



Two new species of the genus *Nigrobaetis* Kazlauskas (in Novikova & Kluge), 1987 (Ephemeroptera: Baetidae) from Southwest China

BO LI¹, WEIFANG SHI², XIANFU LI³ & XIAOLI TONG^{1*}

¹Department of Entomology, College of Plant Protection, South China Agricultural University, Guangzhou 510642, Guangdong Province, China.

<https://orcid.org/0000-0002-1251-854X>

²Guizhou Center for Disease Control and Prevention, Guiyang 550001, Guizhou Province, China.

<https://orcid.org/0000-0002-3846-9280>

³Institute of Eastern-Himalaya Biodiversity Research, Dali University, Dali 671000, Yunnan Province, China.

<https://orcid.org/0000-0002-1258-1573>

*Corresponding author: xtong@scau.edu.cn; <https://orcid.org/0000-0003-1731-229X>

Abstract

Two new species of *Nigrobaetis* Kazlauskas (in Novikova & Kluge), 1987 are described from Southwest China: *Nigrobaetis bilongus* sp. nov. based on larval and imaginal materials which are reared from larvae; *Nigrobaetis trialbus* sp. nov. based on larval stage.

Key words: Mayfly, taxonomy, *Nigrobaetis*, nymph, adult, Oriental realm

Introduction

The genera *Nigrobaetis* Kazlauskas (in Novikova & Kluge), 1987 and *Takobia* Novikova & Kluge, 1987 were initially established as subgenera of *Baetis* Leach, 1815 by Novikova & Kluge (1987). Waltz *et al.* (1994) raised *Nigrobaetis* and *Takobia* to the generic level, and erected *Alainites* as new genus which encompassed the species previously placed in the *muticus* species group of the genus *Baetis*. Later, Waltz and McCafferty (1997) transferred the subgenus *Baetis* (*Margobaetis*) Kang & Yang in Kang *et al.* (1994) and several related species of *Baetis* to *Nigrobaetis*, and moved *Baetis* (*Acerbaetis*) Kang & Yang in Kang *et al.* (1994) to *Alainites*. Kluge and Novikova (2014) treated *Alainites* as a junior synonym of *Takobia* and considered *Alainites* and *Takobia* as subordinated taxa under the genus *Nigrobaetis* or *Nigrobaetis/g1* in the rank-free nomenclature proposed by Kluge. Thus, the taxonomic status of *Nigrobaetis*, *Takobia* and *Alainites* remains controversial. Recently, however, the evidences based on cladistic analyses of the family Baetidae and molecular data of allied genera were suggested to prefer considering *Alainites*, *Nigrobaetis* and *Takobia* as valid genera (Cruz *et al.* 2020, Sroka *et al.* 2021, Phlai-ngam *et al.* 2022, Yanai *et al.* 2022). In the present paper, we focus on *Nigrobaetis* s. str. (sine *Takobia* or *Alainites*) *sensu* Waltz *et al.* (1994) which is diagnosed by the following main characters, in larval stage: (i) presence of a small interantennal carina; (ii) prosthema of right mandible normal or baculiform, inner margin of incisors with one row of fine setae; (iii) labial glossae with a cluster of dorsal setae; (iv) paraproct lacking prolongation, with a restricted number of marginal spines (usually < 30 spines, see discussion below); in imaginal stage: (v) hindwings (if present) with 2–3 longitudinal veins; (vi) terminal segments of male genital forceps elongate.

Currently, *Nigrobaetis* s. str. comprises 28 species in the world, three species in Europe (Müller-Liebenau 1969, Novikova & Kluge 1994, Kluge 2004, Godunko *et al.* 2018), seven species in Africa (Soldán 1977, Soldán & Thomas 1983, Lugo-Ortiz & de Moor 2000, Gattolliat 2004, Kaltenbach *et al.* 2021) and 18 species are recognized in Asia (Müller-Liebenau 1984, 1985, Bae & Park 1998, Bae *et al.* 1998, Kang *et al.* 1994, Kang & Yang 1996, Gattolliat & Sartori 2008, Gattolliat *et al.* 2012, Fujitani *et al.* 2003, Fujitani *et al.* 2017, Kubendran *et al.* 2015, Sivaruban *et al.* 2022). The objective here is to describe two new species of *Nigrobaetis* newly discovered from

Yunnan, Guizhou and Sichuan provinces during the surveys of the mayfly fauna of Southwest China that is currently under way.

Materials and methods

Larvae of the new species were collected with a D-frame net in alpine streams from Southwest China. Some of the larvae were immediately preserved in vials containing 90% ethanol in the fields, while a subset of living individuals with well-developed wing pads were selected for transportation to artificial rearing cages *in situ* and some larvae were taken back to the laboratory for rearing to emergence individually (each reared imago together with its mature larval and subimaginal exuvia were stored in single vial containing 90% ethanol). Preserved specimens of both stages were dissected under the stereo microscope and mounted on slides in sealing solution for examination, illustration and photography using a camera with close-up lens and digital microscope. Measurements and ratio ranges given presented herein are for preserved mature larvae and imagoes reared in the laboratory only. Type specimens have been deposited in the Insect Collection, South China Agricultural University (SCAU), Guangzhou, China.

Results

Nigrobaetis bilongus sp. nov.

(Figs 1–45, 47–48)

Material examined. **Holotype:** male mature larva (in ethanol, deposited in SCAU), **CHINA, Yunnan Province**, Dali City, Mt. Cangshan, Qingbi Stream (2100–2600 m a.s.l.), 2.vi. 2020, collected by Xianfu Li. **Paratypes** (in ethanol): 15 larvae, 10 male imagos, 3 female imagos reared from larvae and 5 specimens on mounted slides (including 2 mature larvae, 2 male imagos and one female imago), 26.v.–2.vi.2020, same data as holotype; 9 larvae (including one on mounted slides), Heilong Stream (2220 m a.s.l.), Mt. Cangshan, Dali City, 15.v. 2020, coll. Xianfu Li; 3 male imagos, 4 male subimagos and 7 female imagos reared from larvae in Heilong Stream, Mt. Cangshan, Dali City, 1.v. 2018, coll. Xianfu Li; 20 larvae (including one on mounted slides), Ziyang River (2500–2600m a.s.l.), West Slope of Mt. Cangshan, Yangbi County, Dali City, 4.xii.2012, coll. Haomiao Zhang. **Sichuan Province:** 2 larvae (including one on mounted slides), Pingwu County, Laohegou Natural Reserve, Shabazi (1400 m a.s.l.), 29.v.2012, coll. Weifang Shi.

Diagnosis. **Larvae:** Body colouration generally brown, immature or female larvae usually predominantly brown dorsally except tergites IX–X yellowish; mature male larvae tergites I–VI light brown or yellowish, tergites VII–X brown (X paler than VII–IX). Antennae extremely long, approximately 3/4–4/5 length of body. Mandible with dense spine-like bristles on margin between incisors and mola. Maxillary palp elongate, much longer than galea-lacinia. Glossae with a cluster of 3–5 dorsal setae subapically. Foreleg tarsi with one row of 24–27 stout pointed setae on ventral margin. Gills on abdominal segments I to VII. Posterior margin of tergites II–X with triangular spines. Inner margin of paraproct with one row of 27–30 pointed spines. Cerci exceptionally long, subequal or slightly longer than body. **Male imagoes:** Head reddish purple; turbinate eyes cylindrical with high stalk, faceted surface rounded and orange red. Thorax purple or dark brown. Forewing transparent with light brownish longitudinal veins; hind wing with costal projection and three longitudinal veins, second vein bifurcate, third vein relatively short, not exceeding midline. All legs cream-coloured. Abdominal segments I–VI white or transparent, VII–X reddish purple. Terminal segment of genital forceps elongate, ca. 4.5–6.0 times longer than wide. Cerci extremely elongated. **Female imagoes:** Head creamy yellow. Thorax yellowish brown. Wing coloration and venation as in male. Legs similar to that of male imago. Abdominal segments uniformly purple except tergites I and X slightly paler than other segments; tergites II–VIII each with a pair of oblique paler short stripes and one small paler spot near anterior margin. Cerci as in male.

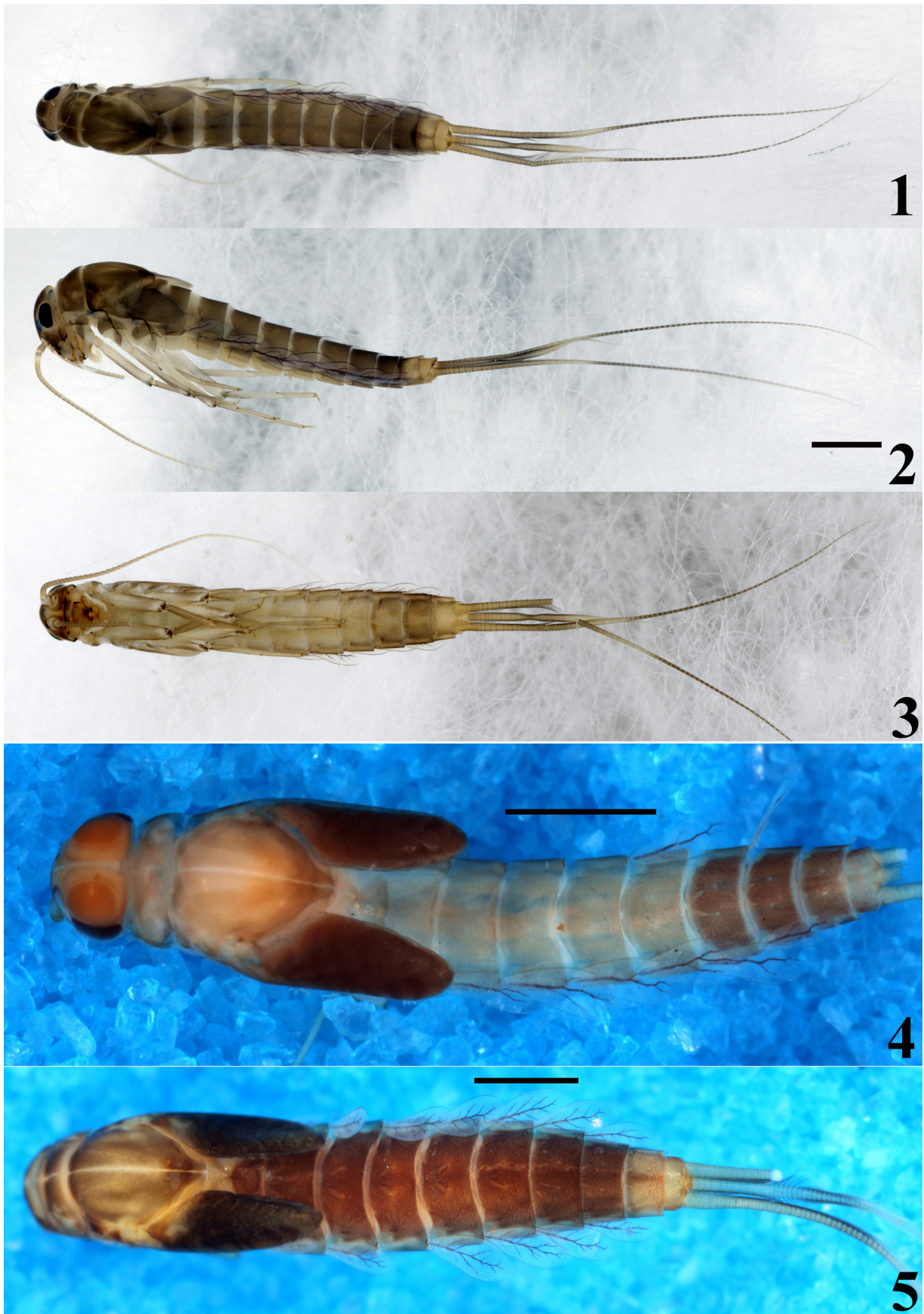
Description of larvae. **Length.** Body length 5.5–8.0 mm; antennae: 4.5–5.0 mm; cerci: 5.0–7.5 mm. **Colouration.** Head and thorax brown; abdominal tergites tend to be change in colouration depending on the life stage and sexes: immature or female larvae usually predominantly brown dorsally except tergites IX yellowish with a narrow “T-shape” brown marking and X yellowish (Figs 1–3, 5); mature male larvae tergites I–VI light brown or yellowish, tergites VII–X brown (Fig. 4); anterior margin of abdominal tergites II–VIII usually each with pair of yellowish

oblique narrow stripes and small spots. Legs largely light brown dorsally, except femora with irregular pale patches on basal and distal areas. Proximal 1/3 of cerci yellowish brown with obscure band, apical 2/3 of cerci brown.

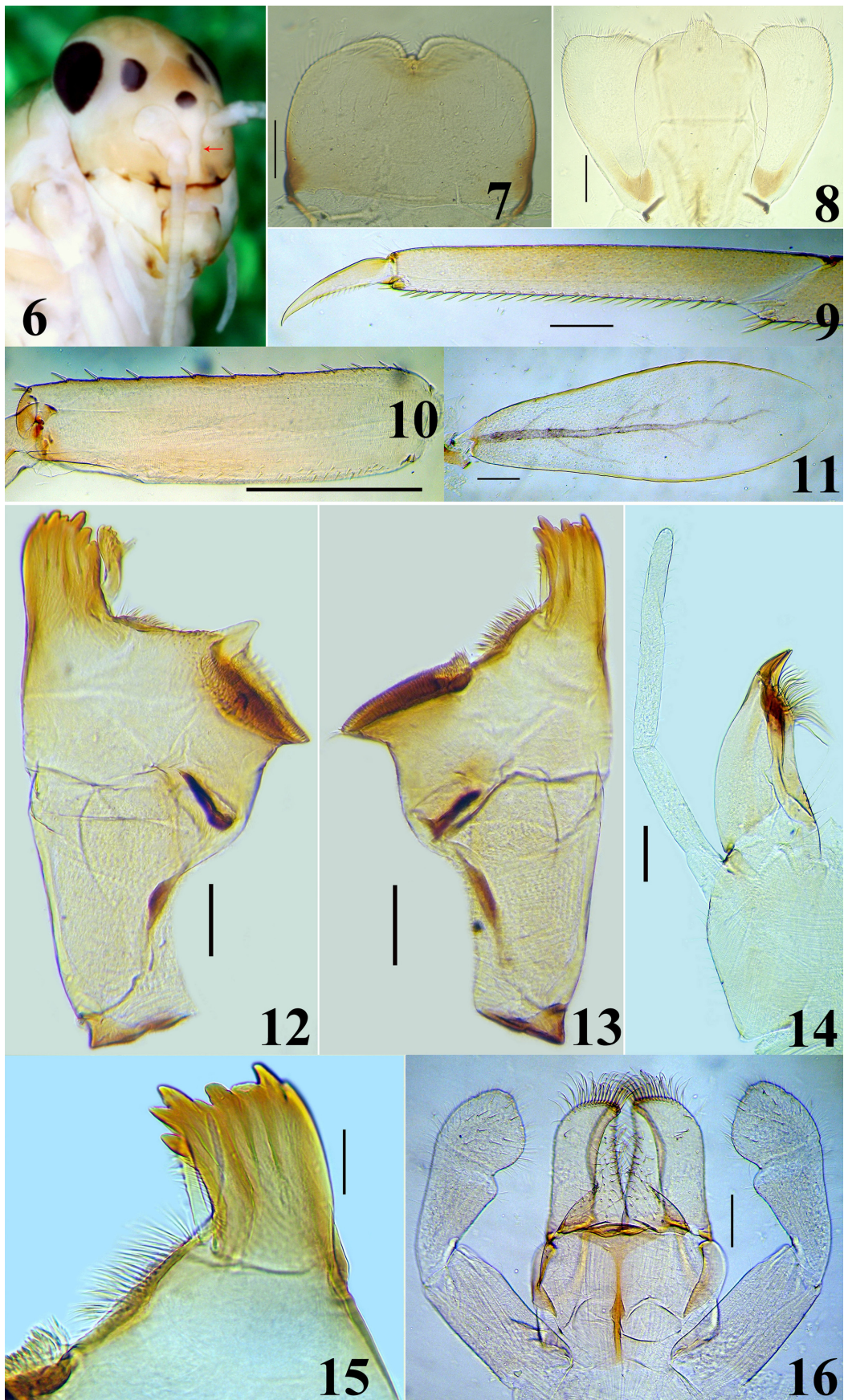
Head. Antennae close to each other, frons with a distinct median carina between bases of antennae (Fig. 6); scape and pedicel without scale-like setae, only bearing sparse hair-like setae. Antennae extremely long, ca. 1/2–3/4 of body length, length of antennae may change depending on the life stage, in mature larvae, antennae tend to shorten as terminal segments becoming as thin as a threadlet which easily lost or broken. Labrum rectangular (Figs 7, 17), ca. 1.5 times wider than long; anteromedian notch deep with a small rounded lobe at base; dorsally in distal half with one long, simple seta near midline and irregular row of 4–5 medium to long, simple setae; in proximal half with fine, simple setae scattered over dorsal surface (Fig. 17); ventrally bordered with feathered setae along anterior margin and 9–10 short, spine-like setae near lateral and anterolateral margin (Fig. 17). Left mandible (Figs 12, 18): incisors fused, with 7 denticles apically; prosthema robust, apically with 3 acute and 6–7 blunt denticles; margin between incisors and mola with dense spine-like bristles (Fig. 18). Incisor of right mandible (Figs 13, 19) fused, with 7 denticles apically; prosthema stick-like with distolateral dentation (Fig. 19); margin between incisors and mola with densely spine-like bristles, inner margin of incisors with one row of very fine setae (Figs 15, 19), tuft of setae at apex of mola present. Hypopharynx apically covered with fine setae, lingua with a median projection (Fig. 8); superlinguae subequal to lingua. Maxillae (Fig. 14): apex of maxillae with three elongated canines, crown of galea-lacinia with three stout feathered dentisetae; base of lacinia with one row of 5–6 simple setae increasing in length upwardly and one short spine-like hump seta. Maxillary palp elongated and 2-segmented, ca. 1.8–2.0 times length of galea-lacinia, segment II apically rounded with hair-like setae and much longer than segment I. Labium (Fig. 16) with glossae basally broad, narrowing toward apex, slightly shorter than paraglossae; inner margin with 12–14 long, simple setae; apex with two long, curved, robust setae and one short, robust, pectinate seta; outer margin with 9–10 long, simple setae; ventral surface of glossae covered with many hair-like setae (Fig. 16), dorsal surface with a cluster of 3–5 fine dorsal setae subapically (Figs 21, 45). Paraglossae sub-rectangular, curved inward, ca. 1.6 times width of glossae; apex with two rows of long, robust setae in apical area; dorsal surface with a slightly curved row of 6–7 long, simple setae near inner margin. Segment I of labial palp shorter than length of segments II and III combined. Segment I ventrally with sparse hair-like setae, dorsal face with many micropores medially. Segment II slightly expanded apico-laterally, dorsal face with one oblique row of 4–5 long pointed setae (Fig. 20), ventrally with many hair-like setae. Segment III truncated apically and slightly expanded inwards; ventrally covered with 12–15 short, simple setae and numerous hair-like setae (Fig. 22), dorsally with 9–12 short, simple setae near apex and many fine hair-like setae.

Thorax. Hind wing pads well developed. Femora of foreleg (Figs 10, 29) dorsally with one row of 10–13 long, stout setae, except proximal part with 4–5 stout setae subparallel to dorsal margin, dorsoapical setal patch formed by 2 stout, bluntly pointed setae; ventrally with many stout pointed setae near ventral margin; anterior face with numerous micropores and scale bases. Tibiae with only sparse hair-like setae on dorsal margin, ventral side with many stout, simple, pointed setae, both faces covered with many scale bases; tibiopatellar suture present (Fig. 29). Tarsi with sparse hair-like setae dorsally, ventral margin with one row of 24–27 stout, simple, pointed setae (Figs 9, 29); both faces with numerous scale bases. Claws (Fig. 29) slender, with one row of 10–12 acute teeth increasing in length toward the apex; subapical setae absent. Middle and hind legs similar to foreleg in structure, except tibiae with 5–6 stout pointed setae dorsally and tarsi with only 12–15 stout, simple, pointed setae along ventral margin.

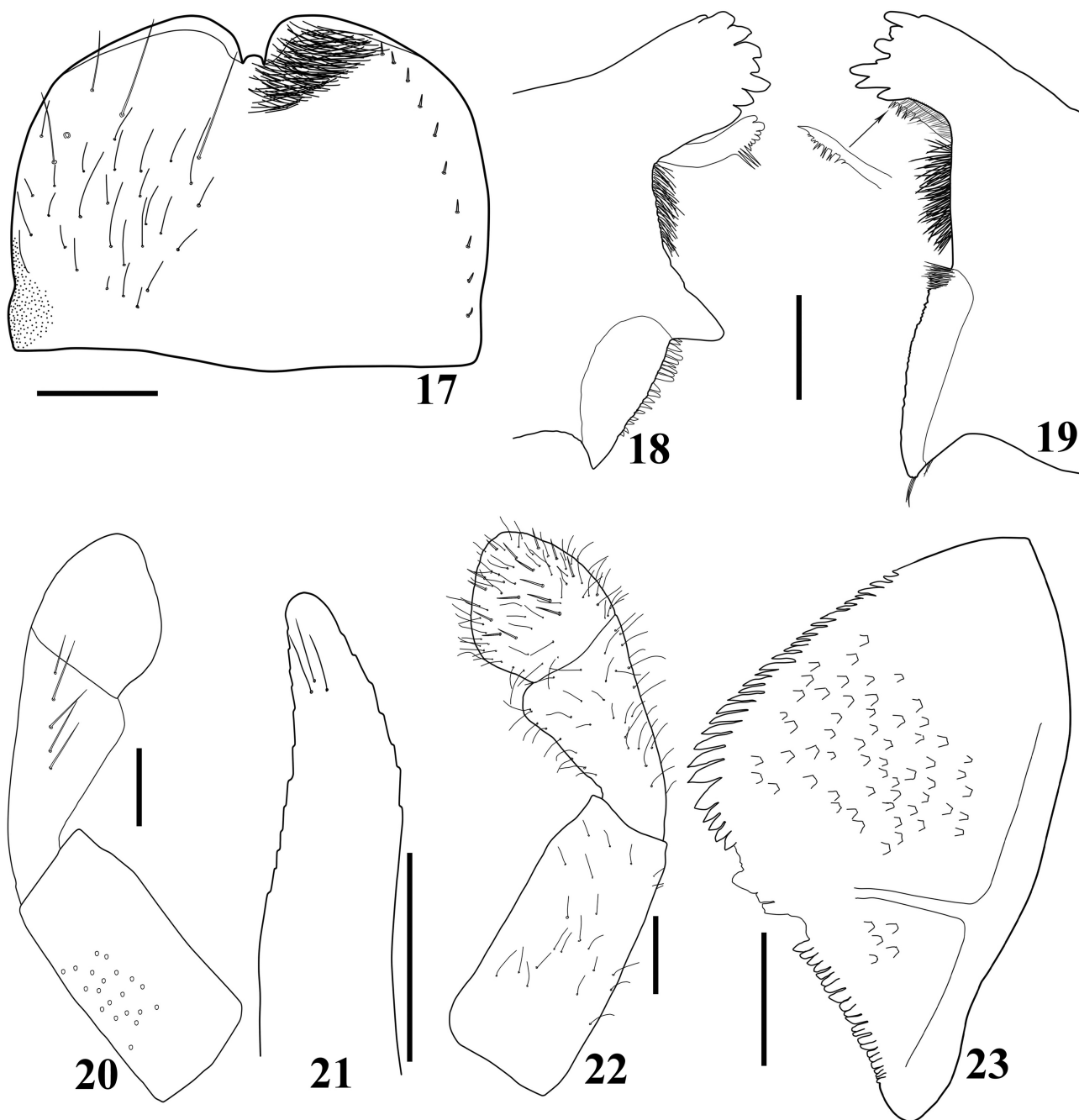
Abdomen. Dorsal surface of tergites scattered with numerous trapezoidal scale bases. Posterior margin of tergite I without spines, tergites II–X with nearly equilateral triangular spines (Fig. 28) except X with isosceles triangular spines, longer than wide. Sternites surface covered with numerous trapezoidal scale bases, posterior margin of sternites VI–IX with triangular spines. Gills present on segments I to VII (Figs 24–27), long elliptic and transparent; margin with a brown band and small denticles intercalating fine hair-like setae (Fig. 25); tracheation well visible (Fig. 11); gill I slender, similar to gill VII in shape and length, gills II–VI alike in shape (Figs 11, 24–27). Paraproct (Fig. 23) subtriangular, without posterior prolongation, surface with many trapezoidal scale bases, inner margin with 27–30 pointed spines; surface of cercotractor with some trapezoidal scale bases, margin with 21–23 blunt spines. Cerci extremely long, subequal or slightly longer than body length, proximal 1/3 of cerci with swimming setae along inner margin, terminal 2/3 of cerci smooth and extremely prolonged (Figs 1–3); median caudal filament ca. 2/3–3/4 of cerci length. Cerci length can be different depending on the instar examined. In mature larvae (with darker wing pads), terminal segments that lacking swimming setae tend to shrink as thin as a threadlet which easily broken, especially when deposited in ethanol.



FIGURES 1–5. Larval habitus of *Nigrobaetis bilongus* sp. nov. 1–3, young larva; 4, mature male larva (dorsal view); 5, mature female larva (dorsal view). (Scale bars = 1mm).



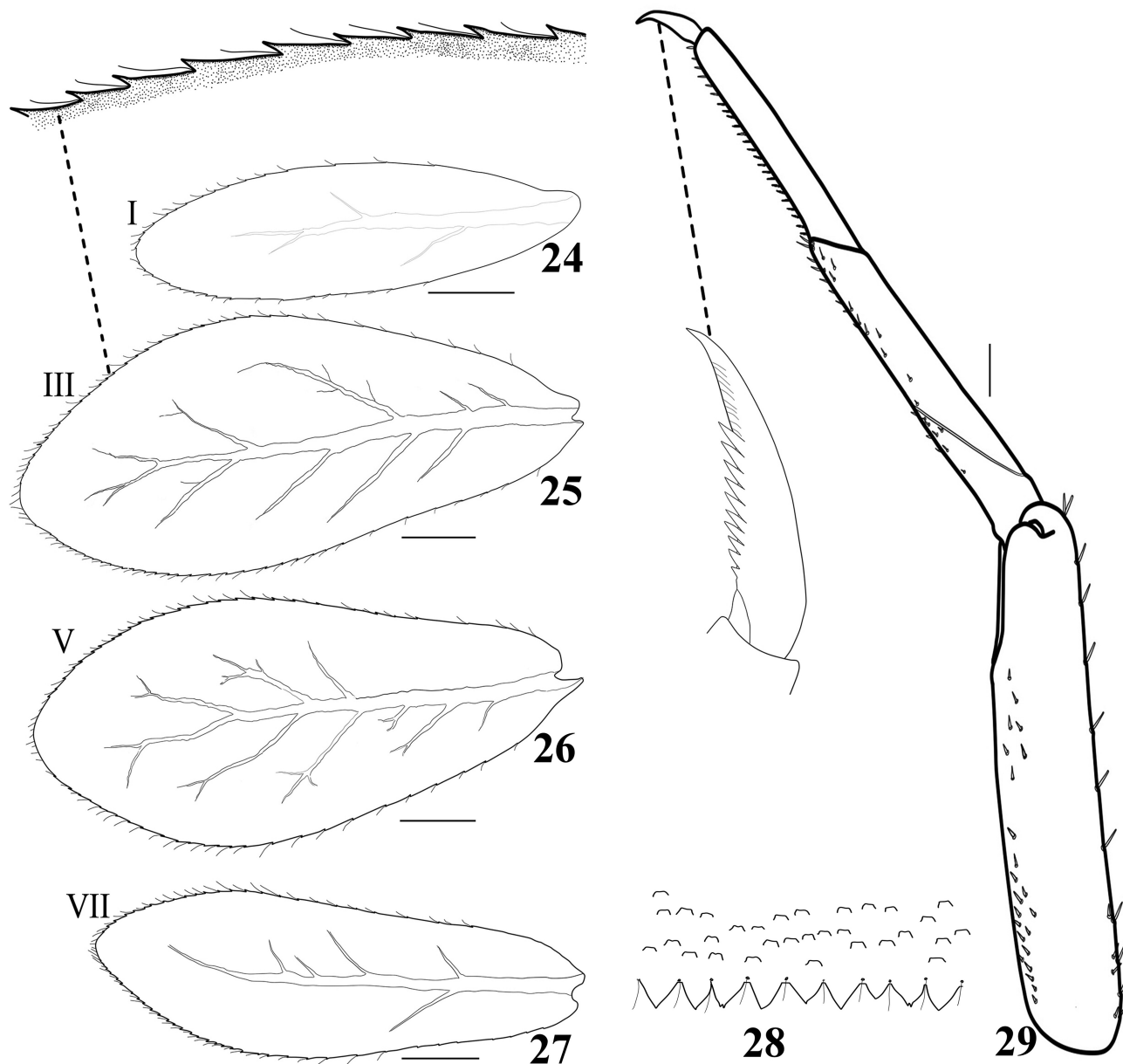
FIGURES 6–16. *Nigrobaetis bilongus* sp. nov. 6, front view of head; 7, labrum; 8, hypopharynx; 9, foreleg tarsus and claw; 10, foreleg femur; 11, gill VI; 12, left mandible; 13, right mandible; 14, maxillae; 15, incisor of right mandible; 16, labium (ventral view). (Scale bars: Fig. 10 = 0.5mm; Fig. 15 = 0.05mm; remains = 0.1mm).



FIGURES 17–23. *Nigrobaetis bilongus* sp. nov. 17, labrum (left, dorsal view; right, ventral view); 18, left mandible; 19, right mandible; 20, labial palp (dorsal view); 21, glossa (dorsal view); 22, labial palp (ventral view); 23, paraproct. (Scale bars = 0.1mm).

Male imago: Body length 5.0–5.5 mm; forewings: 5.8–6.5 mm; hind wings: 0.8–0.85 mm; cerci: 11.0–12.0 mm. Head reddish purple. Antennal scape and pedicel light brown, flagellum cream-coloured (Figs 32–34); median carina between bases of antennae becoming prominent lamellar (Figs 32, 34). Turbinate eyes with high stalk, cylindrical, slightly widened apically; faceted surface rounded and orange red, stalk 2/3 purple basally and 1/3 orange distally (Figs 30, 32–34). Thorax purple. Forewing transparent with light brownish longitudinal veins (Fig. 30), except pterostigma slightly frosted-white with several incomplete short veins (Fig. 36); hind wing with costal projection and three longitudinal veins, second vein forked at basad midline, third vein relatively short, not exceeding midline (Fig. 37). All legs cream-coloured; tarsi of fore leg 5-segmented, without apical spines on tarsal segments (Fig. 38), tarsi of middle and hind legs 4-segmented, with apical spine on fused 1st+2nd and 3rd tarsal segments; all claws with one oval lobe and one pointed curved hook. Ratio of foreleg femur/tibia/tarsus = 1:1.5:2; ratio of foreleg tarsal

segments =1:17:14:9:3. Abdominal segments I–VI white, nearly transparent, VII–X reddish purple (Fig. 30). Cerci extremely elongated, white except segment joinings with light brown (Fig. 30). Genitalia light brownish to white, unistyliger nearly rectangular, without apico-internal process; segments I and II of forceps almost completely fused; segment I conical, tapered apically; segment II cylindrical, weakly contracted proximally; terminal segment narrow and elongate, ca. 4.5–6.0 times longer than wide (Fig. 35).



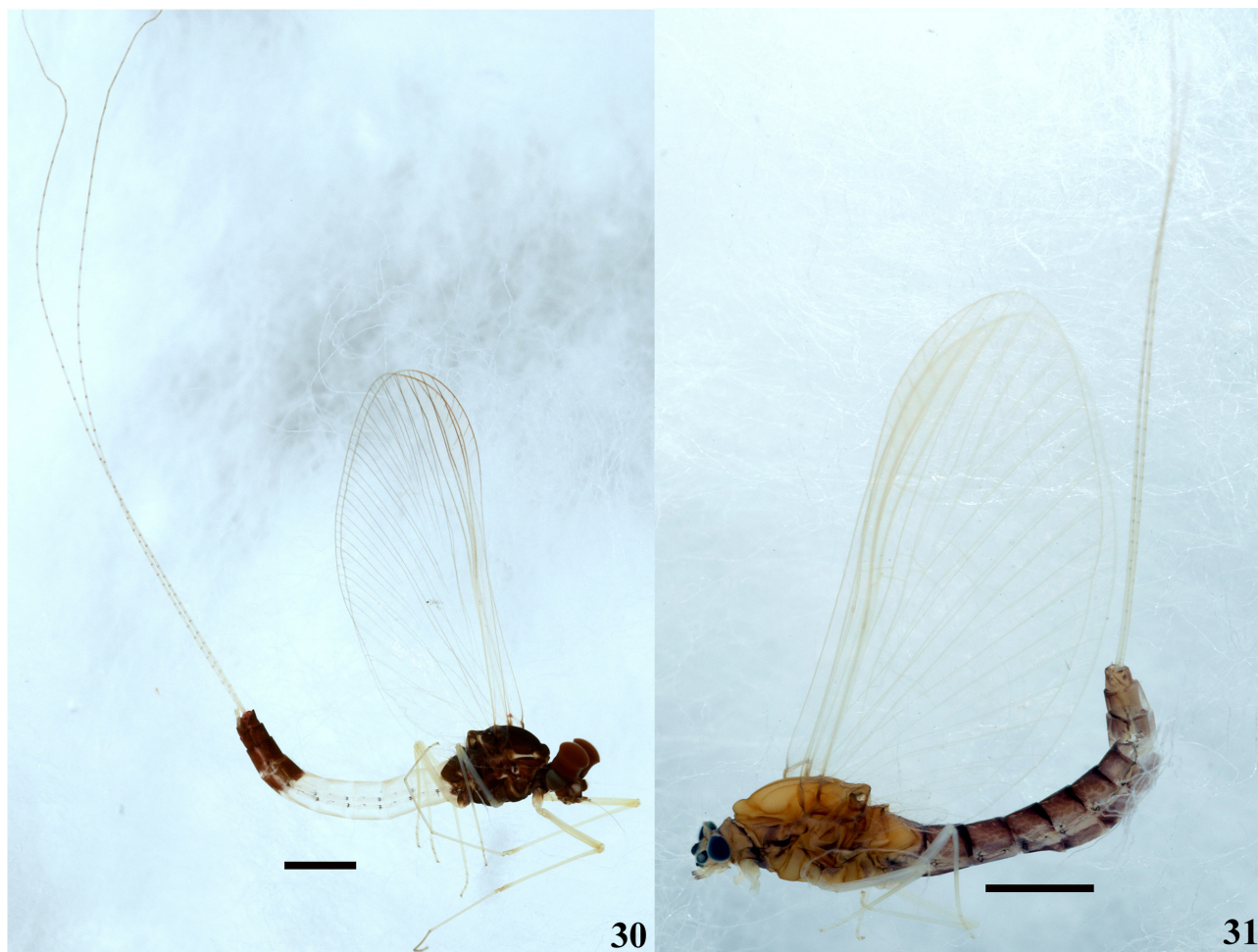
FIGURES 24–29. *Nigrobaetis bilongus* sp. nov. 24, gill I; 25, gill III; 26, gill V; 27, gill VII; 28, posterior margin of tergite V (partly); 29, foreleg. (Scale bars = 0.1mm).

Male subimago (in ethanol). Similar to imago, except following characters: (i) head creamy yellow; (ii) turbinate eyes creamy yellow, stalk shorter with rounded apically; thorax yellowish brown, wings brown with numerous marginal hairs; (iii) all legs except tarsi are covered with microtrichia; in fore legs, tarsal segments I–IV are covered with blunt microlepidies and tarsal segment V is covered with pointed microlepidies (Figs 39, 42); all tarsal segments of middle and hind legs are covered with pointed microlepidies (Figs 40–41, 43–44); (iv) abdominal segments I–VI and X creamy yellow, VII–IX brown.

Female imago (Fig. 31). Body length 5.5–6.5 mm; forewings: 6.0–6.8 mm; cerci: 7.0–10.0 mm. Head wide, creamy yellow, distance between eyes exceeding eye length. Antennal scape and pedicel light brown, flagellum cream-coloured; interantennal carina raised and lamellar. Thorax yellowish brown. Wing colouration and venation as in male. All legs cream-coloured, similar to that of male imago. Abdominal segments uniformly purple except

tergites I and X slightly paler than other segments; tergites II–VIII each with a pair of oblique paler short stripes and one small paler spot near anterior margin. Cerci as in male.

Eggs. Oval, length ca. 200µm, width ca. 120µm; chorion uniformly covered with a small-mesh reticulation and without any ridges when observed under optical microscope (Figs 47–48).



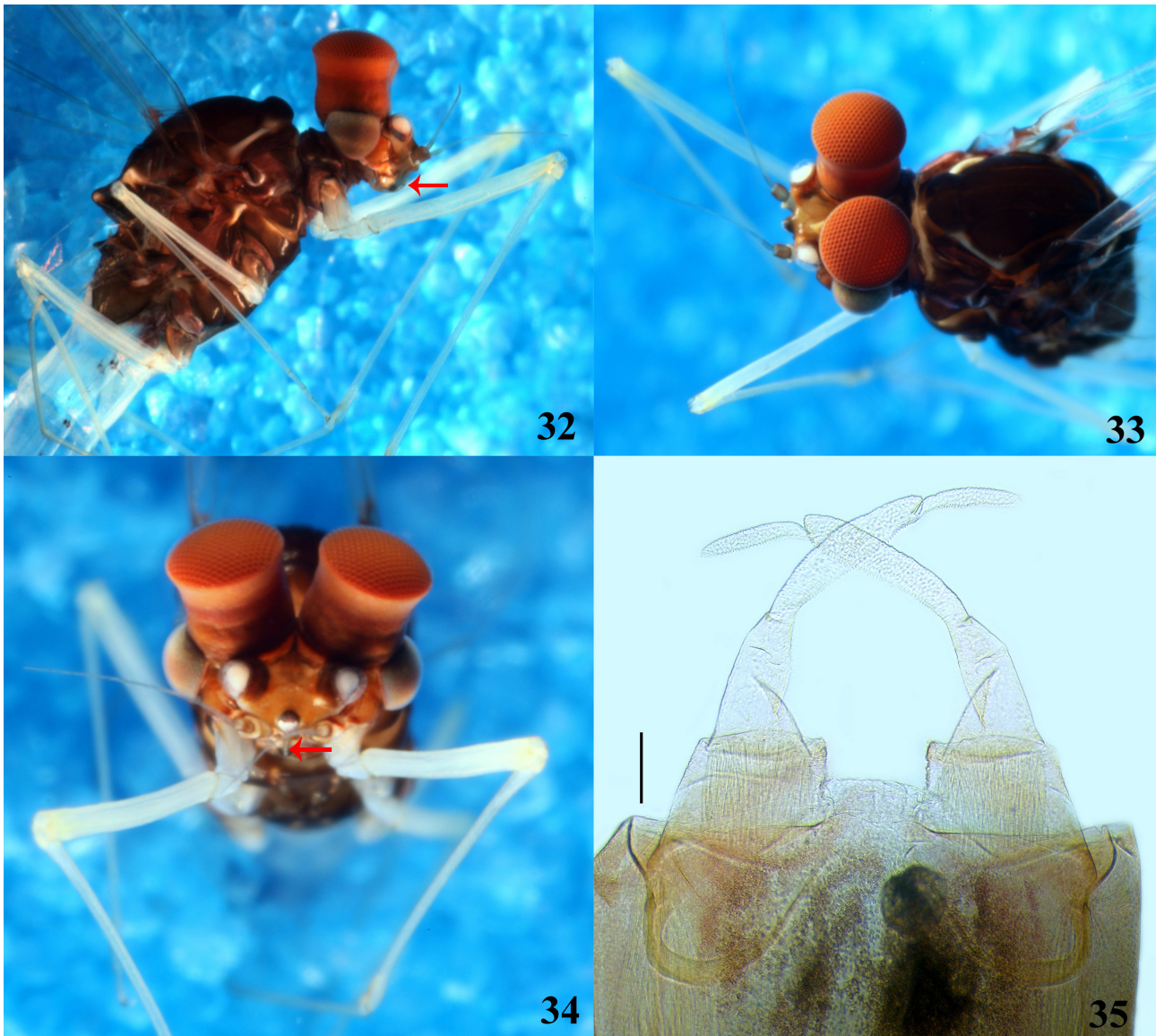
FIGURES 30–31. Imagoes of *Nigrobaetis bilongus* sp. nov. 30, male imago; 31, female imago. (Scale bars = 1mm).

Etymology. The specific epithet is an arbitrary combination of “*bi-*” (derived from Latin, the prefix of two) and “*longus*” (from Latin, meaning long), referring to the larvae having unusually long antennae and cerci, which are conspicuous characters for recognizing this new species in the field.

Distribution. China (Yunnan, Sichuan).

Biology. The larvae of the new species occur in slow-flowing streams or in the margins of pools of high altitude areas (Figs 77–78). They usually climb about surfaces of stones, when disturbed, larvae dart away swiftly by rapidly flipping the long caudal filaments up and down. Emergence occurs in late May and early June in Mt. Cangshan when the average water temperature was around 15 °C. The imagoes have been observed swarming in full sunlight from morning to noon over the pools. The swarms vary in height above the water level from 2.0 m to 3.0 m.

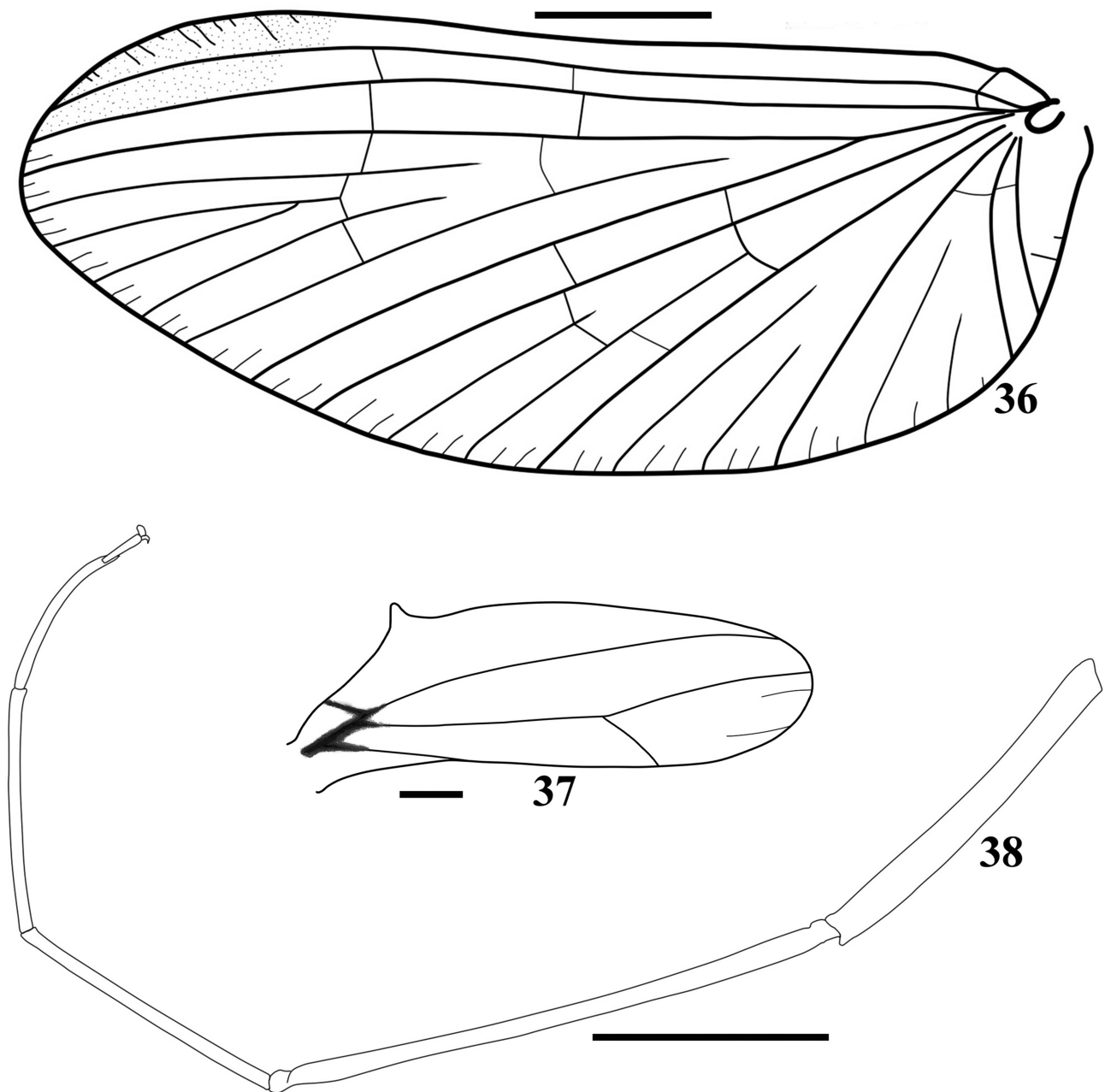
Discussion. The larva of the new species is similar to *Nigrobaetis niger* (Linnaeus, 1761) and *Nigrobaetis gracilentus* (Chang & Yang, 1994) (in Kang *et al.* 1994) in appearance, but it can be distinguished from the latter by the following characters: (1) maxillary palp elongate, ca. 1.8–2.0 times of galea-lacinia while those in *N. gracilentus* ca. 1.5 times as long as galea-lacinia (Kang *et al.* 1994); (2) the articulation between segments II and III of labial palp clearly visible (Figs 16, 20, 22) and segment III truncated apically (vs. the articulation unclear in *N. niger* and *N. gracilentus*); (3) apex of paraglossae with two rows of long, robust setae (vs. three rows in *N. niger* and *N. gracilentus*); (4) dorsal surface of paraglossae with a slightly curved row of 6–7 long, robust setae (Fig. 45), while those in *N. gracilentus* with a strongly arched row of 9–10 long setae submedially (Fig. 46); (5) dorsal surface of glossae with a cluster of 3–5 dorsal setae subapically (Fig. 21) (vs. 0–1 seta in *N. gracilentus* and about 15 setae in *N. niger*); (6) foreleg tibiae with only sparse hair-like fine setae on dorsal margin (vs. foreleg tibiae dorsally with 5–7



FIGURES 32–35. Male imago of *Nigrobaetis bilongus* sp. nov. 32, lateral view of head (arrow indicates interantennal carina); 33, top view of head; 34, front view of head (arrow indicates interantennal carina); 35, genitals. (Scale bar = 0.1mm).

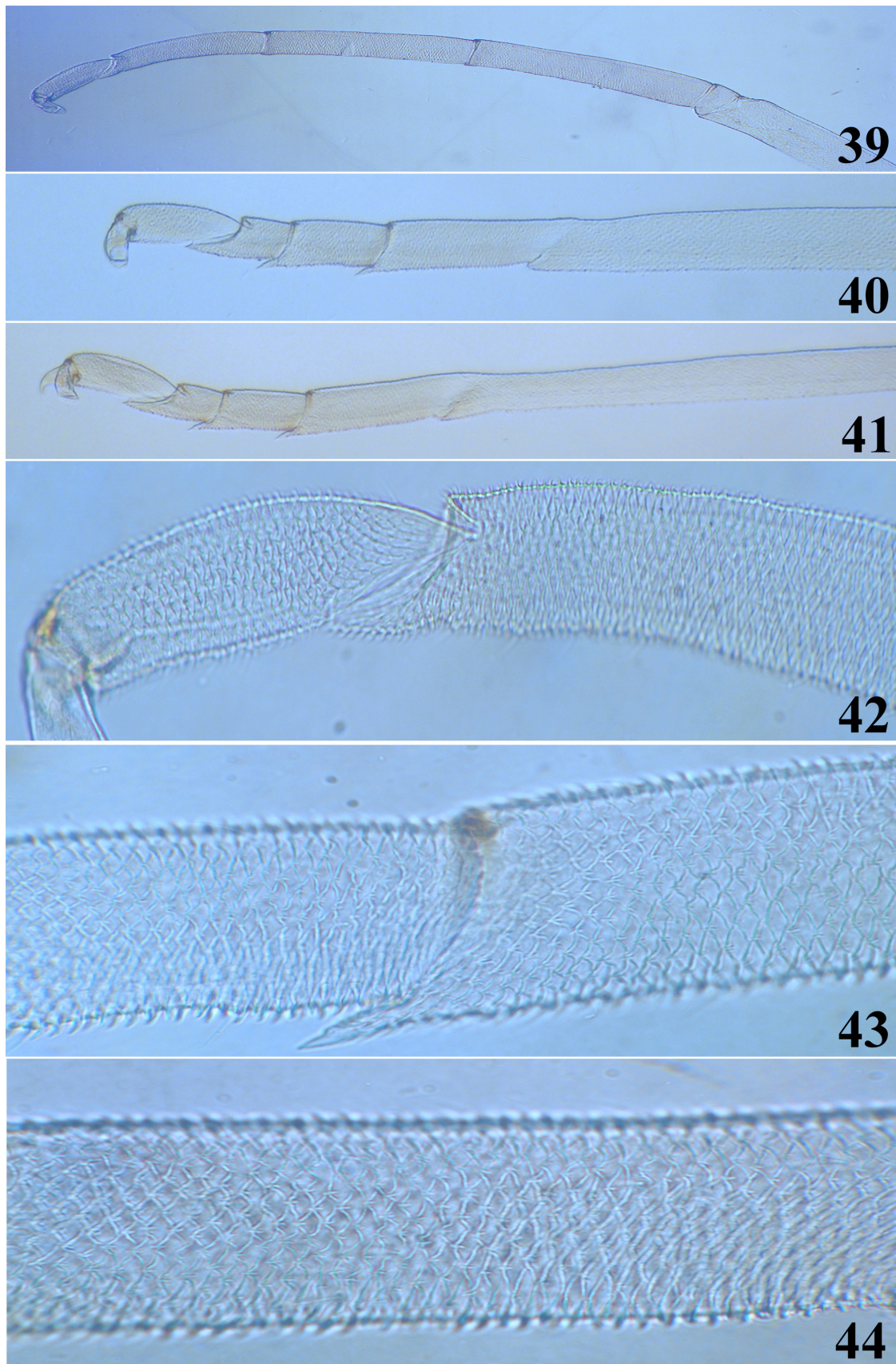
stout, pointed setae in *N. gracilentus*); (7) posterior margins of abdominal tergites with nearly equilateral triangular spines (vs. with long, acute marginal spines in *N. niger* and *N. gracilentus*); (8) inner margin of paraproct with one row of 27–30 pointed spines (vs. about 16–19 spines in *N. niger* and *N. gracilentus*). In addition, this new species resembles to *N. gombaki* (Müller-Liebenau, 1984) from Malaysia by sharing with similar number of marginal spines on paraproct (Müller-Liebenau 1984), but it differs from the latter by the following characters: (i) densely spine-like bristles present on margin between incisors and mola of mandible (Figs 12–13); (ii) maxillary palp elongate, approximately 1.8–2.0 times length of galea-lacinia (Fig. 14); (iii) tarsi bearing a close row of 24–27 stout, simple pointed setae on ventral margin (Fig. 9). In general, the number of marginal spines on paraproct is good diagnosis character which has little intraspecific variation (Müller-Liebenau 1984). The members of *Nigrobaetis* s. str. usually possess a restricted number of marginal spines on paraproct (ranging from 4–16 spines), which is an unique character within the *Alainites/Nigrobaetis/Takobia* complex (Figs 23,71, Müller-Liebenau 1984, Novikova & Kluge 1987, Kang *et al.* 1994, Lugo-Ortiz & de Moor 2000, Gattolliat 2004, Gattolliat & Sartori 2008, Gattolliat *et al.* 2012, Kluge & Novikova 2014, Kubendran *et al.* 2015, Martynov & Godunko 2017, Godunko *et al.* 2018, Sroka *et al.* 2021, Sivaruban *et al.* 2022). There are exceptions, however, apart from the new species and *N. gombaki* above mentioned, *N. rhithralis* (Soldán & Thomas, 1983) and *N. digitatus* (Bengtsson, 1912) also have 28–35 and 17–28 spines respectively on inner margin of paraproct (Godunko *et al.* 2018). Despite that, the number of marginal spines

on paraproct in *Nigrobaetis* s. str. is much less than other members of the *Alainites/Nigrobaetis/Takobia* complex because *Nigrobaetis* s. str. has no prolongation of paraproct (Waltz *et al.* 1994, Kluge & Novikova 2014, Martynov & Godunko 2017, Tong & Dudgeon 2000, Sroka *et al.* 2021, Phlai-ngam *et al.* 2022, Yanai *et al.* 2022). Therefore, the restricted number of marginal spines on paraproct in *Nigrobaetis* could be considered as one of useful characters to distinguish it from *Alainites* and *Takobia* in larval stage.

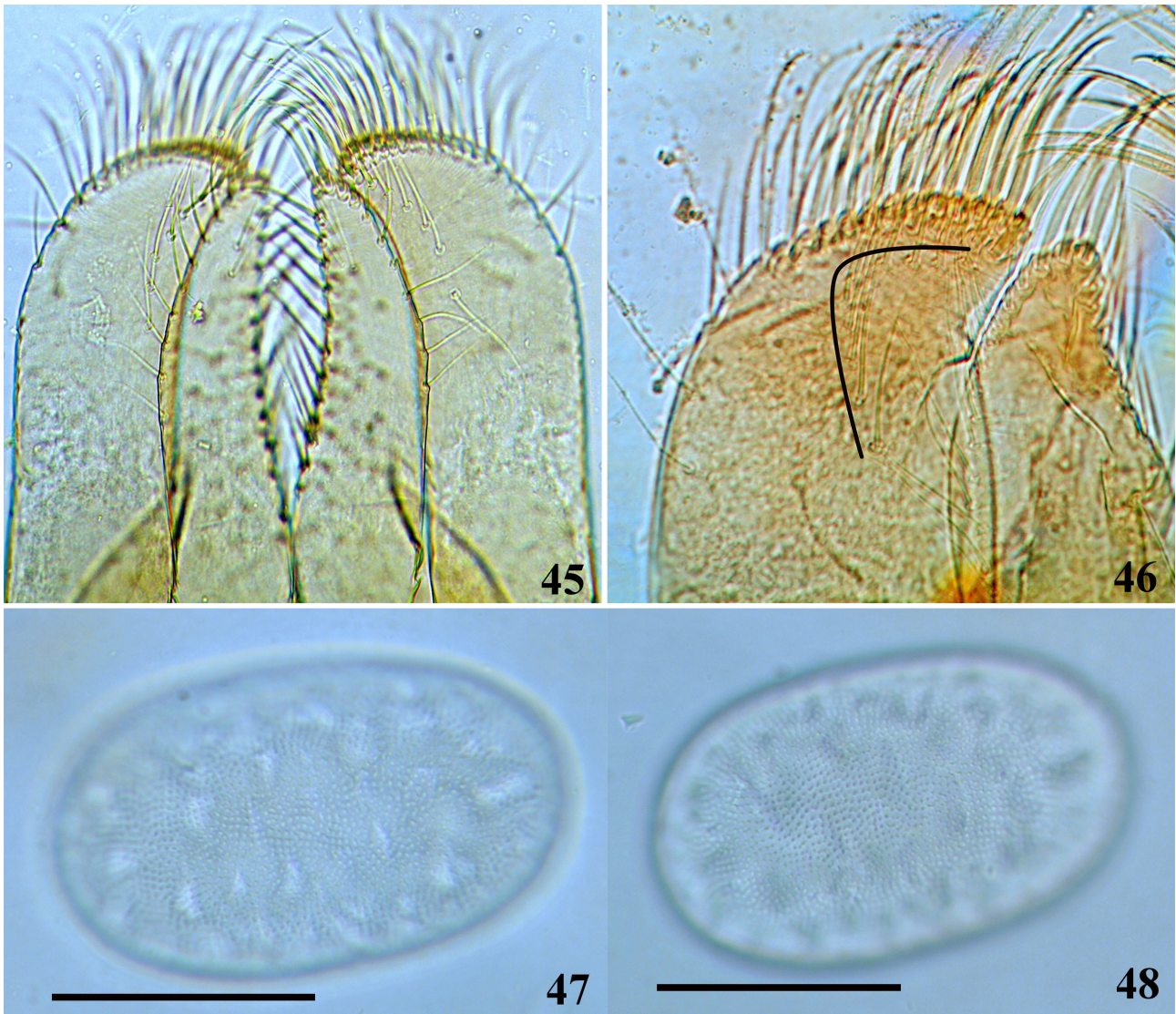


FIGURES 36–38. Male imago of *Nigrobaetis bilongus* sp. nov. 36, fore wing (Scale bar = 1mm); 37, hind wing (Scale bar = 0.1mm); 38, foreleg (Scale bar = 1mm).

Additionally, the eggshell structure of the new species is peculiar and characterized by covering with reticular structure (Figs 47–48) which is similar to those of *Nigrobaetis digitatus* and *N. niger* (Kopelke & Müller-Liebenau 1981). This type of chorionic structure is called the microreticule-type that was found from *Baetis maurus* Kimmins, 1938 by Ubero-Pascal and Puig (2007). In male subimago of the new species (female subimago absent in this study), texture of microlepidés on subimaginal tarsi is the same with those of *N. digitatus* and *N. acinaciger* Kluge, 1983 (Kluge 2022), i.e. fore leg tarsal segments I–IV are covered with blunt microlepidés and tarsal segment V is covered with pointed microlepidés (Figs 39, 42), all tarsal segments of middle and hind legs are covered with pointed microlepidés (Figs 40–41, 43–44). The male imagoes of the new species resemble to those of *N. niger*,



FIGURES 39–44. Male subimaginal tarsi of *Nigrobaetis bilongus* sp. nov. 39, fore leg (5-segmented); 40, middle leg (4-segmented, 1st +2nd fused); 41, hind leg (4-segmented, 1st +2nd fused); 42, tarsal microlepidides of fore leg (blunt microlepidides on I–IV tarsi and pointed microlepidides on V tarsus); 43, tarsal microlepidides of middle leg (all pointed microlepidides); 44, tarsal microlepidides of hind leg (all pointed microlepidides).



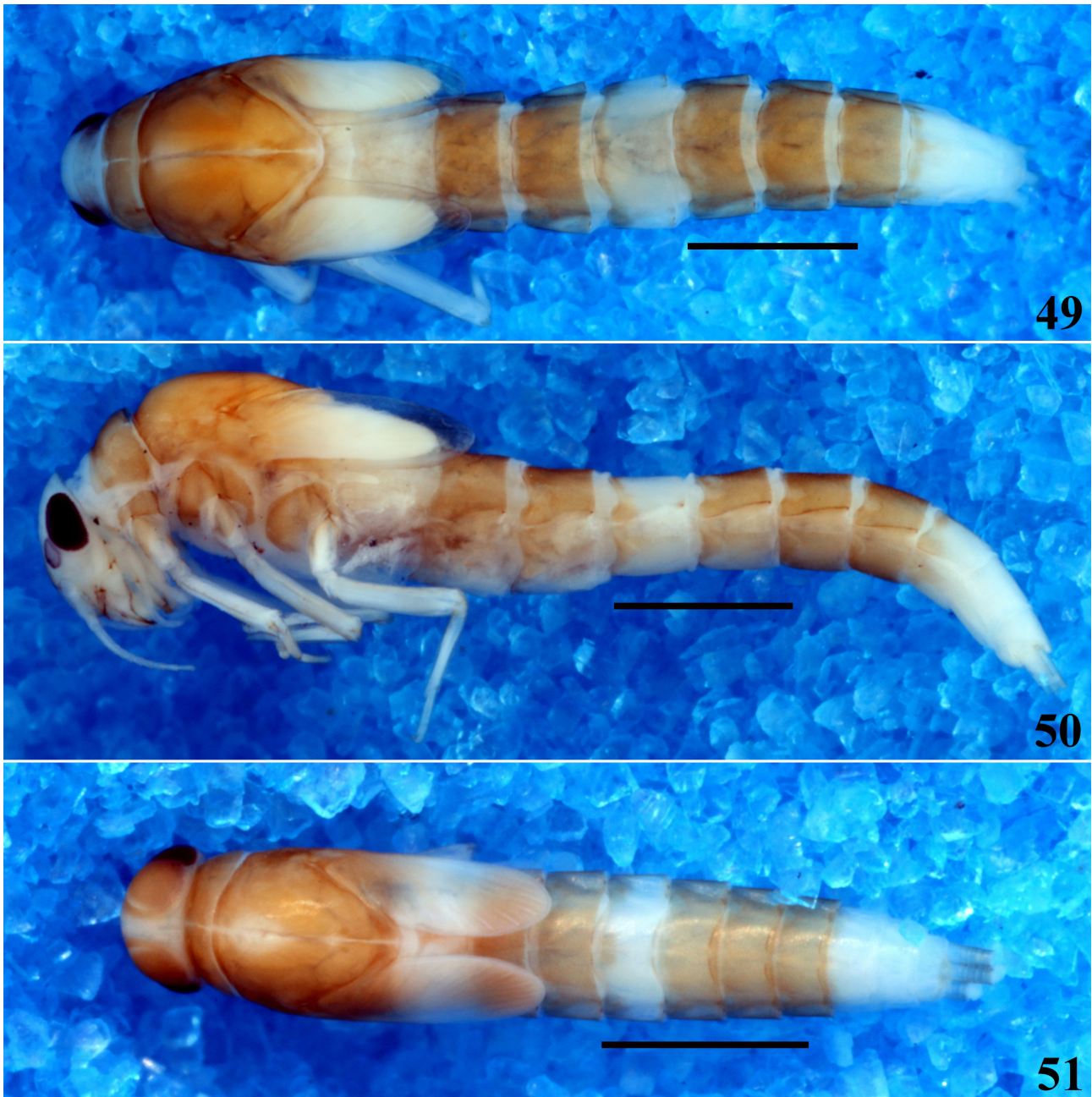
FIGURES 45–48. 45, glossae and paraglossae of *Nigrobaetis bilongus* **sp. nov.** (dorsal view); 46, paraglossa of *Nigrobaetis gracilentus* (dorsal view, black line shows the arched row of dorsal setae); 47–48, two sides of the same egg observed under optical microscope (Bar=0.1mm).

N. ishigakiensis Fujitani, 2017 (in Fujitani *et al.* 2017) and *N. latus* Fujitani, 2017 (in Fujitani *et al.* 2017) in appearance and body colour pattern (i.e., thorax usually dark brown or purple, wings transparent, and abdominal tergites I–VI gray or transparent, VII–X brown) (Fig. 30, Müller-Liebenau 1969, Fujitani *et al.* 2017), but it differs from the latter by the hind wing with three longitudinal veins and the terminal segment of forceps being slender and elongate (ca. 4.5–6.0 times longer than wide), while those in *N. niger*, *N. ishigakiensis* and *N. latus* each with two longitudinal veins, and the terminal segment of forceps being shorter and stockier than that of the new species (Müller-Liebenau 1969, Fujitani *et al.* 2017).

***Nigrobaetis trialbus* sp. nov.**

(Figs 49–72)

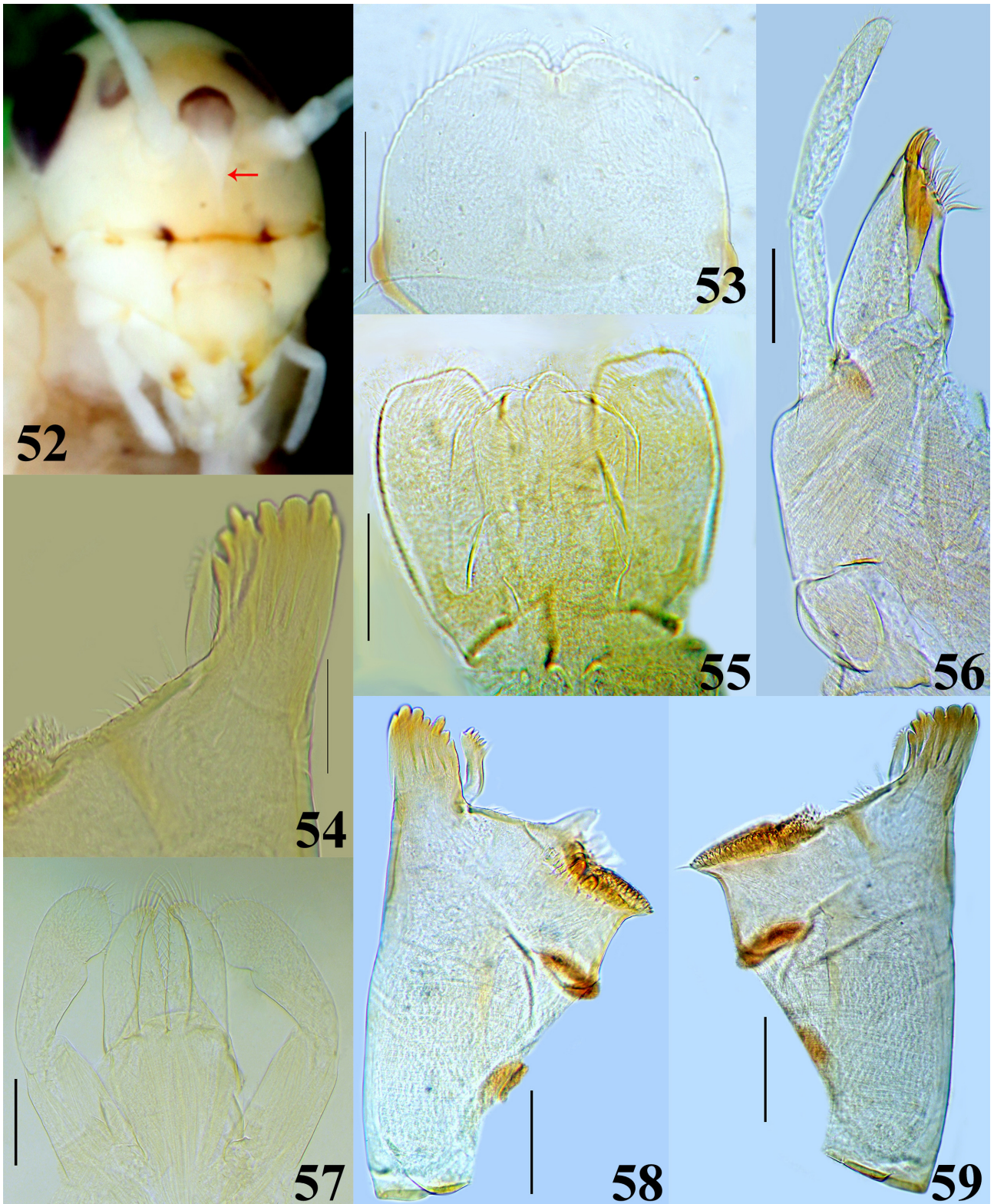
Material examined. Holotype: male mature larva (in ethanol, deposited in SCAU), **CHINA, Guizhou Province**, Daozhen County, Sanqiao Town, Daqiaogou (1400m a.s.l.), 21.viii.2004, coll. Jun Wang. **Paratypes:** 9 larvae in ethanol and 3 specimens on mounted slides, same data as holotype.



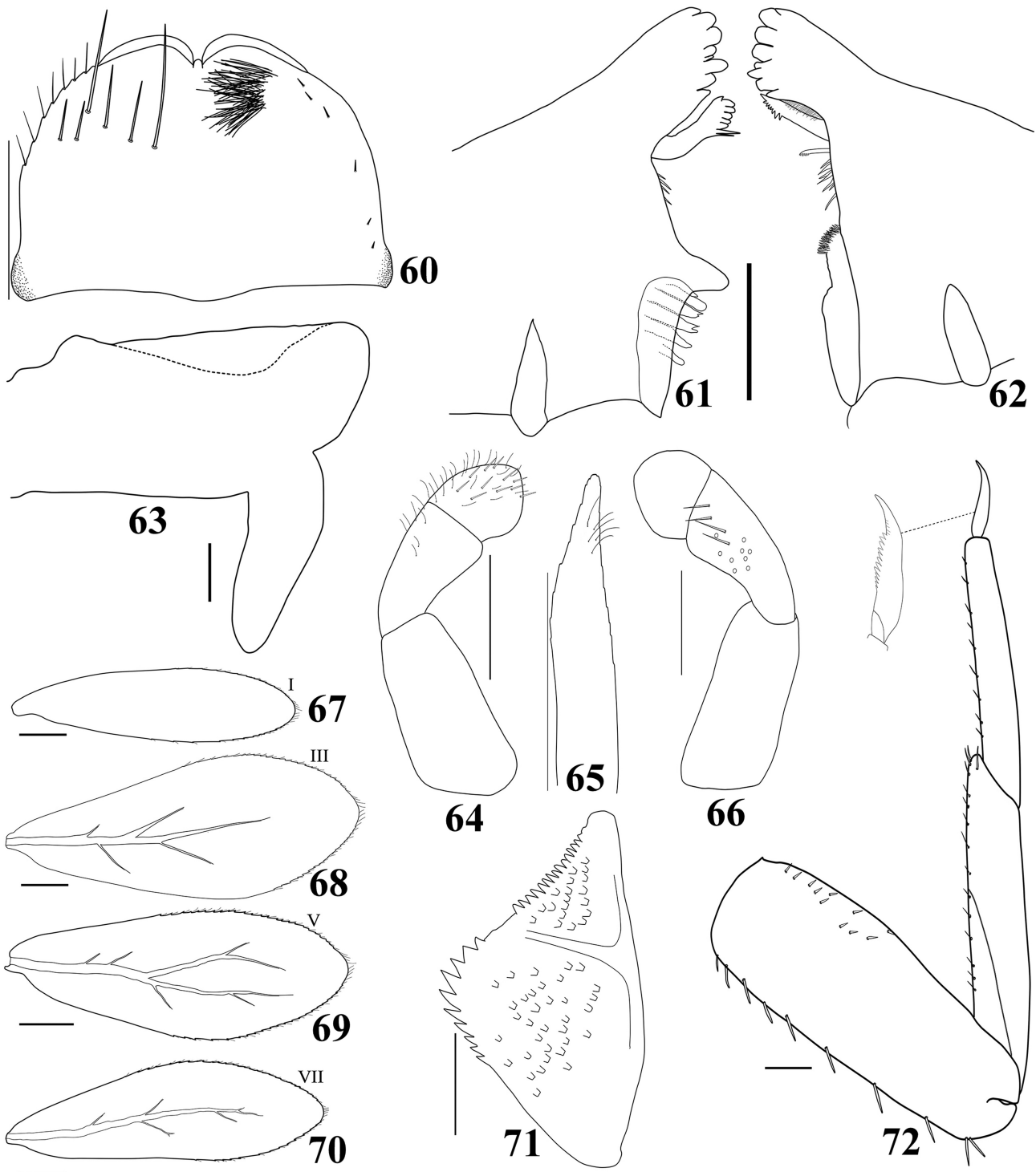
FIGURES 49–51. Larval habitus of *Nigrobaetis trialbus* sp. nov. 49, female larva (dorsal view); 50, female larva (lateral view, arrow indicates hindwing pad); 51, male larva (dorsal view). (Scale bars = 1mm).

Diagnosis of larvae. Body bicoloured. Mesonotum largely brown with a small paler oval spot near anterior margin. Fore wing pads brown with a distinct white transverse band medially. Abdominal tergites brown except tergites I, IV and VIII–X white. Right mandible with one row of fine setae along inner margin of incisors. Glossae with a cluster of 4–5 dorsal setae subapically. Hind wing pads well developed. Patella-tibial suture of foreleg present. Posterior margin of tergites I–X with triangular spines. Gills on abdominal segments I to VII. Paraproct with 9–10 pointed marginal spines.

Description of larvae. *Length.* Body length 5.5–6.0 mm; antennae broken; cerci incomplete. *Colouration.* Body bicoloured. Head yellowish with irregular brown markings. Pronotum brown; mesonotum brown except with a small paler round spot near anterior margin (Figs 49, 51). Fore wing pads brown with a distinct white band medially (Figs 49–51). Abdominal tergites brown except tergites I, IV and VIII–X white (IV and VIII with a narrow light brown band on anterior margins). All legs yellowish.



FIGURES 52–59. *Nigrobaetis trialbus* sp. nov.. 52, front view of head; 53, labrum; 54, incisor of right mandible; 55, hypopharynx; 56, maxillae; 57, labium; 58, left mandible; 59, right mandible. (Scale bars: Fig. 54 = 0.05mm; remains = 0.1mm).

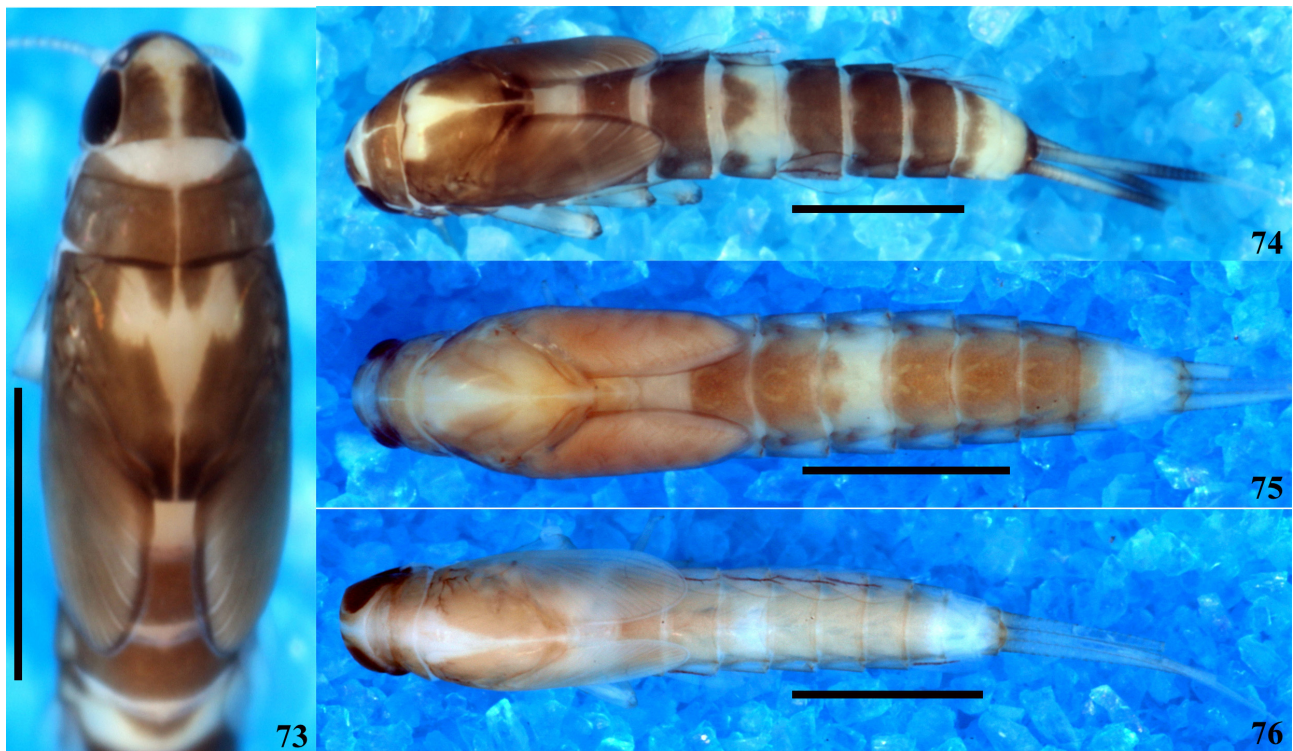


FIGURES 60–72. *Nigrobaetis trialbus* sp. nov. 60, labrum; 61, left mandible; 62, right mandible; 63, hind wing pad; 64, labial palp (ventral view); 65, glossa (dorsal view); 66, labial palp (dorsal view); 67, gill I; 68, gill III; 69, gill V; 70, gill VII; 71, paraproct; 72, foreleg. (Scale bars: Fig. 65 = 0.05mm, remains = 0.1mm).

Head. Antennae close to each other, frons with a distinct median carina between bases of antennae (Fig. 52); scape and pedicel without scale-like setae, only bearing a few sparse hair-like setae. Labrum sub-rectangular (Figs 53, 60), ca. 1.4 times wider than long; anteromedian notch deep with a small rounded lobe at the base; dorsally in distal half with one long, simple seta near midline and irregular row of 5–6 medium to long, simple setae; in proximal half almost smooth, with few sparse hair-like setae on dorsal surface; ventrally bordered with feathered setae along anterior margin and 5–6 short, spine-like setae near lateral and anterolateral margin (Fig. 60). Left

mandible (Figs 58, 61): incisors fused, with 8 denticles apically; prostheca robust, apically with 2 acute and 5–6 blunt denticles; margin between incisors and mola with 4 short acute spines (Fig. 51). Incisor of right mandible (Figs 54, 59, 62) fused, with 8 denticles apically; prostheca stick-like with distolateral dentation (Figs 54, 62); margin between incisors and mola with 7–8 short acute spines and one long, straight spine with distolateral pectinate (Fig. 62); one row of very fine setae along inner margin of incisors (Figs 54, 62), tuft of setae at apex of mola present. Hypopharynx (Fig. 55) apically covered with fine setae, lingua with a median projection; superlinguae slightly longer than lingua. Maxillae (Fig. 56): apex of maxillae with three canines, crown of galea-lacinia with three stout dentisetae; base of lacinia with one row of 4–5 simple setae and one short spine-like hump seta. Maxillary palp 2-segmented, ca. 1.7 times length of galea-lacinia, segment II apically rounded with hair-like setae and longer than segment I. Labium (Fig. 57) with glossae basally broad, narrowing toward apex, subequal to paraglossae in length; inner margin of glossae with 9–10 long, simple setae; apex with two long, curved, robust setae; outer margin with 7–8 long, simple setae; ventral surface of glossae covered with sparse hair-like setae near inner margin and proximally, dorsal surface of glossae with a setal tuft of 4–5 fine setae subapically (Fig. 65). Paraglossae sub-rectangular, curved inward, ca. 1.3 times width of glossae; apex with two rows of long, robust setae in apical area; dorsally with row of 3–4 long, simple setae near inner margin. Segment I of labial palp slightly shorter than length of segments II and III combined. Segment I ventrally with sparse hair-like setae, dorsal face with a few micropores medially. Segment II slightly expanded apico-laterally, dorsal face with one oblique row of 4–5 long pointed setae (Fig. 66), ventrally with several hair-like setae. Segment III truncated apically and slightly expanded inwards; ventrally covered with 10–14 short, simple setae and numerous hair-like setae (Fig. 64), dorsally with 5–7 short, simple setae near apex and many fine hair-like setae.

Thorax. Hind wing pads well developed (Fig. 63). Femora of foreleg (Fig. 72) dorsally with one row of 8–10 long, stout setae, dorsoapical setal patch formed by 2 stout, bluntly pointed setae; ventrally with many stout pointed setae near ventral margin; anterior face with micropores and scale bases. Tibiae with a single stout, apical blunt seta on distal end of dorsal margin, ventral margin and submargin with many stout pointed, partly pectinate setae, both faces covered with many scale bases; tibiopatellar suture present. Dorsal margin of tarsi with a few hair-like setae, ventral margin with one row of 9–10 stout pointed setae (Fig. 72); both faces with numerous scale bases. Claws (Fig. 72) slender, with one row of about 12 acute teeth; subapical setae absent. Middle and hind legs similar to foreleg in structure, except tarsi with about 7 stout pointed setae along ventral margin.



FIGURES 73–76. Larvae of *Nigrobaetis facetus* from different locations in China 73, head and thorax (dorsal view, fresh specimen); 74, thorax and abdomen (dorsal view, fresh specimen); 75, female mature larva; 76, male mature larva. (Scale bars = 1mm).

Abdomen. Dorsal surface of tergites scattered with numerous trapezoidal scale bases; posterior margin of tergites I–X with triangular spines (as Fig. 28). Sternites surface covered with numerous trapezoidal scale bases, posterior margin of sternites VII–IX with triangular spines. Gills present on segments I to VII (Figs 67–70), long elliptic; margin with a light brown band and small denticles intercalating fine hair-like setae (as Fig. 25); tracheation well visible except gill I (Fig. 67); gill I slender, similar to gill VII in shape and length, gills II–VI alike in shape. Paraproct subtriangular, without posterior prolongation (Fig. 71), surface with many trapezoidal scale bases, inner margin with ca. 10 pointed spines; surface of cercotractor with some trapezoidal scale bases, margin with 13–15 blunt spines.

Alate stage: Unknown.

Etymology. The specific epithet is an arbitrary combination of “*tri-*” (derived from Latin, the prefix of three) and “*albus*” (from Latin, meaning white), referring to the larval body dorsally having three distinct white transverse bands: (1) across the median part of forewing pads (including tergite I), (2) tergite IV and (3) tergites VIII–X, which can be easily distinguished from other *Nigrobaetis* species in the field.

Distribution. China (Guizhou).



FIGURES 77–78. Type localities of *Nigrobaetis bilongus* sp. nov. (Qingbi Stream, Mt. Cangshan, Dali City, Yunnan).

Discussion. This new species is most similar to *N. facetus* (Chang & Yang, 1994) (in Kang *et al.* 1994) by the abdomen with alternating brown and white (Figs 73–76), which is easily distinguished them from the other *Nigrobaetis* species with predominately brown abdomen. The new species was found in a high altitude (1400m a.s.l.) mountain stream in the Yunnan-Guizhou Plateau of southwestern China. In contrast, *N. facetus* was originally described from a lowland stream of Taiwan (Kang *et al.* 1994), subsequently, it was found constantly in low altitude (< 200m a.s.l.) streams of the Chinese mainland (Hong Kong SAR, Guangdong and Hunan Provinces etc.). It is worth mentioning that, judging from the position of gill sockets (Kang *et al.* 1994: Fig. 23), Kang *et al.* (1994) mislabeled the abdominal tergite IV as the tergite V in the original figures because the high resolution image of original publication showed that gills I and VII still remain on their sockets. The new species can be distinguished from *N. facetus* by the following combination of characters: (i) labial glossae having a cluster of 4–5 thin dorsal setae subapically (Fig. 65) (vs. having 7–9 dorsal setae in *N. facetus*); (ii) the mesonotum largely brown with a paler small oval spot near anterior margin (Figs 49, 51) (vs. anteromedial margin of mesonotum having a large subtriangular paler patch in *N. facetus*); (iii) the median part of forewing pads having a distinctive transverse white

band (Figs 49, 51) (versus forewing pads uniformly brown in *N. facetus*); (iv) hindwing pads well developed (versus absent in *N. facetus*); (v) posterior margin of tergite I with triangular spines (versus tergite I without such spines in *N. facetus*); (vi) tergite X white while the posterior half of tergite X brown in *N. facetus* (Figs 74–76). In addition, the new species also resembles *N. arabiensis* Gattolliat & Sartori, 2008 from United Arab Emirates and *N. vuatazi* Gattolliat & Sartori, 2012 (in Gattolliat, Vuataz & Sartori 2012) from Jordan by having 7 pairs of gills and similar colour pattern in the abdomen (Gattolliat & Sartori 2008, Gattolliat *et al.* 2012). But it can be separated from them by the following characters: from *N. arabiensis* by the presence of tibiopatellar suture and from *N. vuatazi* by the absence of spatulate setae on the dorsal margin of mid and hind tibiae; maxillary palp elongate, ca. 1.7 times length of galea-lacinia in the new species, while those in *N. arabiensis* and *N. vuatazi* are merely slightly longer than galea-lacinia; paraproct possessing 10 spines on inner margin in the new species, versus *N. arabiensis* and *N. vuatazi* having only 4 and 6 spines respectively.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 31872265 and 31960255). We would like to thank to Dr. N. J. Kluge for providing important references. Thanks are due to the referees for their advice and comments.

References

- Bae, Y.J. & Park, S.Y. (1998) *Alainites*, *Baetis*, *Labiobaetis* and *Nigrobaetis* (Ephemeroptera) in Korea. *Korean Journal of Systematic Zoology*, 14, 1–12.
- Bae, Y.J., Park, S.Y. & Hwang, J.M. (1998) Description of larval *Nigrobaetis bacillus* (Kluge) with the key to the larvae of Baetidae in Korea. *Korean Journal of Limnology*, 31 (4), 282–286.
- Bengtsson, S. (1912) Neue Ephemeriden aus Schweden. *Entomologisk Tidskrift*, 33 (1–2), 107–117.
- Cruz, P.V., Nieto, C., Gattolliat, J.-L., Salles, F.F. & Hamada, N. (2020) A cladistic insight into the higher level classification of Baetidae (Insecta: Ephemeroptera). *Systematic Entomology*, 46 (1), 44–55.
<https://doi.org/10.1111/syen.12446>
- Fujitani, T., Hirowatari, T. & Tanida, K. (2003) Genera and species of Baetidae in Japan: *Nigrobaetis*, *Alainites*, *Labiobaetis*, and *Tenuibaetis* n. stat. (Ephemeroptera). *Limnology*, 4 (3), 121–129.
<https://doi.org/10.1007/s10201-003-0105-2>
- Fujitani, T., Kobayashi, N., Hirowatari, T. & Tanida, K. (2017) Morphological description of four species belonging to the genus *Nigrobaetis* (Ephemeroptera: Baetidae) from Japan. *Limnology*, 18, 315–331.
<https://doi.org/10.1007/s10201-016-0509-4>
- Gattolliat, J.-L. (2004) First reports of the genus *Nigrobaetis* Novikova & Kluge (Ephemeroptera: Baetidae) from Madagascar and La Réunion with observations on Afrotropical biogeography. *Revue Suisse de Zoologie*, 111, 657–669.
<https://doi.org/10.5962/bhl.part.80259>
- Gattolliat, J.-L. & Sartori, M. (2008) Order Ephemeroptera. *Arthropod Fauna of the UAE*, 1, 47–83.
- Gattolliat, J.-L., Vuataz, L. & Sartori, M. (2012) First contribution to the mayflies of Jordan (Insecta: Ephemeroptera). *Zoology in the Middle East*, 56, 91–110.
<https://doi.org/10.1080/09397140.2012.10648945>
- Godunko, R.J., Martynov, A.V. & Gattolliat, J.-L. (2018) Redescription of *Nigrobaetis rhithralis* (Soldán & Thomas, 1983) (Ephemeroptera: Baetidae). *Zootaxa*, 4462 (1), 41–72.
<https://doi.org/10.11646/zootaxa.4462.1.2>
- Kaltenbach, T., Mary, N. & Gattolliat, J.-L. (2021) The Baetidae (Ephemeroptera) of the Comoros and Mayotte. *African Invertebrates*, 62 (2), 427–463.
<https://doi.org/10.3897/AfrInvertebr.62.70632>
- Kang, S.C., Chang, H.C. & Yang, C.T. (1994) A revision of the genus *Baetis* in Taiwan (Ephemeroptera, Baetidae). *Journal of Taiwan Museum*, 47, 9–44.
- Kang, S.C. & Yang, C.T. (1996) Two new species of *Baetis* Leach (Ephemeroptera: Baetidae) from Taiwan. *Chinese Journal of Entomology*, 16, 61–66.
- Kimmins, D.E. (1938) A new Moroccan Ephemeropteron. *Annals and Magazine of Natural History*, Series 11, 1 (3), 302–305, pl. 10.
<https://doi.org/10.1080/00222933808526771>
- Kluge, N.J. (1983) New and little known mayflies of the family Baetidae (Ephemeroptera) from Primorye Territory. *Revue d'Entomologie de l'URSS*, 61 (1), 65–79.
- 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>

- Kluge, N.J. (2022) Taxonomic significance of microlepidies on subimaginal tarsi of Ephemeroptera. *Zootaxa*, 5159 (2), 151–186. <https://doi.org/10.11646/zootaxa.5159.2.1>
- Kluge, N.J. & Novikova, E.A. (2014) Systematics of *Indobaetis* Müller-Liebenau & Morihara, 1982, and related implications for some other Baetidae genera (Ephemeroptera). *Zootaxa*, 3835 (2), 209–236. <https://doi.org/10.11646/zootaxa.3835.2.3>
- Kopelke, J.P. & Müller-Liebenau, I. (1981) Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815. Teil III: *buceratus*-, *atrebatinus*-, *niger*, *gracilis*- und *muticus*-Gruppe (Ephemeroptera, Baetidae). *Deutsche Entomologische Zeitschrift*, 28 (1–3), 1–6. <https://doi.org/10.1002/mmnd.4800280102>
- Kubendran, T., Balasubramanian, C., Selvakumar, C., Gattolliat, J.-L. & Sivaramkrishnan, K.G. (2015) Contribution to the knowledge of *Tenuibaetis* Kang & Yang 1994, *Nigrobaetis* Novikova & Kluge 1987 and *Labiobaetis* Novikova & Kluge 1987 (Ephemeroptera: Baetidae) from the Western Ghats (India). *Zootaxa*, 3957 (2), 188–200. <https://doi.org/10.11646/zootaxa.3957.2.3>
- Leach, W. E. (1815) Entomology. *Brewster's Edinburgh Encyclopedia*, 9 (1), 57–172. [1st Edition]
- Linnaeus, C. (1761) *Fauna Svecica sistens Animalia Sveciae regni: mammalia, aves, amphibia, pisces, insecta, vermes. Distributa per classes & ordines, genera & species, cum differentiis specierum, synonymis auctorum, nominibus incolarum, locis natalium, descriptionibus insectorum. Editio altera, auctior*. Sumtu & Literis Direct. Laurentii Salvii, Stockholmiae, 578 pp. <https://doi.org/10.5962/bhl.title.46380>
- Lugo-Ortiz, C.R. & de Moor, F.C. (2000) *Nigrobaetis* Novikova & Kluge (Ephemeroptera: Baetidae): first record and new species from southern Africa, with reassignment of one northern African species. *African Entomology*, 8 (1), 69–73.
- Martynov, A.V. & Godunko, R.J. (2017) Mayflies of the Caucasus Mountains. IV. New species of the genus *Nigrobaetis* Novikova & Kluge, 1987 (Ephemeroptera, Baetidae) from Georgia. *Zootaxa*, 4231 (1), 70–84. <https://doi.org/10.11646/zootaxa.4231.1.4>
- Müller-Liebenau, I. (1969) Revision der europäischen Arten der Gattung *Baetis* Leach, 1815 (Insecta, Ephemeroptera). *Gewässer und Abwässer*, 1969 (48/49), 1–214.
- Müller-Liebenau, I. (1984) New genera and species of the family Baetidae from West-Malaysia (River Gombak) (Insecta: Ephemeroptera). *Spixiana*, 7, 253–284.
- Müller-Liebenau, I. (1985) Baetidae from Taiwan with remarks on *Baetiella* Ueno, 1931 (Insecta, Ephemeroptera). *Archiv für Hydrobiologie*, 104, 93–110. <https://doi.org/10.1127/archiv-hydrobiol/104/1985/93>
- Novikova, E.A. & Kluge, N.J. (1987) Systematics of the genus *Baetis* (Ephemeroptera, Baetidae), with description of new species from Middle Asia. *Vestnik Zoologii*, 4, 8–19.
- Novikova, E.A. & Kluge, N.J. (1994) Mayflies of the subgenus *Nigrobaetis* (Ephemeroptera, Baetidae, Baetis). *Entomologicheskoe Obozrenie*, 73 (3), 623–644.
- Phlai-ngam, S., Tungpairajwong, N. & Gattolliat, J.-L. (2022) A new species of *Alainites* (Ephemeroptera, Baetidae) from Thailand. *Alpine Entomology*, 6, 133–146. <https://doi.org/10.3897/alpento.6.96284>
- Sivaruban, T., Srinivasan, P., Barathy, S. & Isack, R. (2022) A new species of *Nigrobaetis* Novikova & Kluge, 1987 (Ephemeroptera, Baetidae) from Tamil Nadu, India. *Zootaxa*, 5091 (1), 182–190. <https://doi.org/10.11646/zootaxa.5091.1.8>
- Soldán, T. (1977) Three new species of mayflies (Ephemeroptera) from the mist oasis of Erkwit, Sudan. *Acta Entomologica Bohemoslovaca*, 74, 289–294.
- Soldán, T. & Thomas, A.G.B. (1983) *Baetis numidicus* n. sp., Ephéméroptère nouveau d'Algérie (Baetidae). *Annales de Limnologie*, 19 (3), 207–211. <https://doi.org/10.1051/limn/1983024>
- Sroka, P., Yanai, Z., Palatov, D. & Gattolliat, J.-L. (2021) Contribution to the knowledge of the genus *Takobia* Novikova & Kluge, 1987 (Ephemeroptera, Baetidae) in Central Asia. *ZooKeys*, 1071, 127–154. <https://doi.org/10.3897/zookeys.1071.71582>
- Tong, X. & Dudgeon, D. (2000) Two new species of *Alainites* (Ephemeroptera: Baetidae) from Hong Kong, China. *The Pan-Pacific Entomologist*, 46, 115–120.
- Ubero-Pascal, N., Puig, M.A. (2007) Microscopy and egg morphology of Mayflies. In: Méndez-Vilas, A. & Días, J. (Eds.), *Modern Research and Educational Topics in Microscopy. Formatex Microscopy Book Series*, 3 (1), pp. 326–335.
- Waltz, R.D., McCafferty, W.P. & Thomas, A. (1994) Systematics of *Alainites* n. gen., *Dipheter*, *Indobaetis*, *Nigrobaetis* n. stat., and *Takobia* n. stat. (Ephemeroptera, Baetidae). *Bulletin de la Société d'histoire Naturelle de Toulouse*, 130, 33–36.
- Waltz, R.D. & McCafferty, W.P. (1997) New generic synonymies in Baetidae (Ephemeroptera). *Entomological News*, 108 (2), 134–140.
- Yanai, Z., Sroka, P. & Gattolliat, J.-L. (2022) Two new species of *Alainites* (Ephemeroptera, Baetidae) from the Mediterranean biodiversity hotspot. *ZooKeys*, 1118, 73–95. <https://doi.org/10.3897/zookeys.1118.84643>