



## A new *Cinygmula* McDunnough, 1933 species with distinct imaginal frontal fold from eastern Chinese Himalaya (Ephemeroptera: Heptageniidae)

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### Abstract

In the family Heptageniidae (Ephemeroptera), *Cinygmula hutchinsoni* (Traver, 1939) from central Asia is the only species known to possess pointed claws and a distinct frontal fold. Here, a second similar species with the same combination of characters is described. *Cinygmula longissima* **sp. nov.** is found in the eastern Himalaya of China, shows stouter imaginal penes, darker wingbases, and narrower nymphal gills than the previous one. This discovery indicates that this species of Heptageniidae are not limited to central Asia but live in high-altitude alpine habitats.

**Key words:** transformation, morphology, subimago, biogeography, mayfly

### Introduction

As a general rule, from nymph to subimago and imago, the main imaginal structures of mayfly usually enlarge progressively, such as compound eyes, legs, caudal filaments and genitalia. While some other nymphal structures, like mouthparts, accessory gills and abdominal projections, reduce step by step (Edmunds & McCafferty 1988). In recent years, changing of some other structures, such as head shape, leg position and wing posture have been realized and presented in certain level (Kluge 2015; Ma & Zhou 2021).

The species *Cinygmula hutchinsoni* (Traver 1939), which has remarkable frontal fold on its imaginal head, has another unusual character: two similar pointed claws (Kluge 2015). Traver (1939) even placed it in an independent genus upon those two points. So far, no other similar case has been reported in the genus. Geographically, the species bearing pointed claws are exclusively in central Asia and in high altitude area (Kluge 2015). The occurrence in other region of this phenomenon, if we find, will broaden and deepen our knowledge on this issue.

In summer of 2023, we spent nearly one month to collect mayflies in eastern Qinghai—Xizang Plateau of China. During this collecting, some heptageniid adults with elongated frontal fold and two pointed claws and its nymphs were found. Its morphology shows it is a new species and enriches the diversity of *Cinygmula* and Heptageniidae as well.

### Methods

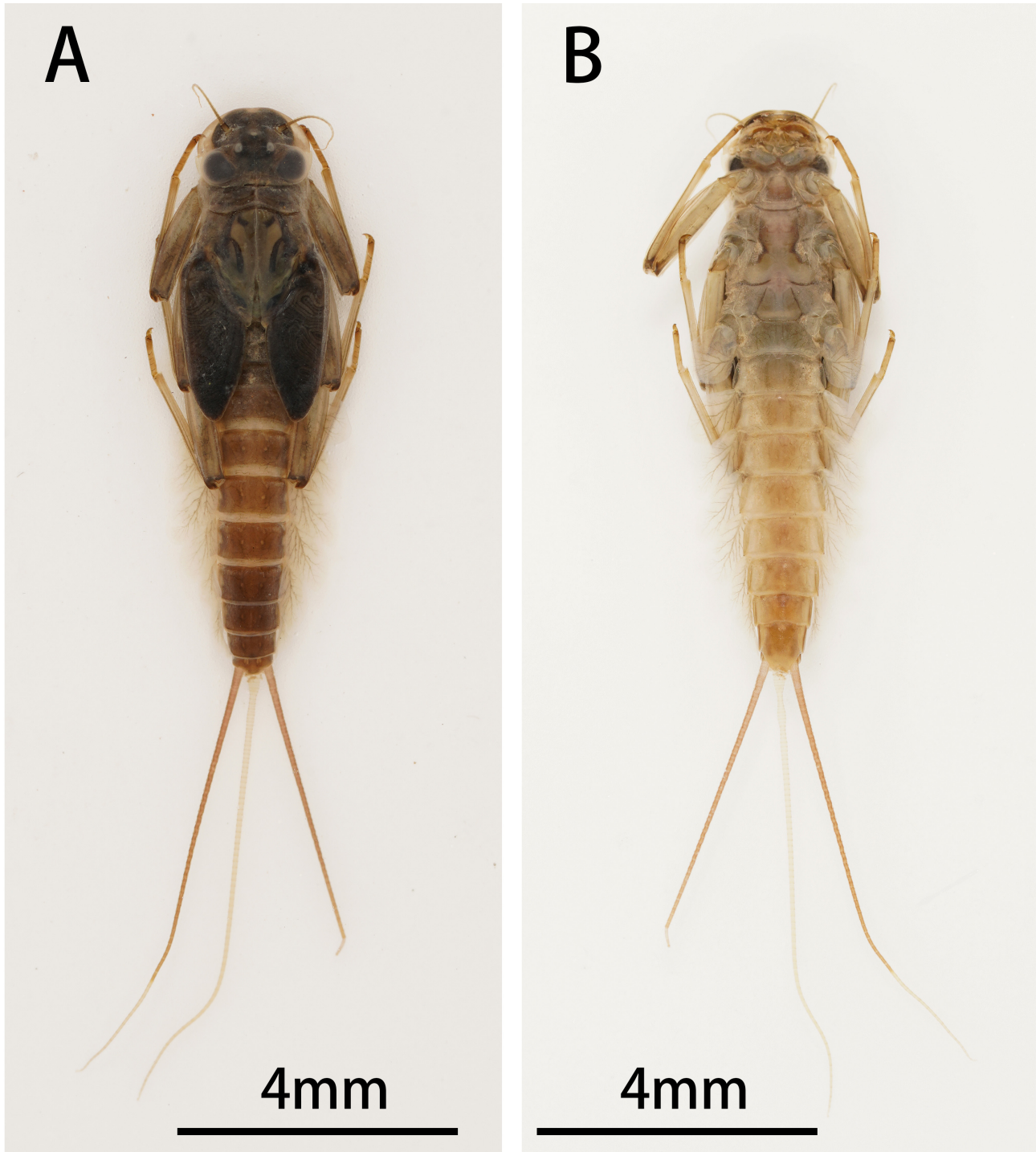
Nymphs were collected from the river by hand screen. Imagos and subimagos were obtained on the stones near the river. All materials used in this study were stored in ethanol (about 85%), deposited in the mayfly collection of College of Life Sciences, Nanjing Normal University.

Specimens were examined under a stereomicroscope (Nikon SMZ 745T). Nymphs and winged stages were photographed by a SONY a7R IV camera with a LAOWA 25mm 5 × macro lenses. Eggs were dissected from female subimagos. The SEM (scanning electronic microscope) samples were prepared with a standard protocol: fixed in 4% glutaraldehyde for 5–8 hours, rinsed with PBS (physiological saline) 2–3 times (10–15 minutes each), dehydrated in concentration gradient acetone (30%, 50%, 70%, 80%, 90%, 100%, 10 to 15 min each), and coated with gold film in a vacuum. Terminology mainly follows that of Kluge (2004).

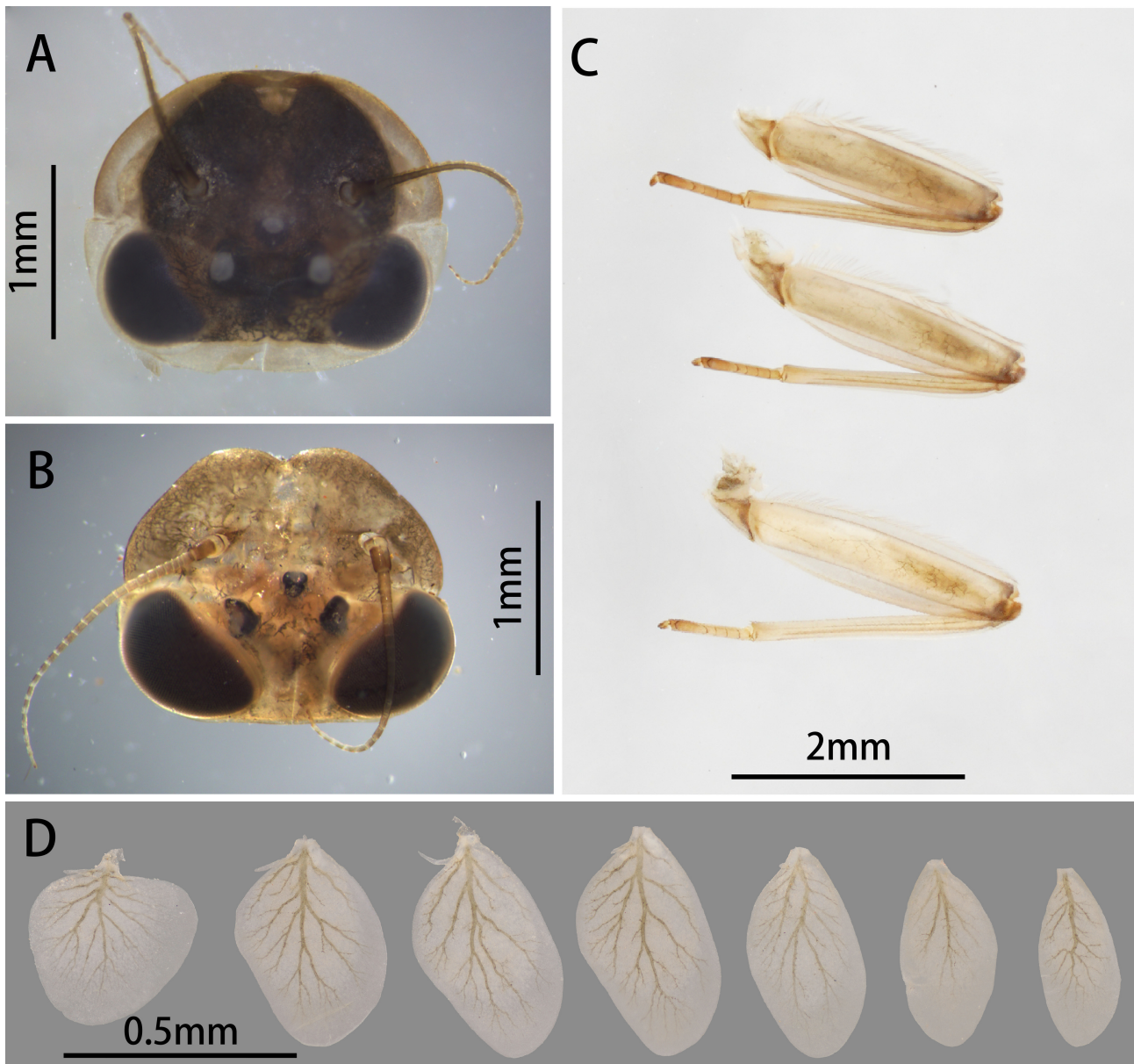
Abbreviations: KCT Knob-terminated coiled thread; MP Medius posterior; MA Medius anterior; Rs Radial sector.

***Cinygmula longissima* sp. nov.**

**Holotype:** male imago, China, Qinghai Province, Menyuan County, Laohugou, 37°46'44" N, 101°20'11" E, 3620 m a.s.l., 25. VII. 2023, leg. De-Wen Gong. Paratypes: 1 male imago, 1 male subimago, 1 female subimago, 7 nymphs, same as the holotype.



**FIGURE 1.** Nymphal habitus of *Cinygmula longissima* sp. nov.  
A: dorsal view; B: ventral view



**FIGURE 2.** Nymphal structures of *Cinygmula longissima* **sp. nov.**

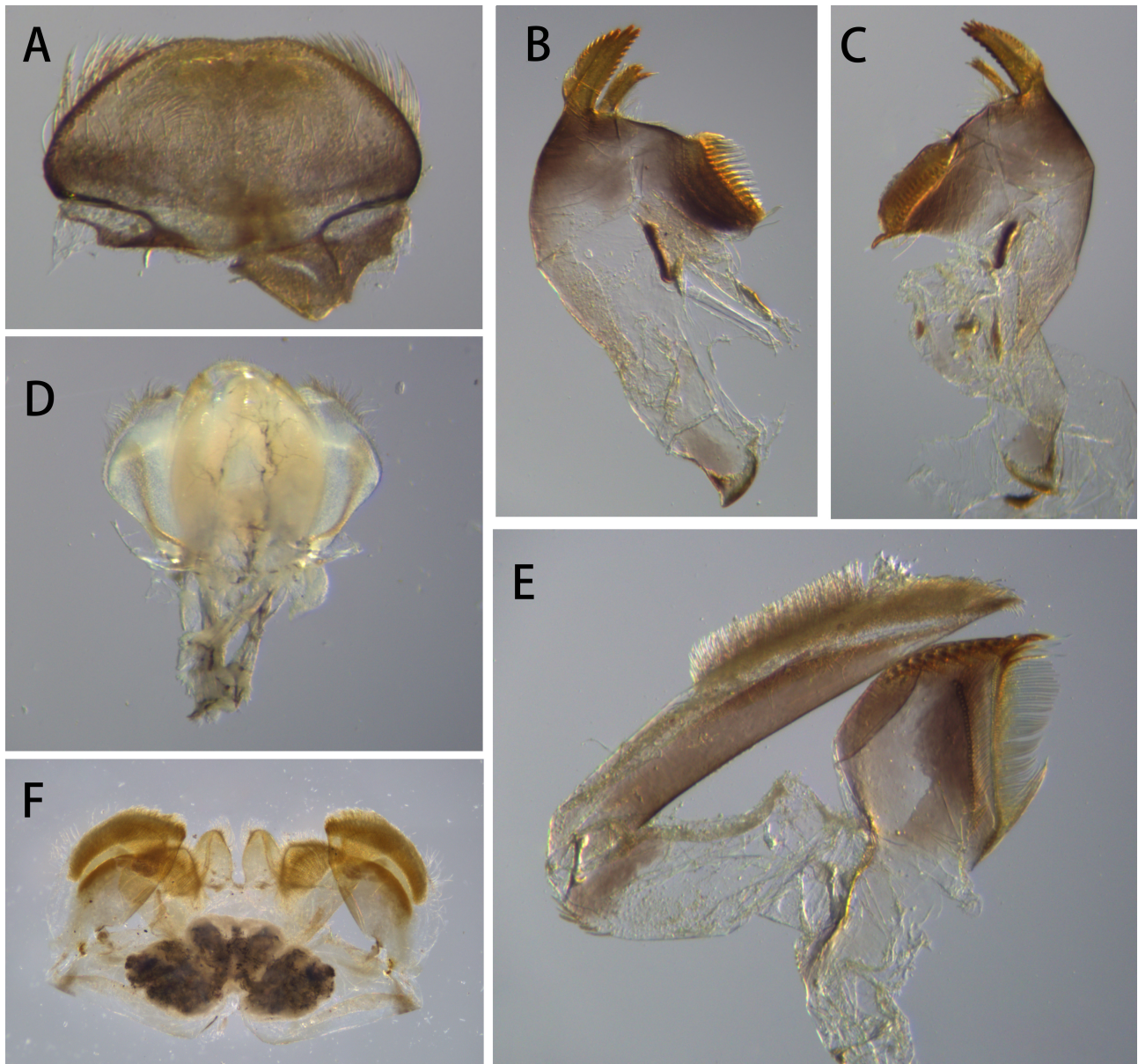
A: head of a mature nymph (showing the frontal fold in the shell, dorsal view); B: head capsule (showing the shape, dorsal view); C: fore-, mid- and hindleg (from above to bottom); D: Gills I–VII (from left to right)

**Mature nymph** (Figs. 1–3): body 9.0–10.0 mm, caudal filaments 7.5–8.0 mm; body slim, grey to pale brown (Fig. 1). Anterior half of head extended forward, median notch shallow “v” shaped (Figs 2A–B). Labium and maxillary palp partially visible in dorsal view. Antennae 2.5 mm, slightly longer than head width, with very tiny almost invisible setae (Figs 2A–B). Posterior margin of head almost straight (Figs 2A–B).

**Mouthparts** (Fig. 3): labrum semi-circular, with hair-like setae on dorsal and ventral surfaces, and margins, those setae on dorsal surface longer but fewer (Fig. 3A). Structures of left and right mandibles similar, both outer and inner incisors serrated, prostheca presented by row of setae, a few spine-like setae on mesal margin near molar (Figs 3B–C); right mola with distinct denticle on mesal corner (Fig. 3C). Maxillae with row of spine-like setae on mesal margin and another row on ventral surface; line of comb-shaped spines on crown. Apical segment of maxillary palp ca. 1.5× basal one, apex narrowed and pointed; row of setae brush on ventral surface (Fig. 3E). Lingua larger than superlinguae, with hair-like setae on margins and tips (Fig. 3D). Glossae of labium oval, paraglossae heart-shaped, slightly wider than the former; both of them with hair-like setae on margins and dense setae on dorsal surfaces; two



segments of labial palpi subequal in length and width; basal segment with several hair-like setae near apex, apical segment with row of setae on outer margin, and with two setae brushes on both dorsal and ventral surfaces, ventral ones larger (Fig. 3F).

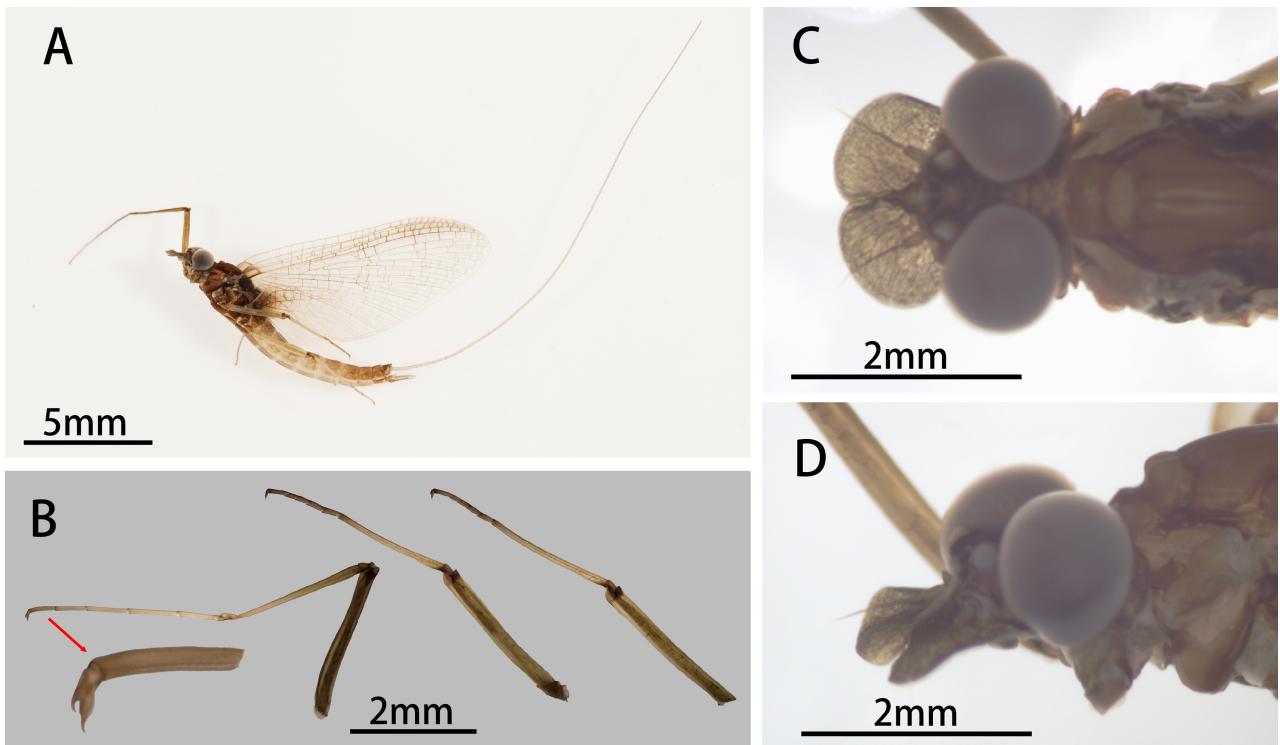


**FIGURE 3.** Nymphal mouthparts of *Cinygmula longissima* sp. nov.

A: labrum (dorsal view); B: left mandible (dorsal view); C: right mandible (dorsal view); D: hypopharynx (dorsal view); E: left maxilla (ventral view); F: labium (ventral view)

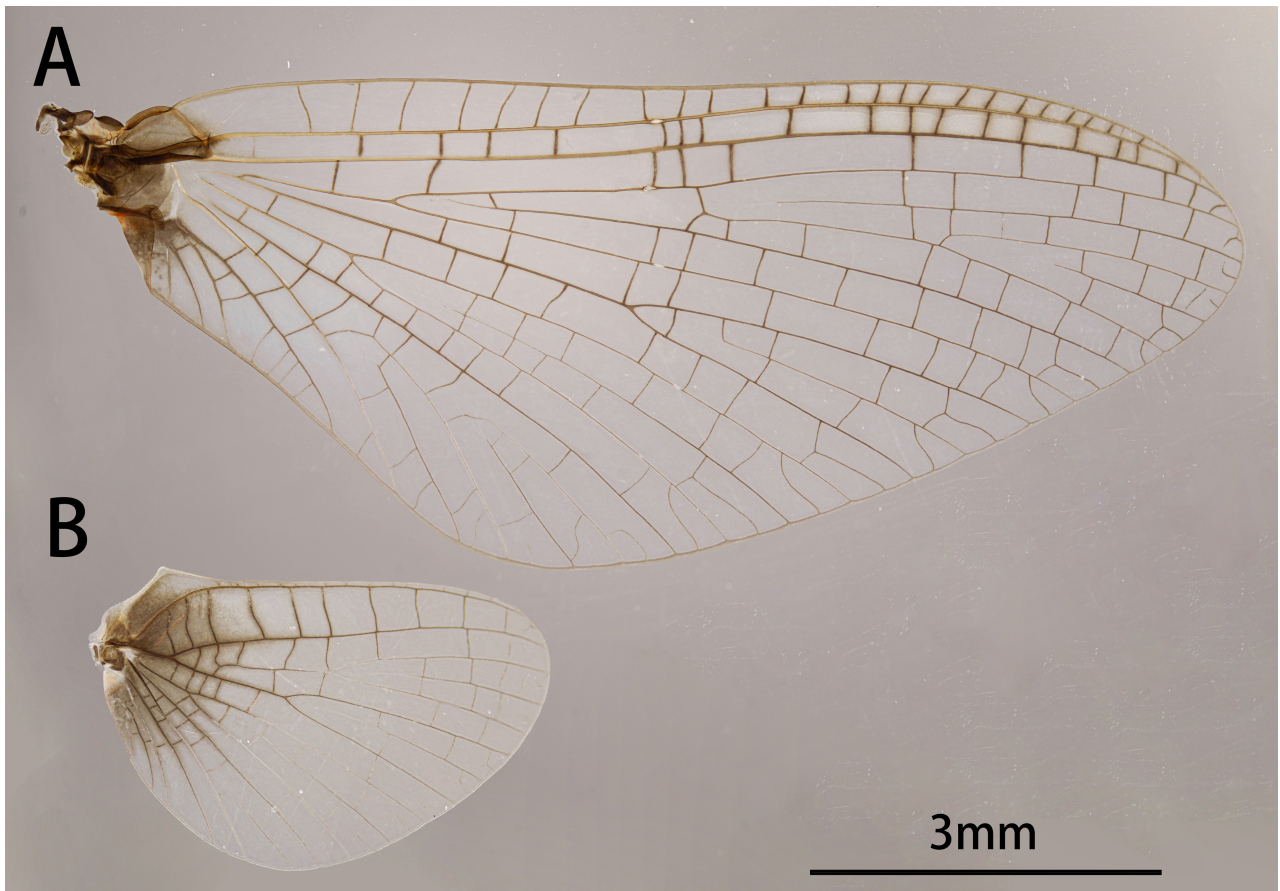
**Thorax:** Pronotum slightly extended laterally (Fig. 1A). Three pairs of legs similar to each other; foretibiae subequal to forefemora; tibiae of mid- and hindlegs shorter than their femora. Median portion of femora paler than surrounding area; tibiae and tarsi uniformly pale to grey, with apex slightly darker (Fig. 1A). Femora with a row of setae on posterior margin and some scattered spine-like setae on dorsal surface. Tibiae with some spine-like setae on anterior margin and some setae on dorsal margin, these setae much shorter than those of femora. Tarsi with setae on dorsal and apical margins (Fig. 2C). Claws of all legs deep brown to dark, with 5 subapical denticles (Fig. 8A).





**FIGURE 4.** Male imago of *Cinygmula longissima* sp. nov.

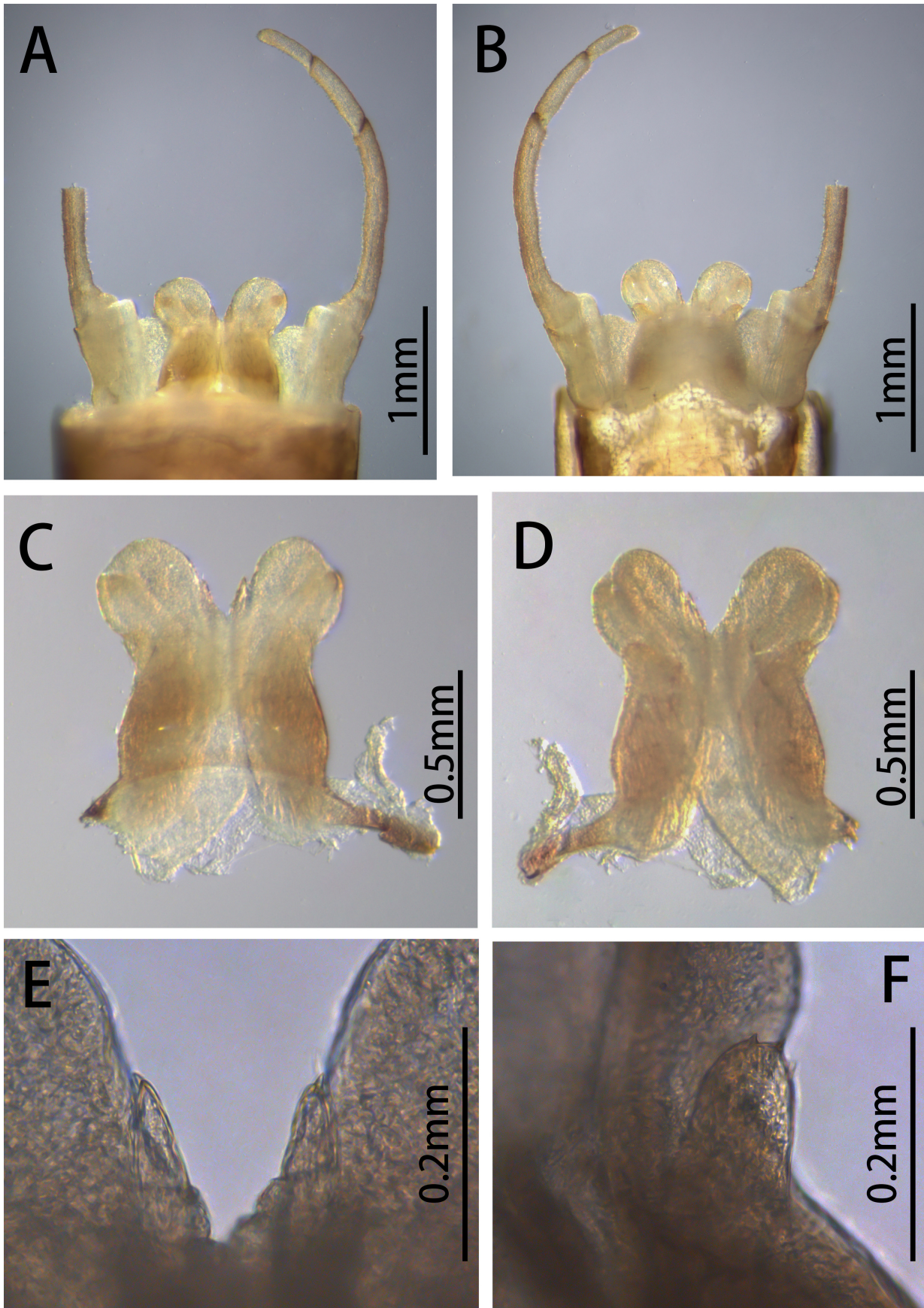
A: habitus; B: legs (fore-, mid- and hindlegs from left to right and foreclaws enlarged); C: head (dorsal view); D: head (lateral view)



**FIGURE 5.** Wings of male *Cinygmula longissima* sp. nov.

A: forewing; B: hindwing





**FIGURE 6.** Male genitalia of *Cinygmula longissima* sp. nov.

A: genitalia (dorsal view); B: genitalia (ventral view); C: penes (dorsal view); D: penes (ventral view); E: titillators of penes; F: lateral spine

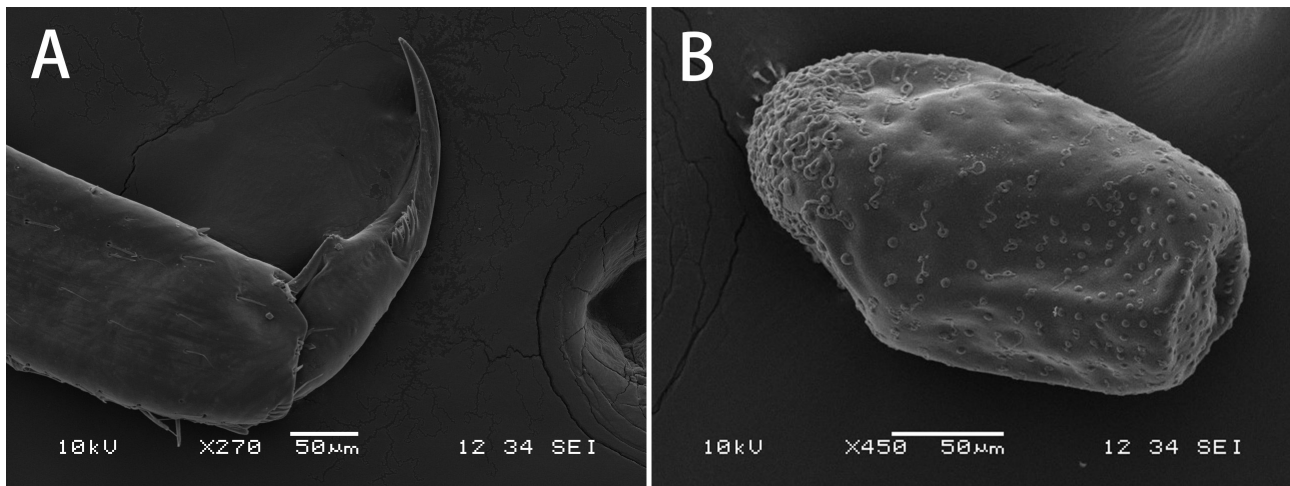




**FIGURE 7.** Subimaginal habitus of *Cinygmula longissima* sp. nov.

A: male; B: female

**Abdomen:** glabrous, with pair of submedian pale stripes and pair of pale dots, sometimes merged together (Fig. 1A); middle portions of abdominal sterna blacker than lateral portions. (Fig. 1B). Gill plates semi-hyaline with clear brown to dark tracheae. Gills I heart shaped, with single filament; gills II–V similar to each other, plate oval, with single filament; gills VI–VII with plate only (Fig. 2D). Cerci with very tiny setae on articulations, nearly glabrous (Fig. 1).



**FIGURE 8.** Nymphal claw and egg of *Cinygmula longissima* sp. nov.

A: claw; B: egg

**Male imago** (Figs 4–6): body length 11.0–12.0 mm, forewing 11.5 mm, hindwing 4.0 mm, foreleg 9.0 mm, cerci 20.0–21.0 mm. Head and thorax brown to chocolate, abdomen whitish to grey (Fig. 4A). Tergites with a pair of submedian pale stripes and a pair of pale dots but most of them unclear; each sternite with brown to black dot near anterior margin; cerci grey (Fig. 4A).

Frontal fold distinct, anterior margin with a shallow “V” shaped notch (Figs 4C–D). Distance between two compound eyes subequal to width of median ocellus (Fig. 4C). Wings transparent (Figs 4A, 5), veins brown, tinged with brown clouds; forewings with pigmented base, Rs and MP forked subequally to wingbase and symmetrically (Fig. 5A). Basal pigmentation of hindwings wider and darker than forewings, MA and MP forked symmetrically, costal projection nearly acute (Fig. 5B). Legs uniformly grey to dark brown, femora darker. Length ratio of forefemora: tibiae: tarsi 10.0: 9.0: 12.0; tarsal segments 2.5: 3.0: 2.5: 2.0: 1.5. Mid- and hindlegs, with femora slightly longer than tibiae, tibiae longer than tarsi; all tarsal segments subequal in length but apical one usually longer than others (Fig. 4B). Articulation between tibia and first tarsal segment distinct. Two claws of all legs similar, acute (Fig. 4B).

**Genitalia** (Fig. 6): Posterior margin of styliger convex, posterolateral angles of styliger projected (Figs 6A–B). Gonostyli with combined length of segments III and IV slightly shorter than segment II, segment III slightly longer than segment IV (Figs 6A–B). Penes fused at basal 2/3 and separated at apical 1/3, apex round (Figs 6C–D); each penis with pointed median titillators (Figs 6A–E) and lateral spine with 3–4 tiny protuberances on apex (Figs 6A–D, F).

**Male subimago** (Fig. 7A): body length 9.0 mm, cerci 9.0 mm, forewings 10.5 mm, hindwings 4.0 mm. Similar to male imago but abdomen darker, pale stripes and dots on abdominal tergites clearer. Frontal fold of head and pointed claws of legs as in imago (Fig. 7A).

**Female imago** (unknown).

**Female subimago** (Fig. 7B): body length 13.0 mm, forewings 13.0 mm, hindwings 5.0 mm, similar to male subimago but slightly larger and darker. Veins of wings clearer and darker. Frontal fold and claws same as the male (Fig. 7B).

**Egg:** about 0.2 mm length. One pole flat, other convex. Convex pole with KCTs, flat pole covered with fine protuberances, median portion with both KCTs and protuberances but fewer than on two poles (Fig. 8B).

## Biology

The materials in this study were collected from Qilianshan Mt., Qinghai province, border of northeastern Himalaya (Fig. 9A). The nymphs of this species *Cinygmula longissima* sp. nov. were collected by hand screen in a valley stream, latitude about 3620 m. The bottom of the stream consists of different sizes of stones (diameters of them from



0.1 m to 1.0 m, with some very big rocks). (Fig. 9B). Banks of the stream have some sand and peddle. No aquatic plant was seen in the water. Other mayflies collected from same place include some *Epeorus* (Heptageniidae) and *Baetis* (Baetidae). The imago were found and gathered on the stones at the banks of stream.



**FIGURE 9.** Collecting site map and habitat of *Cinygmula longissima* sp. nov.

A: location map (red dot indicating the collecting locality); B: habitat

The air temperature of collecting time is about 10°C, water 7°C. The current velocity is about 3 m/s.

## Diagnosis

Besides the new species *Cinygmula longissima* **sp. nov.** described here, only one species *C. hutchinsoni* has two pointed claws and distinct frontal fold. The general body and color patterns of those two species are similar. However, the male genitalia of them are different: the penes of a new species is stout, outline is nearly round (Fig. 6). Differently, two penes of *C. hutchinsoni* are slimmer and their outline is waved (Kluge 2015). In addition, the shapes of titillators of these species are different too: the titillators of *C. longissima* **sp. nov.** are sharper (Fig. 6E) and the lateral spines are wider (Fig. 6F) than those of *C. hutchinsoni*.

In nymphal stages, the gills I of new species *C. longissima* **sp. nov.** are slightly narrower than those of *C. hutchinsoni*. The tracheae of gills are dark in the new species but colorless in *C. hutchinsoni* (Kluge 2015).

One egg pole of *C. longissima* **sp. nov.** is convex and the other one is flat (Fig. 8B). But two poles of *C. hutchinsoni* egg are flat. The eggs of the *C. hutchinsoni* also have more numerous and larger KCTs and bumps than in *C. longissima* **sp. nov.**

## Etymology

The specific Latin name *longissima* means extremely long, indicating the distinct frontal fold of its adults.

## Discussion

The origin of large frontal fold in heptageniid imagos has been discussed by Kluge (2015) and we agree to that this character is a relic of their nymphs. Traver (1939), Pescador & Edmunds (1994), Zhou & Peters (2003), Si *et al.* (2017), Ma & Zhou (2021) and Qiang & Zhou (2023) respectively showed some species in the families Heptageniidae, Oligoneuriidae, Siphuriscidae, Palingeniidae, Neoephemeridae and Isonychiidae have distinct frons, frontal fold or produced epicranium on imaginal head. At the same time, the nymphs of them also have elongated head or frons.

Two kinds of heptageniid claws (“ephemeropteran claw” and “pointed claw”) were mentioned and compared by Kluge (2015) too. Actually, there is one more type besides the vestigial claws in the families Polymitarcyidae, Behningiidae and Palingeniidae. This type is the blunt claw of forelegs inflated into a small balloon or bubble-like structure (Zhou & Peters 2003; Braasch & Boonsoong 2009). In our view, this phenomenon comes from the different transformations of soft tissue of claws: it can shrink or enlarge in different sizes in different species but keep normal in most species. Unfortunately, the functions of those legs are almost unknown besides the holding female during mating.

Previously, the mayflies with frontal fold and pointed claws were reported exclusively from alpine streams of Central Asia (Kluge 2015). The present finding in China shows this kind of mayflies can also be found in eastern Himalaya (Fig. 10A). Our finding confirms that this kind of mayflies live in rapid and strong current, especially alpine areas (Fig. 10B). In this type of habitats, they have extremely modified head and gill suck which help them to stay and feed on stones in water current.

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