digital photos): A. Foreleg (dorsal view); B. Midleg (dorsal view); C. Hindleg (dorsal view); D. Fore femur (dorsal view); E. Middle femur (dorsal view); F. Hind femur (dorsal view); G. Gill I (ventral view); H. Gill II (ventral view); I. Gill III (ventral view); J. Gill IV (ventral view); K. Gill V (ventral view); L. Hindleg claw.



FIGURE 3. Imagos and subimagos of *Cincticostella gosei* (digital photos): A. Male imago; B. Female imago; C. Male subimago; D. Female subimago.



**FIGURE 4.** Male and female structures of *Cincticostella gosei* (digital photos): A. Forewing; B. Hindwing; C. Male head and thorax (ventral view); D. Female abdominal terga (ventral view); E. Genitalia (dorsal view); F. Genitalia (ventral view); G. Genitalia (lateral view). Scale bar: A–C. 1.0 mm; D–G. 0.5 mm.



FIGURE 5. Male of Cincticostella gosei: A. Forewing; B. Hindwing.



FIGURE 6. Egg morphology of Cincticostella gosei (SEM photos): A. Whole egg; B. Egg surface enlarged.

**Female imago:** body length 6.5–8.0 mm, caudal filaments 7.0–9.0 mm, forewing 7.5–9.0 mm, hindwing 1.5–2.0 mm. Color pattern similar to male (Fig. 3B). Lengths of femur: tibia: tarsus of foreleg=1.0: 1.3: 0.7, tarsal segments arranged in decreasing order of length=5, 2, 3, 4, 1; femur: tibia: tarsus of midleg=1.1: 0.9: 0.5, tarsal segments arranged in decreasing order=5, 3, 2, 4, 1; femur: tibia: tarsus of hindleg=1.4: 1.1: 0.5, tarsal segments arranged in decreasing order=5, 3, 2, 4, 1; femur: tibia: tarsus of hindleg=1.4: 1.1: 0.5, tarsal segments arranged in decreasing order=5, 3, 2, 4, 1. Sterna of segment VII extended into narrow lobe, subanal plate with depressed posterior margin (Fig. 4D).

**Male subimago** (Fig. 3C): body length 6.0–7.5 mm, caudal filaments 6.0–8.0 mm, forewing 7.0–8.5 mm, hindwing 1.4–1.8 mm. Body general brown to gray. Scutellum with long and pointed posterior prolongation. Forewings and hindwings semi-hyaline, with tiny setae on hind margins. Lengths of femur: tibia: tarsus of foreleg=0.8: 1.0: 0.9, tarsal segments arranged in decreasing order of length=3, 2, 4, 5, 1; femur: tibia: tarsus of midleg=0.9: 0.7: 0.4, tarsal segments arranged in decreasing order=5, 1, 2, 3, 4; femur: tibia: tarsus of hindleg=1.0: 0.8: 0.4, tarsal segments arranged in decreasing order=5, 1, 2, 3, 4.

**Female subimago** (Fig. 3D): body length 5.5–7.0 mm, caudal filaments 6.0–7.5 mm, forewing 7.0–8.0 mm, hindwing 1.4–1.8 mm. Body general yellowish to brown. Lengths of femur: tibia: tarsus of foreleg=0.8: 0.8: 0.5, tarsal segments arranged in decreasing order of length=5, 2, 3, 1, 4; femur: tibia: tarsus of midleg=0.9: 0.7: 0.4, tarsal segments arranged in decreasing order=5, 1, 2, 3, 4; femur: tibia: tarsus of hindleg=1.2: 1.0: 0.5, tarsal segments arranged in decreasing order=5, 1, 2, 3, 4; femur: tibia: tarsus of hindleg=1.2: 1.0: 0.5, tarsal segments arranged in decreasing order=5, 1, 2, 3, 4; femur: tibia: tarsus of hindleg=1.2: 1.0: 0.5, tarsal segments arranged in decreasing order=5, 1, 2, 3, 4; femur: tibia: tarsus of hindleg=1.2: 1.0: 0.5, tarsal segments arranged in decreasing order=5, 1, 2, 3, 4; femur: tibia: tarsus of hindleg=1.2: 1.0: 0.5, tarsal segments arranged in decreasing order=5, 1, 2, 3, 4.

**Egg.** Scanned egg is 0.13 mm length and 0.08 mm width. Oval, with one polar cap, surface sculptured with hexagonal structures and decorated with sparse tubercle-like projections (Fig. 6).

**Distribution.** China (Yunnan, Guizhou, Sichuan, Anhui, Gansu, Jiangxi, Zhejiang, Jiangsu, Shaanxi, Henan provinces), Thailand, India (Fig. 7).

**Ecology.** The stream where the *C. gosei* nymphs were collected is 4.0–8.0 m wide, with water depth 0.2–0.5 m, and contains stones of various sizes (Fig. 8). The nymphs were collected under stones. The nymphs emerged at about 4:00 PM to 8:00 PM local time in April, most around 5:00 PM; before molting to subimago, the nymph were seen climbing on the substrates underwater or floating on water surface for about 30 minutes. Subimagos persisted for 24–36 hours, most of them molted around 1:00 AM to 6:00 AM. The observed lifespan of imagos was about 3 days.

**Molecular similarity:** The COI gene sequence of our *C. gosei* is most similar to Thai materials under the same species name. The distance between them is 0.170 while the average distance of known species in the genus *Cincticostella* is 0.217–0.270 (Table 2).

	Cfusca	C.tornata	C.levanidovae	C.orientalis	C.nigra	C.elongatula	Cfemorata	<i>C.gosei</i> (Thailand)	
C. fusca									
C. tornata	0.229								
C. levanidovae	0.229	0.221							
C. orientalis	0.228	0.234	0.218						
C. nigra	0.226	0.270	0.246	0.252					
C. elongatula	0.239	0.256	0.239	0.233	0.146				
C. femorata	0.235	0.144	0.231	0.237	0.242	0.215			
C. gosei	0.239	0.225	0.229	0.222	0.218	0.245	0.236		
(Thailand)									
C. gosei	0.250	0.235	0.236	0.269	0.251	0.270	0.217	0.170	
(China)									

TABLE 2. Mean values of K2P genetic distance among the Cincticostella DNA barcodes (COI)



**FIGURE 7.** Distribution map of *Cincticostella gosei* (the Thai data from Gose 1969 and Edmunds & Murvosh 1995, the Indian data from Martynov *et al.*, 2021; blue asterisk indicates the adults collecting site, red dots represent other localities of our materials, yellow ones are in India and Thailand).



FIGURE 8. Habitat of Cincticostella gosei in Tianmu mountain (Zhejiang Province, Eastern China).

#### Discussion

Because of short COI gene distance (0.170), at this point, we treat our species as the same *C. gosei* of Thailand awaiting more COI sequences of the genus, especially that of India or other places.

Both the imagos and nymphs of *Cincticostella gosei* are easily distinguished from other species in the same genus by the following characters. For male imago: (1) The shape of penes (progressively narrowed from base to apex and without any projections) is a good diagnostic character of this species. (2) The forceps are unique: they have a slight crease at apical 1/3, making them look like bending inwards smoothly. The forceps of all other species in the same genus are abruptly bent inwards subapically, making the forceps seem to have an articulation (Ishiwata, 2003; Zhang *et al.*, 2020; Zheng & Zhou, 2021). (3) This species is smaller than others (body size less than 10.0 mm). In contrast, imagos of most known species (such as *C. nigra*, *C. femorata*, *C. fusca*) are longer than 15.0 mm. (4) The tibiae are deep dark, while the counterparts of others are yellowish to pale, although the tarsi of *C. fusca* are dark brown. (5) Except grey and semi-hyaline stigmatic area, wings of this species do not have any markings or color and are totally transparent; this situation is similar to *C. femorata*, while the C and Sc areas of *C. fusca* are brown (Zhang *et al.*, 2020; Zheng & Zhou, 2021). The smaller body, almost transparent wings and dark tibiae are good diagnostic characters of the female *C. gosei*, too.

We agree with the diagnostic characters provided by Martynov *et al.* (2021) for the nymphs of this species, which include the small body, pairs of small tergal spines on abdomen, and slightly expanded femora having clear middle longitudinal ridges on legs. Further, the maxillae without palpi, slim and long labial palpi, long apical segments of labial palpi are additional identification characteristics of the *C. gosei* nymphs.

On the other hand, we also find three fine differences between the Chinese nymphs and Indian materials of *C. gosei*. First, the labra of them are different. The Indian form has a deeper emargination on the front margin, while the Chinese form has a shallow median notch (Fig. 1C). Second, the number and shape of claw denticles are not the same. The Chinese nymphs have seven denticles (Fig. 2L), but the Indian counterparts have 5–6. Third, the femora of midlegs are also different. The Chinese nymphs have slight serrations and spines on their outer margins (Fig. 2E), while the Indian nymphs have spines only (Martynov *et al.*, 2021). Those three nymphal characters of Chinese *C. gosei* are also present in the original description and figures of Thailand *C. gosei* provided by Gose (1969).

Kluge (2004), Jacobus & McCafferty (2008) and Ogden *et al.* (2009) considered the genus *Cincticostella* to be close to *Ephacerella* because they share the synapomorphy of having nymphal mesothoracic projections. Here we can see that the male penes of *C. gosei* (narrowed slightly from base to apex) and forceps (bent inwards gently) are very similar to those of *Ephacerella longicaudata* (Uéno, 1928), the single species in the genus (see fig. 85 of Jacobus & McCafferty, 2008). These may be additional evidences to strengthen the sister-group relationship between *Cincticostella* and *Ephacerella*.

Jacobus & McCafferty (2008) attributed the imagos of *C. gosei* (as *N. nigromaculata*) to the genus *Notacanthella* Jacobus & McCafferty, 2008, but confident stage associations for *Notacanthella* species are still needed at this point, and the association of *Notacanthella* sp. A stages (Jacobus & McCafferty, 2008) should be considered tentative (L. M. Jacobus, pers. comm.).

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#### References

Allen, R.K. (1975) *Ephemerella (Cincticostella*): A revision of the nymphal stages. *Pan-Pacific Entomologist*, 51 (1), 16–22.
Allen, R.K. (1980) Geographic distribution and reclassification of the subfamily Ephemerellinae (Ephemeroptera: Ephemerellidae). *In*: Flannagan, J.F. & Marshall, K.E. (Eds.), *Advances in Ephemeroptera Biology*. Plenum Press, New

York, pp. 71-91.

https://doi.org/10.1007/978-1-4613-3066-0\_6

- Edgar, R.C. (2004) MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput. *Nucleic Acids Research*, 32, 1792–1797.
  - https://doi.org/10.1093/nar/gkh340

Edmunds, G.F. & Murvosh C.M. (1995). Systematic changes in certain Ephemeroptera studied by R.K. Allen. *Pan-Pacific Entomologist*, 71 (3), 157–160.

- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA Primers for Amplification of Mitochondrial Cytochrome C Oxidase Subunit I from Diverse Metazoan Invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Gose, K. (1969) Mayflies (Ephemeroptera) from Thailand. *In*: Kira, T. & Iwata, K. (Eds.), *Nature and Life in Southeast Asia. Vol. VI.* Japan Society for the Promotion of Science, Tokyo, pp. 125–138.
- Gui, H. (1985) A catalog of the Ephemeroptera of China. *Journal of Nanjing Normal University (Natural Science)*, 4, 79–97. [in Chinese]
- Hrivniak, L., Sroka, P., Godunko, R.J. & Žurovcovà, M. (2017) Mayflies of the Genus *Epeorus* Eaton, 1881 s.l. (Ephemeroptera: Heptageniidae) from the Caucasus Mountains: A New Species of *Caucasiron* Kluge, 1997 from Georgia and Turkey. *Zootaxa*, 4341 (3), 353–374. https://doi.org/10.11646/zootaxa.4341.3.2
- Ishiwata, S. (2003) A revision of the genus *Cincticostella* (Insecta: Ephemeroptera: Ephemerellidae) from Japan. *Species Diversity*, 8, 311–346.

https://doi.org/10.12782/specdiv.8.311

- Jacobus, L.M. & McCafferty, W.P. (2008) Revision of Ephemerellidae genera (Ephemeroptera). *Transactions of the American Entomological Society*, 134 (1–2), 185–274.
  - https://doi.org/10.3157/0002-8320(2008)134[185:ROEGE]2.0.CO;2
- Kluge, N.J. (2004) *The Phylogenetic System of Ephemeroptera*. Kluwer Academic Publishers, Dordrecht, 442 pp. https://doi.org/10.1007/978-94-007-0872-3
- Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33, 1870–1874. https://doi.org/10.1093/molbev/msw054
- Martynov, A.V., Selvakumar, C., Palatov D.M., Subramanian K.A., Sivaramakrishnan K.G., Vasanth, M. & Jacobus, L.M. (2021) Overview of Indian and Nepali representatives of the *Cincticostella nigra* (Uéno, 1928) complex (Ephemeroptera, Ephemerellidae), with discussion about *Cincticostella* Allen, 1971 species complexes. *ZooKeys*, 1040, 123–166. https://doi.org/10.3897/zookeys.1040.64280
- Martynov, A.V., Selvakumar, C., Subramanian, K.A., Sivaramakrishnan, K.G., Chandra, K., Palatov, D.M., Sinha, B. & Jacobus, L.M. (2019) Review of the *Cincticostella insolta* (Allen, 1971) complex (Ephemeroptera: Ephemerellidae), with description of three new species from northern India and Nepal. *Zootaxa*, 4551 (2), 147–179. https://doi.org/10.11646/zootaxa.4551.2.2
- Ogden, T.H., Osborne, J.T., Jacobus, L.M. & Whiting, M.F. (2009) Combined molecular and morphological phylogeny of Ephemerellinae (Ephemerellidae: Ephemeroptera), with remarks about classification. *Zootaxa*, 1991 (1), 28–42. https://doi.org/10.11646/zootaxa.1991.1.2
- Xie, H., Jia, Y.Y., Chen, P., Jacobus, L.M. & Zhou, C.F. (2009) Two new *Cincticostella* species from China with a larval key to species of the genus (Ephemeroptera: Ephemerellidae). *Zootaxa*, 2299 (1), 53–61. https://doi.org/10.11646/zootaxa.2299.1.5
- Xu, J.Z., You, D.S., Su, C.R. & Xu, Y.Q. (1980) Two new species of genus *Ephemerella* (Ephemeroptera: Ephemerellidae). *Journal of Nanjing Normal University (Natural Science)*, 2, 5–8. [in Chinese]
- You, D.S. & Gui, H. (1995) Ephemeroptera. In: Economic Insect Fauna of China. Fasc. 48. Science Press, Beijing, 152 pp. [in Chinese]
- Zhang, W., Han, N., Zhang, M., Wang, Y.F. & Zhou, C.F. (2020) The Imaginal and Detailed Nymphal Characters of *Cincticostella fusca* (Kang & Yang, 1995) (Ephemeroptera: Ephemerellidae). *Zootaxa*, 4729 (2), 277–285. https://doi.org/10.11646/zootaxa.4729.2.8
- Zheng, X.H.E. & Zhou, C.F. (2021) First detailed description of adults and nymph of *Cincticostella femorata* (Tshernova, 1972) (Ephemeroptera: Ephemerellidae). *Aquatic Insects*, 1–13.
  - https://doi.org/10.1080/01650424.2020.1871026
- Zhou, C.F. (2013) A species list of Chinese mayflies (Insecta: Ephemeroptera). In: Tojo, K., Tanida, K. & Nozaki, T. (Eds.), Biology of Inland Waters. 2. Supplement. Proceedings of the first symposium of the Benthological Society of Asia, 2013, pp. 167–225.
- Zhou, C.F., Gui, H. & Su, C.R. (1997) Insects of Funiu Mountain (Ephemeroptera). A study on the taxonomy and fauna of insects in Henan, 2, 221–222. [in Chinese]

Zhou, C.F., Su, C.R. & Gui, H. (2015) Outline of Chinese Mayflies. Science Press, Beijing, 310 pp. [in Chinese]