Zootaxa 4965 (3): 541–557
https://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4965.3.8
http://zoobank.org/urn:lsid:zoobank.org:pub:A42D0F3D-3145-4540-82A4-E936ADBDDD8C

Baetis majus sp. nov., new species of mayfly (Ephemeroptera: Baetidae) from Far East of Russia

TATIANA M. TIUNOVA[1*] & ALEXANDER A. SEMENCHENKO[2] & XIAOLI TONG[3]

[1]*Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, Vladivostok,690022, Russia.*

[2]*Laboratory of Ecology and Evolutionary Biology of Aquatic Organisms, School of Natural Sciences, Far Eastern Federal University, Suhanova St. 8, 690950 Vladivostok, Russia.*

semenchenko_alexander@mail.ru; https://orcid.org/0000-0001-7207-9529

[3]*Department of Entomology, College of Plant Protection, South China Agricultural University, Guangzhou 510642, Guangdong, China.*

xtong@scau.edu.cn; https://orcid.org/0000-0003-1731-229X

[*]*Corresponding author. tiunova@biosoil.ru; https://orcid.org/0000-0002-9586-1502*

Abstract

A new species, *Baetis majus* Tiunova **sp. nov**., is described and illustrated based on larvae and reared adults discovered in the Russian Far East. The differential identification of this species was determined by the characteristics of other representatives of the genus *Baetis* Leach, including subgenera *Baetis* Leach and *Tenuibaetis* Kang & Yang from Eastern and Western Palaearctic, Nearctic and Oriental regions. In addition to morphological studies, DNA barcoding of the described species with average intraspecific K2P distances to nearest neighbours is documented. We reconstructed the phylogenetic relationships of all available *cytochrome c oxidase subunit I* (COI) sequences of the subgenera of *Baetis* and *Tenuibaetis* from four regions. Bayesian analysis using 47 morphological characters additional to partial COI sequences did not allow to determine the species-group of the *Baetis* genus to which the described species belongs.

Key words: Mayflies, taxonomy, DNA barcoding, Baetidae, *Baetis*, *Tenuibaetis*, new species, Asia

Introduction

In the Russian Far East, the genus *Baetis* Leach 1815 is represented by the following subgenera *Baetis* Leach, 1815; *Rhodobaetis* Jacob, 2003; and *Tenuibaetis* Kang & Yang, 1994. On one hand, several authors ranked the subgenus *Tenuibaetis* as genus (Fujitani *et al*. 2003, 2011; Kaltenbach & Gattolliat 2019). The genus *Baetis* is determined by the following morphological characteristics: the presence of a femoral patch (villopore); larval antennae bases never brought together, frons between them does not form a keel; prostheca of right and left mandibles are stick-shape; inner margin of mandibles between prostheca and mola without setae; labial palp 3-segmented, with 2nd segment widened or with inner-apical projection (Waltz & McCafferty 1997; Kang & Yang 1994; Fujitani *et al*. 2003; Kluge & Novikova 2011; Sroka 2012; Martynov & Godunko 2017).

At the present time, in the Far East of Russia, there are 11 species of mayflies belonging to the genus *Baetis*: East-Asian *B*. (*B*.) *feles* Kluge and East-Asian Island *B*. (*R*.) *thermicus* Ueno; East Palaearctic *B*. (*B*.) *ussuricus* Kluge, *B*. (*T*.) *ursinus* Kazlauskas, *B*. (*R*.) *silvaticus* Kluge and *B*. (*R*.) *molecularis* Tiunova & Semenchenko; Transpalaearctic *B*. (*B*.) *fuscatus* (Linnaeus) and *B*. (*B*.) *vernus* Curtis; Circum-Boreal *B*. (*B*.) *macani* Kimmins (Kluge 1997; Tiunova & Semenchenko 2019, 2020). At the same time, for such species as *B. pseudothermicus* Kluge and *B. pentaphyllus* Tiunova & Semenchenko the subgeneric affiliation is still unclear (Kluge 2020).

The DNA barcodes correspond to the 650-bp fragment of the mitochondrial gene *cytochrome c oxidase subunit I* (COI) for animal species delimitation (Hebert *et al.* 2003). DNA barcoding has been useful for species delimitation in the subfamily Baetinae (Telfer *et al*. 2015; Hebert *et al*. 2016; Morinière *et al*. 2017; Stauffer *et al*. 2017). To increase the accuracy of phylogenetic analysis and dividing *Baetis* species into species-groups, morphological characters of larva and adults can be used in addition to COI sequences (Sroka 2012).

Accepted by M. Sartori: 1 Apr. 2021; published: 29 Apr. 2021

During 2014–2018, the first author collected a new species of the genus *Baetis,* which is named *Baetis majus* Tiunova, **sp. nov**., the description and illustration below are based on adults and larvae.

DNA barcoding of the new species and phylogenetic relationships of subgenera *Baetis* and *Tenuibaetis* form Eastern and Western Palearctic, Oriental and Nearctic are provided. We also present a Bayesian tree reconstruction using a dataset of 47 morphological characters and partial COI sequences to increase the phylogenetic signal and relate the described species to the recognised species-groups of the genus *Baetis* (Sroka 2012).

Material and methods

The material was collected in a river of southern Primorye during 2014–2018. Material for DNA analysis was preserved in 96% ethanol, and other material was preserved in 75% ethanol. The material was deposited in the collection of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, Vladivostok.

Larvae of *B. maculosus* Tong *et al.* were also examined (China, Guangdong Province, Yadong River, 18.X.2013, 3 larvae, and leg. Wang Zhaohong).

The total DNA was extracted from the thorax of adult mayflies or larvae using PureLink Genomic DNA Mini Kit (Invitrogen) according to the manufacturer protocol with final elution in 100 µL. Partial fragment of mitochondrial *cytochrome c oxidase subunit* I (658bp) was amplified by using usual primers LCO1490 and HCO2198 (Folmer *et al*. 1994). The optimized PCR conditions (per 10 µL) using GoTaq Green Master Mix (2x) (Promega corp, Madison, WI, USA) were 0.5µL of each primer (10 pmol/µl), 1 µl of total DNA and 3 µL of UltraPure water. Thermocycler conditions were as follows: initial denaturation for 5 min at 95°C followed by 35 cycles of denaturing for 30 s at 95°C, annealing for 30 s at 50°C and an extension time of 60 s at 72°C, with a final extension for 5 min at 72°C. PCR products were visualized on an 1.5% TBE agarose gel with Ethidium bromide after electrophoresis. Single bands were purified using a Exonuclease I (20 U/µl) and Alkaline Phosphatase (1 U/µl) (ThermoFisher, Waltham, MA, USA). Purified PCR products were sequenced directly in both directions using an automated sequencer (ABI prism® 3130 XL DNA Analyzer; Applied Biosystems, USA) at department of Cell biology and Genetics of Far Eastern Federal University, Russia. Forward and reverse sequences were aligned and manually edited in MEGA 7 (Kumar *et al*. 2016) and FinchTV (Geospiza Inc., Seattle, WA) and then checked for the presence of stop codons. The interspecific K2P distances were estimated using MEGA 7.

To reconstruct the phylogenetic tree, we use all available COI sequences of subgenera *Baetis* and *Tenuibaetis* from Eastern and Western Palearctic, Nearctic and Oriental regions. Some species (for example *B. vernus*, *B. intercalaris* McDunnough, and *B. flavistriga* McDunnough) consisted of several molecular operational taxonomic units (mOTU) and belongings to different BOLD BIN numbers (v4.boldsystems.org). We included in the analysis only specimens that were collected closer to the type habitat and COI sequences were longer than 600 bp. To increase the representativeness of the tree, we sequenced and added East-Palaearctic species *B. pseudothermicus*, *B. ursinus* and *B. ussuricus*.

In addition to the DNA barcoding data, a matrix of 36 morphological larval and 11 adult characters was compiled according to Sroka (2012). The final matrix of morphological characters of *Baetis majus* Tiunova **sp. nov**. was 00001011000002200200100011111011010110010100211, and the list of characters can be found in Sroka (2012). We combined morphological matrix and COI sequence of *Baetis majus* Tiunova **sp. nov**. with data of Sroka (2012) and Tiunova & Semenchenko (2019) to reconstruct the second phylogenetic tree.

Phylogenetic analyses were conducted using Bayesian inference (BI) analyses in MrBayes 3.2.7. (Ronquist *et al*. 2012). The optimal nucleotide substitution model for BI was selected using PartitionFinder 2.1.1 (Lanfear *et al.* 2012). The best fit models for the first, second and third codon positions were GTR (Tavare 1986) plus G (Gamma distribution), F81 (Felsenstein 1981) plus I (a proportion of invariable sites) and HKY (Hasegawa *et al*. 1985) plus G respectively. The Metropolis-coupled Markov chain Monte Carlo analysis was run for 5 million generations and sampled every 500 generations. A burnin of 1 250 000 generations (or 25% of the sampled trees) was used. Moreover, trace files were visually inspected in Tracer 1.7 (Rambaut *et al.* 2018). ML analysis was performed in RAxML v. 8.2.4 using bootstrapping with 1000 replications (Stamatakis 2006). Consensus tree was visualized in FigTree v. 1.4.4. Bayesian posterior probabilities and ML bootstrap values were used to evaluate branch support. DNA sequences were uploaded to the GenBank by accession number MT945949 (*Baetis majus* Tiunova **sp. nov**.), MT945950–MT945961 (*B. pseudothermicus*), MT945962–MT945965 (*B. ursinus*), and MW147509–MW147510 (*B. ussuricus*).

Results

Baetis majus **Tiunova sp. nov.**
urn:lsid:zoobank.org:pub:2A2F1007-BEEA-48D9-BEE3-D64D055E4BB0
(Figs 1–41)

Material. Holotype male imago (reared from larva, with larval skin), **RUSSIAN FEDERATION:** PRIMORSKIY KRAY, Shkotovskiy district, Shkotovka River, bridge, Shkotovo village, 43°04´40.83´´ N 132°21´31.72´´E, 22.V.2014, T. Tiunova. **Paratypes**: 2 male, 1 female imagines, 2 larvae, and one larva **(BM175)** same data as holotype; same place, 11.V.2015, 5 larvae, T. Tiunova; same place, 15.V.2018, 27 larvae, T. Tiunova.

Description. ***Male imago*** (in alcohol) (Fig. 1). Length (mm): body 4.7–5.2; forewings 5.5–5.6; cerci 12.0–13.0; femora 1.1; tibia 1.7–1.8. **Head:** light brown or brownish. Antennae yellowish.

Turbinate eyes moderately high (Fig. 1); faceted surface oval in dorsal view, 1.2 times longer than wide; facetted surface of turbinate eyes light orange; the shaft grayish with a yellowish ring.

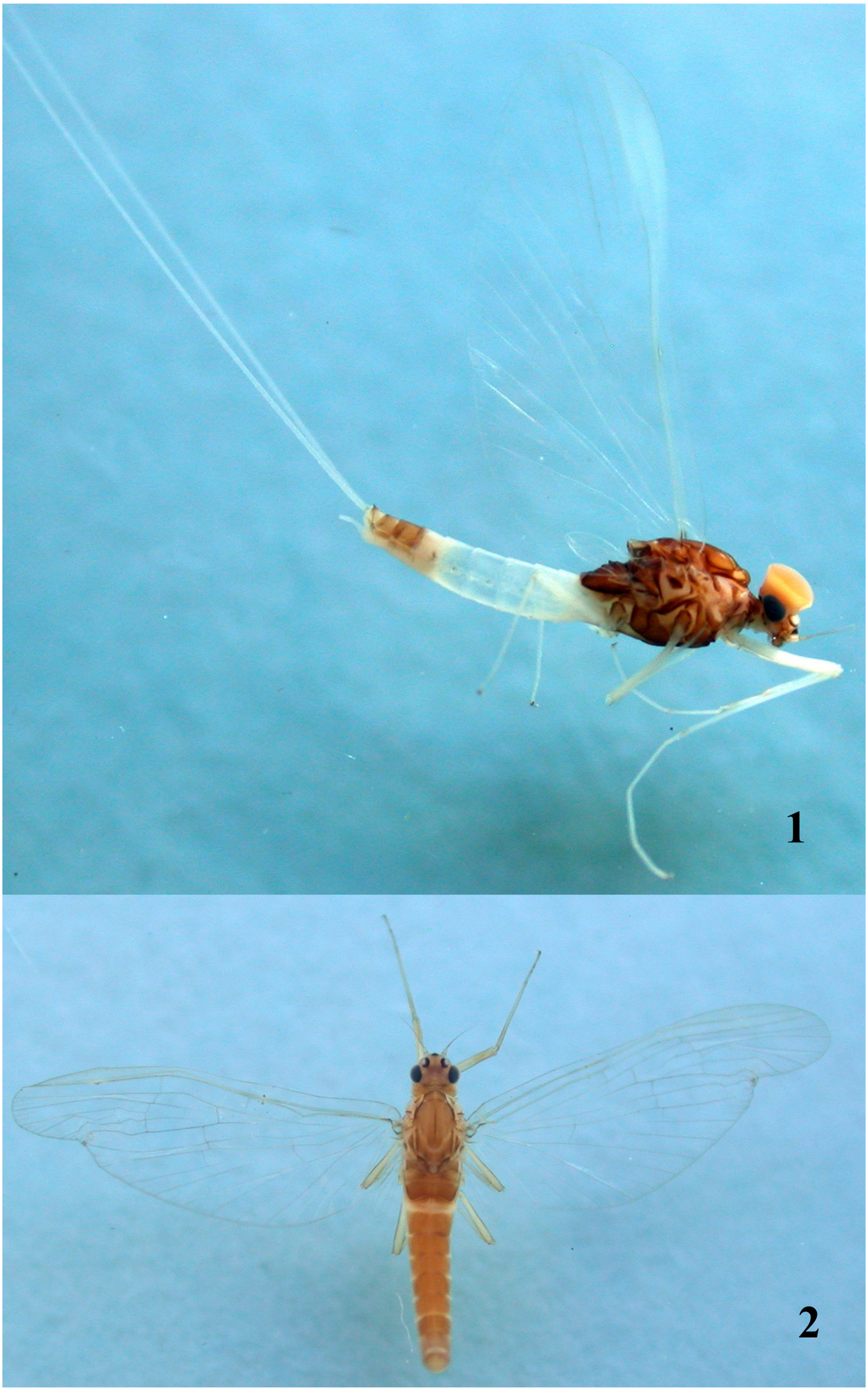
Thorax: Medioscutum and submedioscutum light brown; posterior scutal protuberance dark brown with light lateral sides; scutellum grayish; scuto-scutellar impression white; median longitudinal suture dark brown; sublateroscutum dark brown; anterior phragma dark brown. All legs white (Fig. 1). Lengths ratio of individual foreleg segments: 1.1:1.8:0.8:0.5:0.35:0.2. Forewing transparent, all veins pale; pterostigma milky on a dark background, with 5–6 cross veins. Hind wing hyaline, transparent, and approximately 2 times longer than wide, rounded apex and two simple longitudinal veins; cross veins absent; costal projection present (Fig. 3).

Abdomen: Tergum I brownish; terga II–VI yellowish or whitish, translucent (Fig. 1); terga VIII–X brown with darker lateral margins. Sterna I–VI white, translucent; sterna VII–IX brown, opaque. Styliger whitish (Fig. 4). Unistiliger and segment I of forceps brownish; segments II and III whitish; unistiliger with parallel margins, slightly elongated, almost as long as wide, with a distinct small projection at the apex of the inner edge; segment I of forceps conical and elongate; segment II relatively narrow, slightly widening towards the apex and with beveled inner apical margin; segment III small, well defined, strongly expanded to the apex, its length only slightly exceeds its maximum width (Fig. 4). Caudal filaments whitish (Fig. 1).

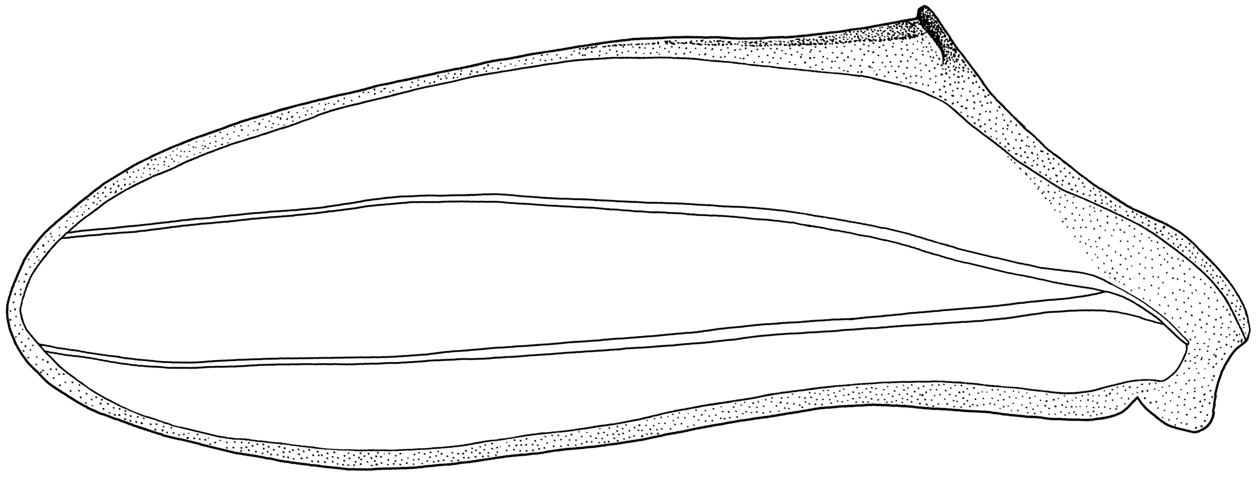
Female imago. Length (mm): body 5.5; forewings 6.5; cerci lost; foreleg: femora 1.0; tibia 1.3. General color of body brown (Fig. 2). Head grayish, posterior margin brownish; antennae whitish. Thorax light brown, with brown median longitudinal suture. Wings transparent; all veins brownish. Legs yellowish or brownish. Abdomen terga I–VIII (with eggs) light brown without maculation; terga IX–X lighter.

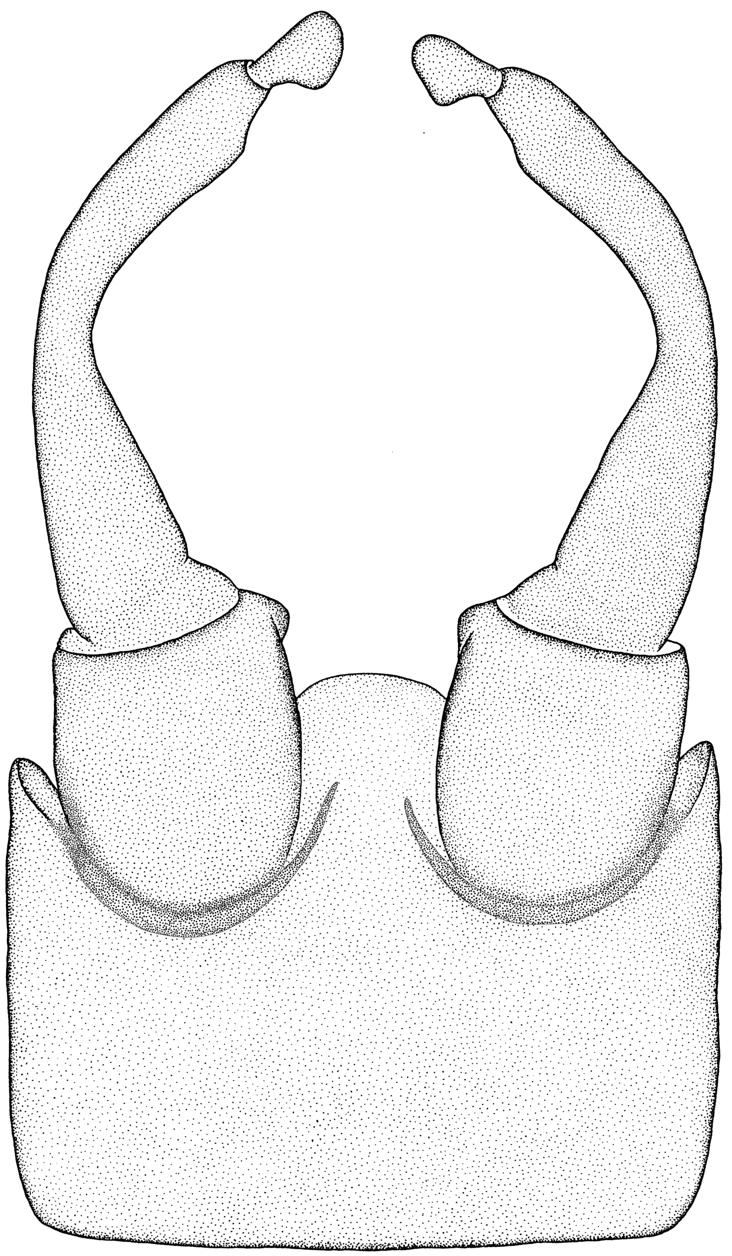
Mature larvae (in alcohol): Length (mm): body 4.7–5.3; cerci 2.5–3.1. General body color brown with light maculation (Figs 5–7)**.**

Head: antennae brownish; antennal pedicel and scape without spatulas, with rare fine hair-like setae only (Fig. 8); head brown with light maculation on vertex; surface of the head covered numerous strong spatulas and fine hair-like setae (Figs 9–10); frons, clypeus and labrum white; fronto-clypeal suture black; gena with a pair of dark brown stripes. Eyes of female black (Fig. 7), larval turbinate eyes of male light orange (Fig. 5). Labrum more or less rounded (width/length ratio of 1.3); dorsal surface with 1 + 2–3 long submarginal setae, arranged in one regular row, and row of long pointed setae laterally on both margins; posterior area with rare thick and short setae (Fig. 14). Canines of right mandible with 7 teeth divided into two groups; incisor with three large teeth of relatively equal size; kinetodontium with four teeth; the first, second and third relatively equal size, fourth smallest (Figs 11, 15); prostheca toothbrush-like. Left mandible canines with 7 teeth; incisor with three teeth, first two the largest; kinetodontium with four teeth, first and fourth the smallest and second the largest (Figs 11, 16); prostheca brush-like, with mostly short blunt appendages and two or three pointed ones on inner side. Maxillary palp two-segmented, surface covered with hair-like setae, more densely located on distal part of second segment; second segment longer than first segment (1.7 times) (Fig. 17). Labium with paraglossae concave in middle, 1.4 times wider than glossae; apical part of paraglossae with two regular rows of long setae; 6 long bristles located along outer margin in ventral surface (Fig. 18); distal part of dorsal surface of paraglossa covered with small stout setae (Figs 12–13).



FIGURES 1–2. *Baetis majus* Tiunova **sp. nov.**, 1, male imago, holotype, lateral view; 2, female imago, dorsal view.





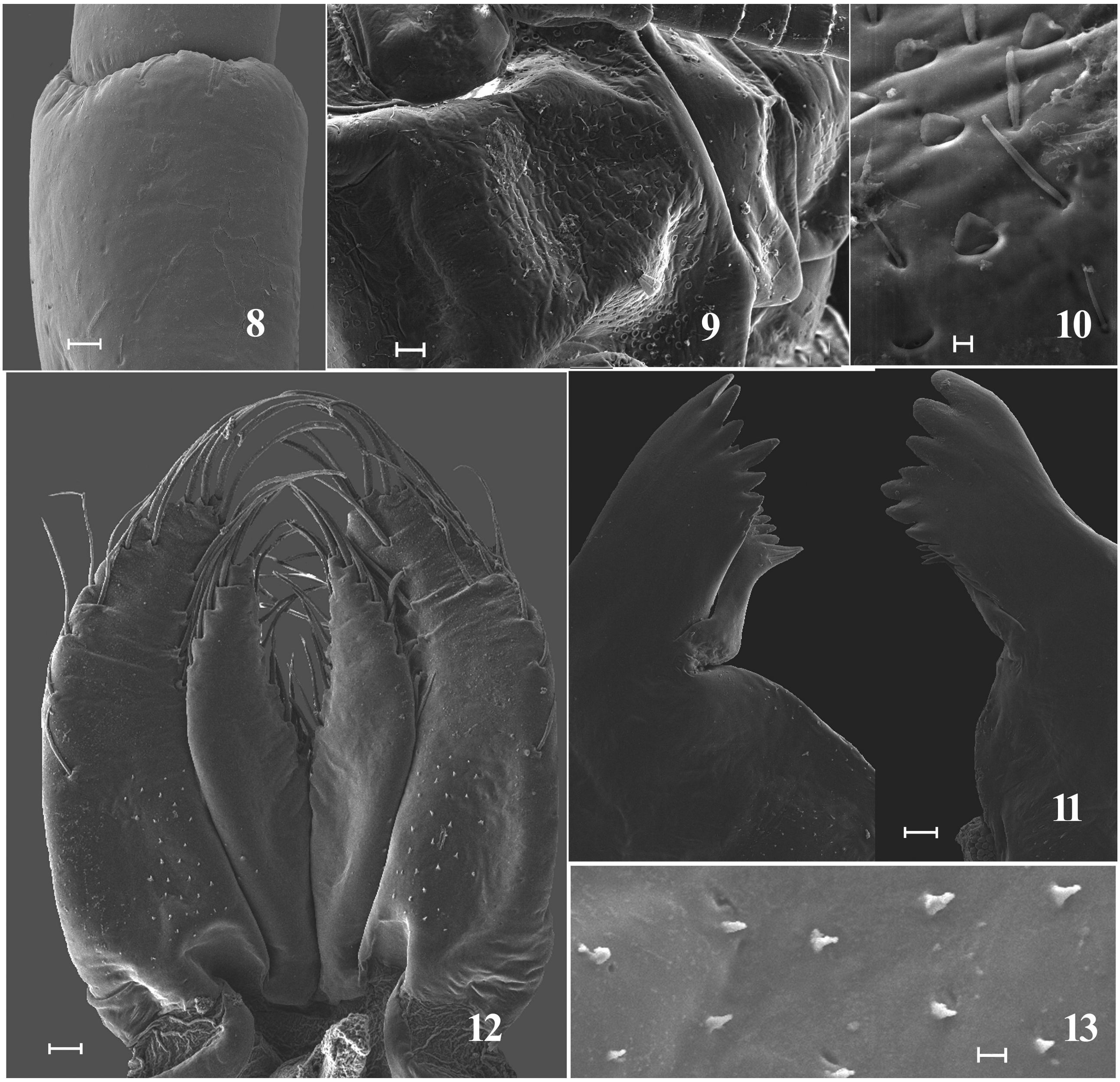
FIGURES 3–4. *Baetis majus* Tiunova **sp. nov.**, male imagoes, holotype: 3, hind wing; 4, unistyliger and forceps.

Glossae triangular with row of 9–10 stout setae on outer and 5–6 long setae on inner margins (Fig. 18). General shape of the second and third segments of the labial palp rather stocky (Figs 19–20); third segment wide, its apex truncated; 1.3 times wider than the second segment; ventral surface covered with long stout setae (Fig. 19); second segment with rounded apicomedial projection; dorsal surface with a row of 4–5 long pointed setae (Fig. 20).

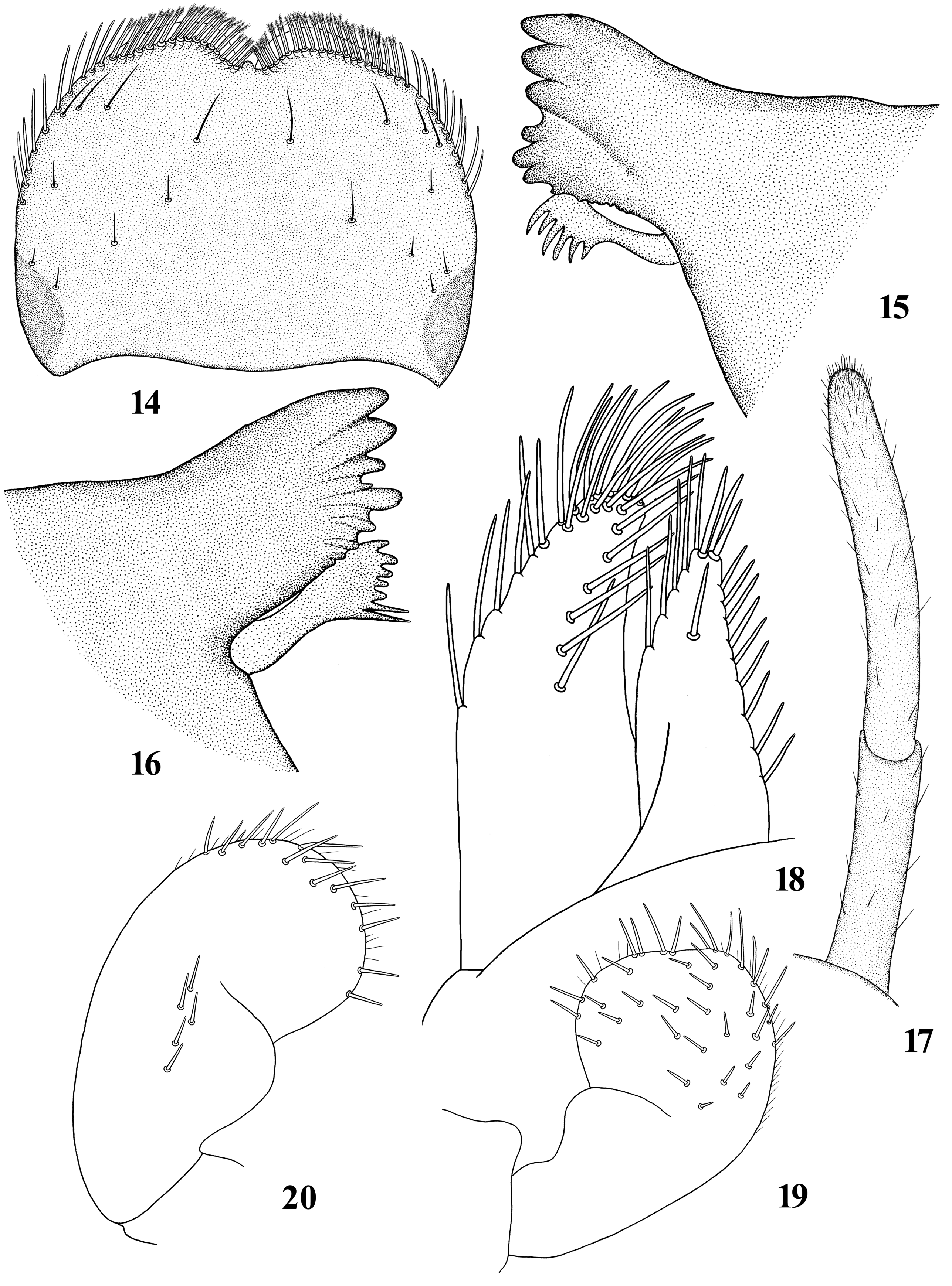
FIGURES 5–7. Color pattern of *Baetis majus* Tiunova **sp. nov.**, larvae, paratypes: 5, male, dorsal view; 6, ventral view; 7, female, dorsal view.

Thorax: brown with contrasting whitish or brownish stripes on the mesonotum (Figs 5, 7). Mesonotum with a V-shaped white spot near anterior margin. Pronotum with a pair of light spots laterally. Legs white, joints of leg segments brown (Fig. 6). Femora with brown rectangular spot near inner margin (Fig. 21); outer edge with row of long, apically widened setae in distal part (Fig. 22) and rounded setae in basal part; inner margin with a irregular row of small pointed setae in basal part; femoral villopore present (Fig. 23). Tibia and tarsi brown in apical part; stout setae arranged in an irregular rows along outer margin of tibia (Figs 21, 27, 29) and a regular row on tarsi (Fig. 21, 28); smaller setae located along inner margin of tibia and tarsi; dorsal surface of femora, tibia and tarsi covered with small bluntly rounded spatulas (Figs 27–29). Patella-tibial suture present (Figs 21, 27). Tarsal claws brown, with row of 13–15 teeth increasing in length toward apex. Apex of tarsal claw without subapical setae (Figs 24, 30). Lengths (mm) of the leg segments as follows: Foreleg: femur 0.9–0.95; tibia 0.5–0.6; and tarsus 0.5–0.55. Middle leg: femur 0.9–0.95; tibia 0.5–0.6; and tarsus 0.5. Hind leg: femur 0.9–1.05; tibia 0.55–0.9; and tarsus 0.45–0.5.

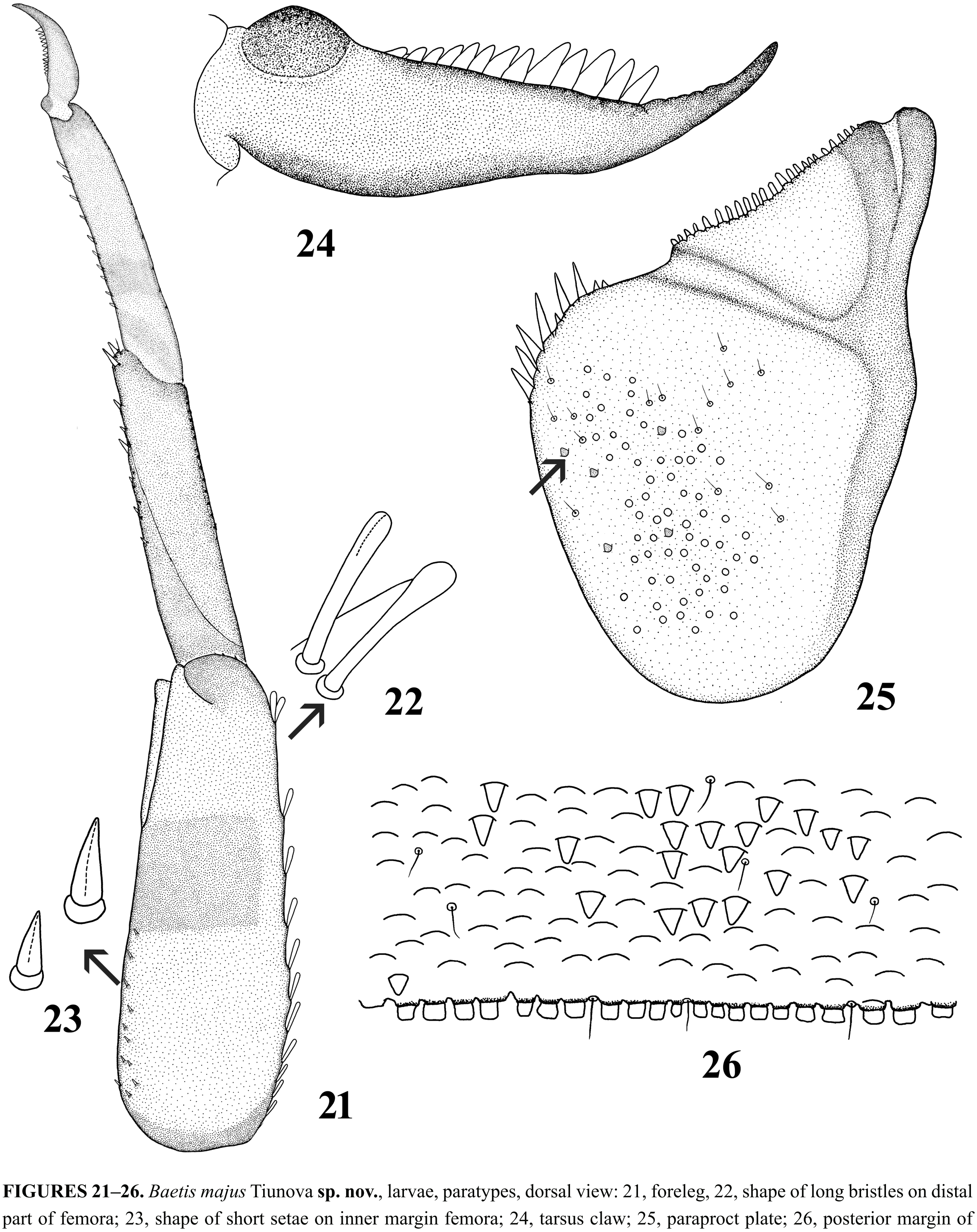
Abdomen: Terga with contrasted white and black or dark brown maculations (Figs 5, 7). Tergum I brownish, posterior margin darker; terga II–VIII with a pair of antero-median light stripes and spots contoured with dark brown; terga II–III and VII darker than terga IV and VI with a pair of large white spots joining along the middle part at the posterior margin; antero-lateral corners and lateral margins white; postero-lateral corners black; terga IX and X completely white (Figs 5, 7); posterior margins of tergum VI with regular row of quadrangular teeth of various sizes; surface of terga densely covered with numerous semilunar impressions, rare hair-like setae and conical spatulas (Fig. 26). Sterna generally dirty white; sterna VII–VIII brownish (Fig. 6). Six pairs of gills, present on abdominal segments II–VII; gills elongated, oval-shaped; all gills matt, with dark band surrounding margin and apparent tracheation; margins with numerous hair-like setae inserted at the base of small teeth (Figs 33–38). Gills II and VI almost equal in length and only slightly smaller than gill III; gills IV–V equal in length, 2 times longer than wide (Figs 35–36); gill VII the smallest,1.3 times shorter than gill II and 1.6 times shorter than gill V (Fig. 38). Paraproct with 9–10 large pointed teeth like spines of different size (Fig. 25); surface of paraproct without notched scales, with fine hair-like setae located disorderly and 3–5 rounded scales, scattered in middle area (Figs 25, 31–32). Cerci brownish with dark band at the middle and dark tips (Figs 5–7).



FIGURES 8–13. *Baetis majus* Tiunova **sp. nov.**, larvae, dorsal view, paratypes: 8, scape; 9, surface of the head; 10, spatulas on head; 11, canines of left and right mandible; 12, glossae and paraglossae; 13, setae on paraglossa. Scale bar: 8, 11–12, 0.01 mm; 9, 0.02 mm; 10, 13, 0.002 mm.



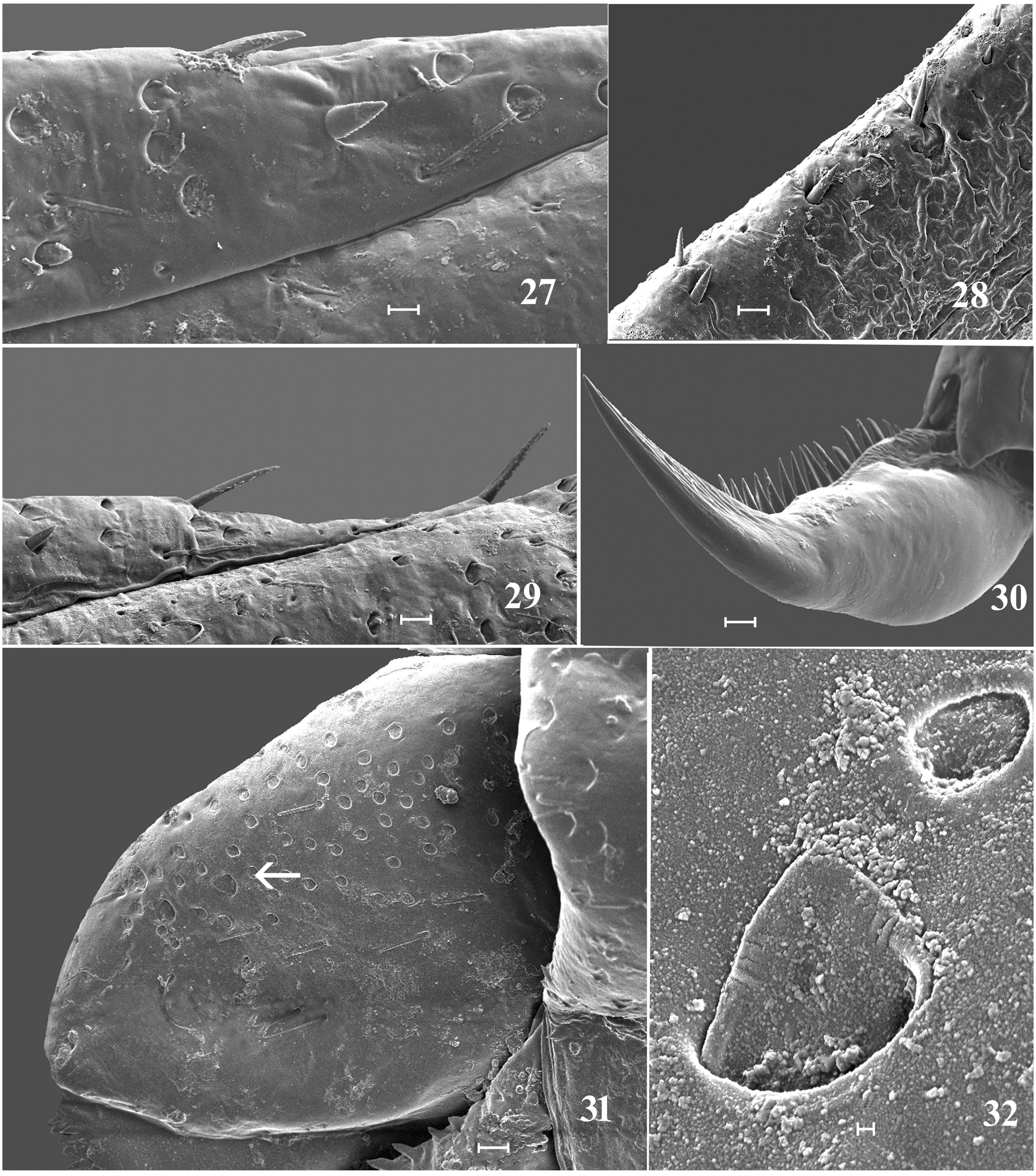
FIGURES 14–20. *Baetis majus* Tiunova **sp. nov.**, larvae, details of mouthparts, paratypes: 14, labrum, dorsal view; 15, canines and prostheca of right mandible, dorsal view; 16, canines and prostheca of left mandible, dorsal view; 17, maxillary palp, dorsal view; 18, glossa and paraglossa, ventral view; 19, labial palp, ventral view; 20, labial palp, dorsal view.



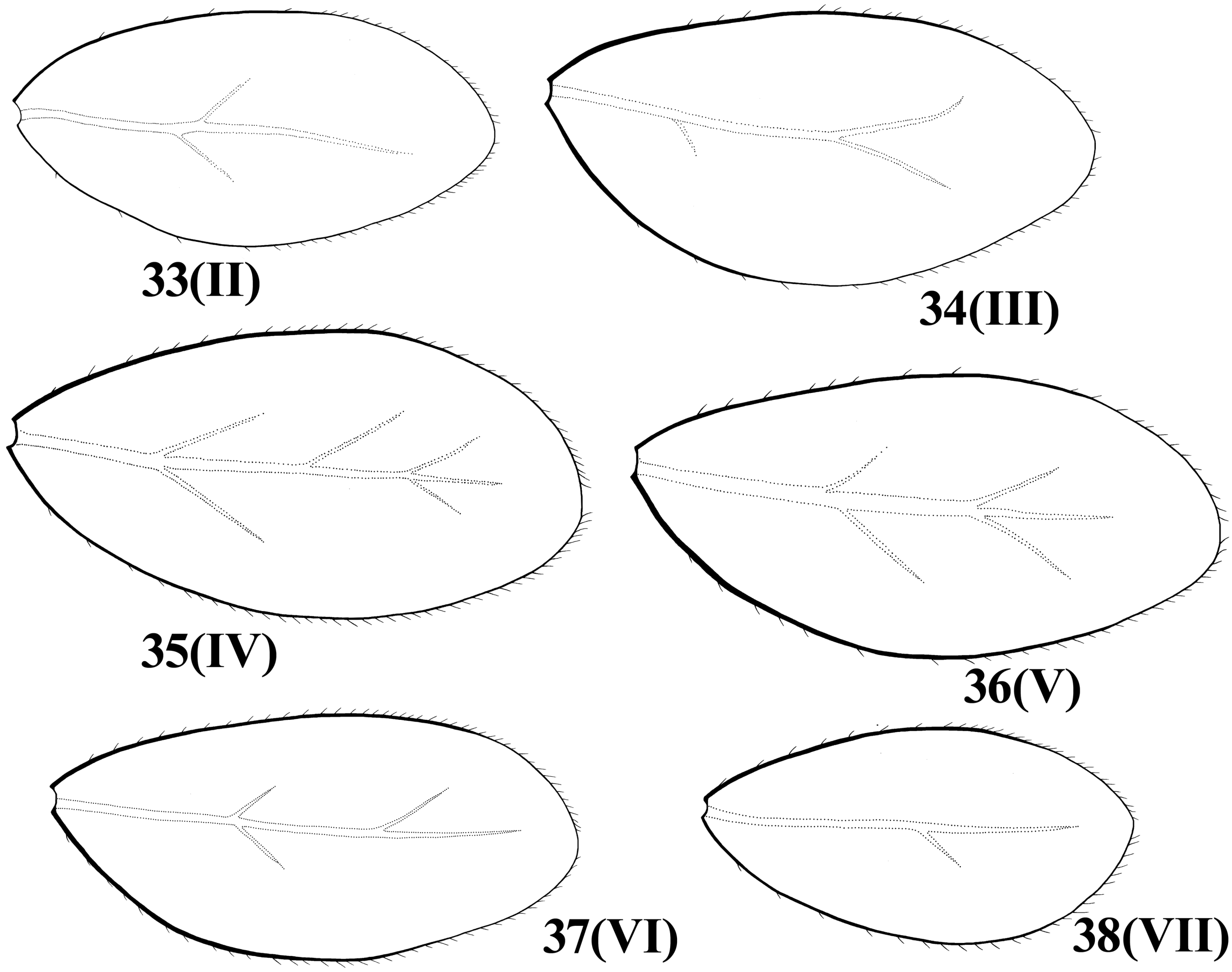
FIGURES 21–26. *Baetis majus* Tiunova **sp. nov.**, larvae, paratypes, dorsal view: 21, foreleg, 22, shape of long bristles on distal part of femora; 23, shape of short setae on inner margin femora; 24, tarsus claw; 25, paraproct plate; 26, posterior margin of tergum VI.

Eggs. General form oval 102–106 µm length and 62–68 µm width (Fig. 39). Chorion corrugated and densely wrinkled, with numerous small granules and with small fossae (Fig. 40). One or two small and round micropyles with a collar-like ring located in the equatorial area (Fig. 41).

Diagnosis. Larva. Six pairs of gills, present on abdominal segments II–VII; dorsal surface of the head, femora, tibia and tarsi covered with numerous small bluntly rounded spatulas and fine hairs (Figs 9–10, 27–29); the first tree teeth of kinetodontium and incisor of right mandible of relatively equal size (Fig. 15); third segment of labial palp wide, its apex truncated; 1.3 times wider than the second segment; second segment of labial palp with rounded apicomedial projection (Figs. 25, 31–32); posterior margins of tergum VI with regular row of quadrangular teeth of various sizes (Fig. 26); paraproct without notched spatulas, with fine hairs located disorderly and 3–5 rounded spatulas, scattered in middle area. Imago: hind wings with two simple longitudinal veins (Fig. 3); unistiliger with parallel margins, slightly elongated, with a distinct small projection at the apex of the inner edge (Fig. 4); segment III of forceps small, well defined, strongly expanded to the apex, its length only slightly exceeds its maximum width.



FIGURES 27–32. *Baetis majus* Tiunova **sp. nov.**, larvae, paratypes: 27, 29, inner margin of fore tibia; 28, inner margin of fore tarsus; 30, tarsal claw; 31, paraproct; 32, spatulas on paraproct. Scale bar: 27–31, 0.01 mm; 32, 0.001 mm.



FIGURES 33–38. *Baetis majus* Tiunova **sp. nov.**, larvae, gills shape, dorsal view, roman numbers belong to the respective gill pairs.

Distribution. Russian Far East. *Baetis majus* Tiunova **sp. nov**. is known from its type locality only (Fig. 42). The larvae of the new species were found in the lower part of the river on swift riffles with cobble and gravel substrate. No larvae were found at other sites upstream of the river. Water temperature was 12–15°C, depth 20–60 cm. According to our data, the adult emergence period is May.

Etymology. The species name is derived from the Latin word *majus*, which means May. All the examined material was collected in May.

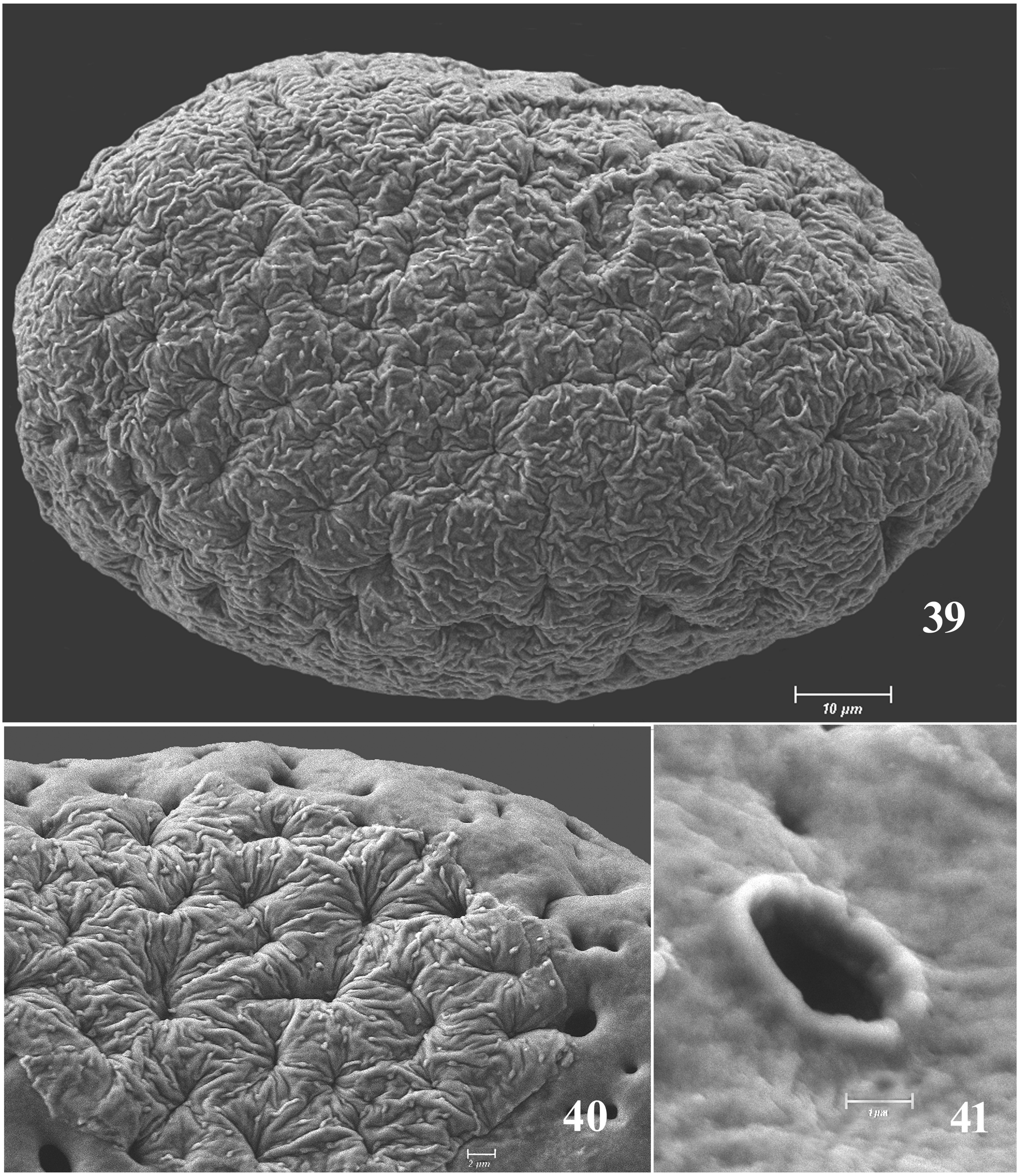
Results of DNA barcoding. We obtained partial COI sequences (657 bp) of one specimen (BM175) of *Baetis majus* Tiunova **sp. nov.,** twelve specimens (BP30, BP34, BP35, BP178, BP179, BP190, BP358, BP387, BP388, BP672, BP674 and BP794) of *B. pseudothermicus*; four specimens (BU29, BU177, BU542 and BU552) of *B. ursinus*; and two specimens (BU543 and BU556) of *B. ussuricus.* The mean nucleotide base compositions of *Baetis majus* Tiunova **sp. nov.** were A 23.6%, C 18.4%, G 21.8% and T 36.2% across all sites for all sequences.

We used nucleotide BLAST algorithm in NCBI GenBank (nucleotide collection database) and animal identification in BOLD systems to find the nearest neighbours to *Baetis majus* Tiunova **sp. nov**. A search in GenBank showed that the nearest neighbours have a similarity less than 85% and relate to unidentified *Baetis* species (KP970694, KP970695) and several species of genus *Acentrella*. Highly similar (98.75–100%) sequences of unidentified *Baetis* species from China (Beijing and Hebei provinces) were found in the BOLD system, but these data was unavailable and set to private status. Probably, *Baetis majus* Tiunova **sp. nov**. inhabits also China (in provinces of Beijing and Hebei).

Bayesian phylogeny revealed two well-supported sister clades, one of which includes *B. bundyiae* Lehmkuhl*, B. hudsonicus* Ide*, B. macani* Kimmins, *B. subalpinus* Bengtsson, *B brunneicolor* McDunnough, *B. liebenauae*

Keffermüller, *B. vernus*, *B. ursinus*, *B. maculosus, B. majus* Tiunova **sp. nov.,** and *B. michaelohubbardi* Selvakumar *et al*.; the second clade includes the remaining species. *B. majus* Tiunova **sp. nov.** was the earliest branching lineage in the clade and includes *B. maculosus* and *B. ursinus;* however, support for this clade was low (Bayesian posterior probability, PP = 0.52).

Morphological and molecular data were used to construct a Bayesian tree, which revealed a polytomy node including *B. majus* Tiunova **sp. nov**. as the first lineage; *B. vardarensis* + *B. lutheri* + *B. pentaphyllus* (*Baetis lutheri* species-group) as the second lineage; and *B. braaschi* + *B. ilex* + *B.* cf. *gadeai* + *B. rhodani* + *B. baksan* (*Baetis rhodani* species-group) as the third lineage. Thus, the species-group to which *B. majus* Tiunova **sp. nov.** belongs remains unidentified (Figs 43, 44).



FIGURES 39–41. *Baetis majus* Tiunova **sp. nov.**, egg: 39, general view; 40, structure of chorion; 41, micropyle.

FIGURE 42. Locality of *Baetis majus* Tiunova **sp. nov.,** Shkotovka River (Primorskiy Kray).

Discussion

The proposed assignment of *Baetis majus* Tiunova **sp. nov**. to the subgenus *Baetis* was based on a combination of the following characters: spatulas absent on scape, pedicel and paraproct (this excluded *B*. *majus* Tiunova **sp. nov**. from the subgenus *Rhodobaetis*) (Müller-Liebenau 1969; Jacob 2003; Godunko *et al*. 2004 a, b); apex of labial palpus not pointed; and paraproct without a patch on the notched spatulas (this excluded *B*. *majus* Tiunova **sp. nov**. from the subgenus *Tenuibaetis*) (Kang *et al.* 1994; Kluge & Novikova 2011; Fujitani *et al*. 2011).

Among more than 80 species of the subgenera *Baetis* and *Tenuibaetis* in Eastern and Western Palaearctic, Nearctic and Oriental regions (excluding representatives of the subgenus *Rhodobaetis*), only larvae of *B. maculosus* (from China), *B. acceptus* Müller–Liebenau & Hubbard, *B. conservatus* Müller–Liebenau (from Sri-Lanka) and *B. mogui* Wiersema *et al*. (from south-central Utah) have six pairs of gills on abdominal segments II–VII, which is similar to those found on *Baetis majus* Tiunova **sp. nov**.

Distinctive features that can distinguish the larva of *Baetis majus* Tiunova **sp. nov**. from other representatives of *Baetis* that have six pairs of gills are as follows: (1) The first three teeth of kinetodontium and incisors of the right mandible are of relatively equal size (Fig. 15). In *B. maculosus* and *B. acceptus,* all teeth differ in size. In *B. maculosus*, the second tooth of kinetodontium is the biggest (Tong *et al.* 2014: fig. 4), whereas in *B. acceptus*, the first tooth is the largest (Müller-Liebenau & Hubbard 1985: fig. 2e), and in *B. moque* the first incisor tooth is broad, approximately as wide as teeth 2 and 3 combined (Wiersema *et al*. 2004: figs 2–3). (2) The third segment of the labial palp is wide; its apex is truncated (Fig. 19). In *B. acceptus* and *B. conservatus*, the third segment is relatively wide; its apex is rounded (Müller-Liebenau & Hubbard 1985: figs 2b and 3a). In *B. mogui*, the third segment is nearly symmetrical or is slightly rounded or elongated (Wiersema *et al*. 2004: fig. 4). (3) The posterior margin of tergum VI has a regular row of quadrangular teeth of various sizes (Fig. 26). In *B. acceptus*, the posterior margin

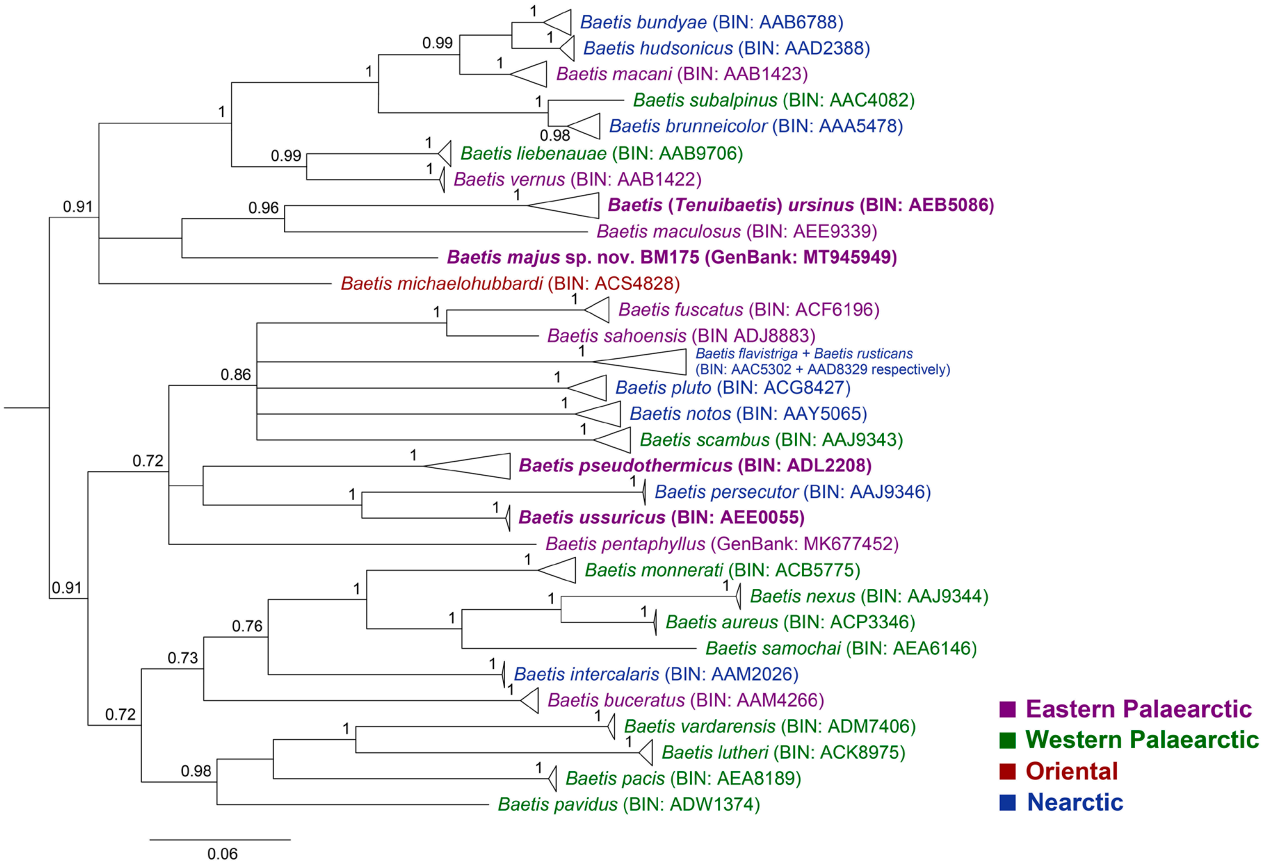


FIGURE 43. Bayesian tree based on mitochondrion COI gene for subgenera *Baetis* and *Tenuibaetis* form Eastern and Western Palearctic, Nearctic and Oriental regions. Specimens obtained in this study are in bold. Bayesian posterior probabilities (PP) above 0.7 are given above tree nodes.

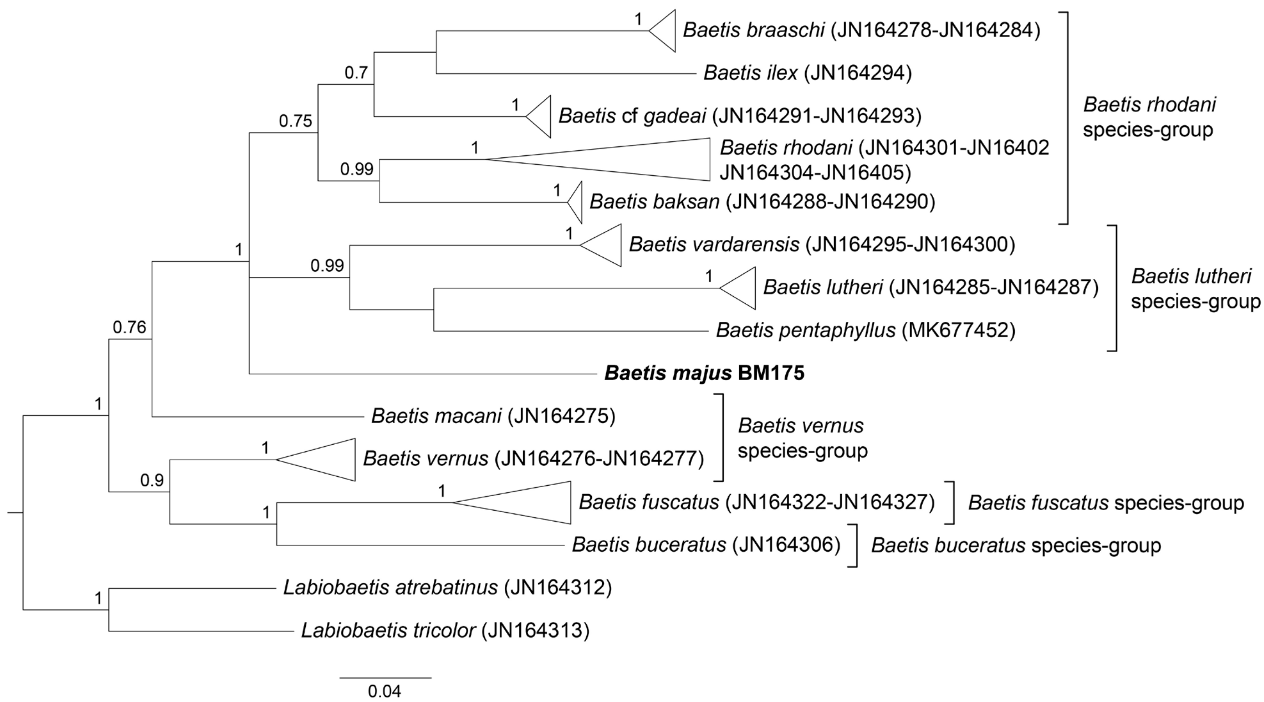


FIGURE 44. Bayesian tree of the genus *Baetis* inferred from the cytochrome c oxidase I (COI) nucleotide sequence data (466 bp) and 47 morphological characters. Two species of *Labiobaetis* were used as outgroup. All data including species-groups are taken from Sroka (2012). Specimen obtained in this study is in bold. Bayesian posterior probabilities (PP) above 0.7 are given above tree nodes.

has a regular row of pointed and elongated teeth (Müller-Liebenau & Hubbard 1985: fig. 19). In *B. conservatus*, teeth on the posterior margin are pointed and short, without any triangulation (Müller-Liebenau & Hubbard 1985: fig. 20). (4) In body coloration, only terga IX and X are pale without maculation (Figs 5, 7). In *B. maculosus*, body colour is contrasted; terga IV–VI and IX–X are completely pale with small dark spots (Tong *et al.* 2014: fig. 10). In *B. acceptus* and *B. conservatus*, all terga are dark (Müller-Liebenau & Hubbard 1985: figs 11–12).

Male imago of *Baetis majus* Tiunova **sp. nov**. has a hind wing with two simple longitudinal veins, which also appears in representatives of the subgenus *Baetis*: *B. maculosus, B. ursinus* and *B. rutilocylindratus* Wang *et al*., and in representatives of the subgenus *Tenuibaetis: B. parvipterus* Fujitani *et al*., *B. flexifemora* Gose, and *B*. *pseudofrequentus* Fujitani *et al*. The male imago of *Baetis majus* Tiunova **sp. nov**. can be differentiated by the following features: (1) Body size: 4.7–5.2 mm (by contrast, *B. maculosus* and *B*. *pseudofrequentus* are 3.0 mm and 3.4–4.2 mm, respectively); (2) unistiliger shape, with parallel margins and slightly elongated, with a distinct small projection at the apex of the inner edge (Fig. 4). In *B. rutilocylindratus,* the unistiliger is broad with a well-defined projection (Wang *et al*. 2011: fig. 4C); in *B. maculosus,* the unistiliger is of trapezoidal shape without projection (Tong *et al* 2014: Fig. 15) ; in *B. parvipterus, B. flexifemora*, and *B*. *pseudofrequentus,* the unistiliger is a cylindrical shape without projection (Fujitani *et al*. 2011); in *B. ursinus,* the unistiliger is broad with a bevelled inner-apical margin (Kluge 1983: fig. 46); and (3) the third segment of the forceps expands strongly to the apex (Fig. 4); in other species, listed above, the third segment has an oval or ellipse shape.

Acknowledgement

The scanning electron micrographs were prepared with the help of Vitaliy Kazarkin (Federal Scientific Center of the East Asia Terrestrial Biodiversity Far Eastern Branch, Russian Academy of Sciences, Vladivostok). Thanks are due to Lili Wang and Yanping Luo (South China Agricultural University) for providing COI sequence of *Baetis maculosus*.

References

Felsenstein, J. (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution*, 17, 368–376.
https://doi.org/10.1007/BF01734359

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.

Fujitani, T., Hirowatari, T. & Tanida, K. (2003) Genera and species of Baetidae in Japan: *Nigrobaetis*, *Alainites*, *Labiobaetis*, and *Tenuibaetis* n. stat. (Ephemeroptera). *Limnology*, 4, 121–129.
https://doi.org/10.1007/s10201-003-0105-2

Fujitani, T., Kobayashi, N., Hirowatari, T. & Tanida, K. (2011) Three species of a genus *Tenuibaetis* (Ephemeroptera: Baetidae) from Japan, with description of a new species. *Limnology*, 12, 213–223.
https://doi.org/10.1007/s10201-010-0342-0

Godunko, R.J., Prokopov, G.A. & Soldán, T. (2004a) Mayflies of the Crimean Peninsula III. The description of *Baetis milani* sp. n. with notes on taxonomy of the subgenus *Rhodobaetis* Jacob, 2003 (Ephemeroptera: Baetidae). *Acta Zoologica Cracoviensia*, 47, 231–248.
https://doi.org/10.3409/173491504783995799

Godunko, R.J., Prokopov, G.A., Kluge, N.J. & Novikova, E.A. (2004) Mayflies of the Crimean Peninsula. II. *Baetis braaschi* Zimmermann, 1980 (= *B. stipposus* Kluge, 1982 syn. n.) (Ephemeroptera: Baetidae). *Acta zoologica cracoviensia*, 47, 155–166.
https://doi.org/10.3409/173491504783995807

Hasegawa, M., Kishino, H. & Yano, Ta. (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution,* 22, 160–174.
https://doi.org/10.1007/BF02101694

Hebert, P., Cywinska, A., Ball, S. & DeWaard, J. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London, Series B-Biological Sciences*, 270, 313–321.
https://doi.org/10.1098/rspb.2002.2218

Hebert, P.D.N., Ratnasingham, S., Zakharov, E.V., Telfer, A.C., Levesque-Beaudin, V., Milton, M.A., Pedersen, S., Jannetta, P. & deWaard, J.R. (2016) Counting animal species with DNA barcodes: Canadian insects. *Philosophical Transactions of the*

Royal Society B, Biological Sciences, 371, 1–10.
https://doi.org/10.1098/rstb.2015.0333

Jacob, U. (2003) *Baetis* Leach 1815, sensu stricto or sensu lato. Ein Beitrag zum Gattungskonzept auf der Grundlage von Artengruppen mit Bestimmungsschlüsseln. *Lauterbornia*, 47, 59–129.

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(2), 9–44.

Kluge, N. Yu. (1997) Order Ephemeroptera— mayflies. *In*: Tsalolikhin, S.J. (Ed.), *Key to Freshwater Invertebrates of Russian and Adjacent Lands, St. Petersburg*, 1997, pp. 175–220 + 304–329. [in Russian]

Kaltenbach, T. & Gattolliat, J.-L. (2019) A new species of *Tenuibaetis* Kang & Yang, 1994 from Indonesia (Ephemeroptera, Baetidae). *ZooKeys*, 820, 13–23.
https://doi.org/10.3897/zookeys.820.31487

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. (2020) Ephemeroptera of the world. http://insecta.bio.spbu.ru/z/Eph-spp/index.htm (accessed 3 May 2020)

Kluge, N.J. & Novikova, E.A. (2011) Systematics of the mayfly taxon *Acentrella* (Ephemeroptera, Baetidae), with description of new Asian and African species. *Russian Entomological Journal*, 20 (1), 1–56.
https://doi.org/10.15298/rusentj.20.1.01

Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular evolutionary genetics analysis, ver. 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 870–874.
https://doi.org/10.1093/molbev/msw054

Lanfear, R., Calcott, B., Ho, S.Y. & Guindon, S. (2012) Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29 (6), 1695–1701.
https://doi.org/10.1093/molbev/mss020

Leach, W.E. (1815) Entomology. *Brewster's Edinburgh Encyclopaedia*, 9, 57–172.

Martynov, A. & Godunko, R. (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

Morinière, J., Hendrich, L., Balke, M.T., Beermann, A.J., Koenig, T., Hess, M., Koch, S., Müller, R., Leese, F., Hebert, P.D., Hausmann, A., Schubart, C.D. & Haszprunar, G. (2017) A DNA barcode library for Germany's mayflies, stoneflies and caddisflies (Ephemeroptera, Plecoptera and Trichoptera). *Molecular Ecology Resources*, 17 (6), 1293–1307.
https://doi.org/10.1111/1755-0998.12683

Müller-Liebenau, I. (1969) Revision der europäischen Arten der Gattung Baetis Leach, 1815 (Insecta, Ephemeroptera). *Gewässer und Abwässer*, 28/29, 1–214.

Müller-Liebenau, I. & Hubbard, M.D. (1985) Baetidae from Sri Lanka with some general remarks on the Baetidae of the Oriental Region (Insecta: Ephemeroptera). *Florida Entomologist*, 68 (4), 537–561.
https://doi.org/10.2307/3494855

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67 (5), 901–904.
https://doi.org/10.1093/sysbio/syy032

Ronquist, F., Teslenko, M., Mark, P.V.D., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*, 61, 539–542.
https://doi.org/10.1093/sysbio/sys029

Sroka, P. (2012) Systematics and phylogeny of the West Palaearctic representatives of subfamily Baetinae (Insecta: Ephemeroptera): combined analysis of mitochondrial DNA sequences and morphology. *Aquatic Insects*, 34 (1), 23–53.
https://doi.org/10.1080/01650424.2012.718081

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.
https://doi.org/10.1093/bioinformatics/btl446

Stauffer, N.J, O'Grady, P.M. & Resh, V.H. (2017) Temporal patterns of genetic diversity in *Baetis tricaudatus* (Ephemeroptera: Baetidae) from the Russian River, Northern California. *Freshwater Science*, 36 (2), 351–363.
https://doi.org/10.1086/691973

Tavaré, S. (1986) Some Probabilistic and Statistical Problems in the Analysis of DNA Sequences (PDF). Lectures on Mathematics in the Life Sciences. *American Mathematical Society*, 17, 57–86.

Telfer, A., Young, M., Quinn, J., Perez, K., Sobel, C., Sones, J., Levesque-Beaudin, V., Derbyshire, R., Fernandez-Triana, J., Rougerie, R., Thevanayagam, A., Boskovic, A., Borisenko, A., Cadel, A., Brown, A., Anais, P., Castillo, A., Nicolai, A., Mockford, G. & Dewaard, J. (2015) Biodiversity inventories in high gear: DNA barcoding facilitates a rapid biotic survey of a temperate nature reserve. *Biodiversity Data Journal*, 3, 6313.
https://doi.org/10.3897/BDJ.3.e6313

Tiunova, T.M. & Semenchenko, A.A. (2019) *Baetis pentaphyllus* sp. nov., a new species of mayfly (Ephemeroptera: Baetidae) from the Russian Far East. *Zootaxa*, 4679 (2), 341–352.

https://doi.org/10.11646/zootaxa.4679.2.7
Tiunova, T.M. & Semenchenko, A.A. (2020) *Baetis* (*Rhodobaetis*) *molecularis* sp. nov., a new mayfly species (Ephemeroptera: Baetidae) from the Russian Far East. *Zootaxa*, 4820 (2), 287–304.
https://doi.org/10.11646/zootaxa.4820.2.4
Tong, X.-L., Dudgeon, D. & Shi, W. (2014) A new species of the genus *Baetis* from China (Ephemeroptera: Baetidae). *Entomological News*, 123 (5), 333–338.
https://doi.org/10.3157/021.123.0503
Waltz, R.D. & McCafferty, W.P. (1997) New generic synonymies in Baetidae (Ephemeroptera). *Entomological News*, 108 (2), 134–140.
Wang, Y.Y., Qin, J.Z., Chen, P. & Zhou, C.F. (2011) A new species of *Baetis* from China (Ephemeroptera: Baetidae). *Oriental Insects*, 45, 72–79.
https://doi.org/10.1080/00305316.2011.579406
Wiersema, N.A., Nelson, C.R. & Kuehnl, K.F. (2004) A new small minnow mayfly (Ephemeroptera: Baetidae) from Utah, U.S.A. *Entomological News*, 115 (3), 139–145.