Zootaxa 4679 (2): 341–352
https://www.mapress.com/j/zt/

Article

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

https://doi.org/10.11646/zootaxa.4679.2.7
http://zoobank.org/urn:lsid:zoobank.org:pub:44D197DB-E48A-4FCC-9B58-0B397D03880D

Baetis pentaphyllus sp. nov., a new species of mayfly (Ephemeroptera: Baetidae) from the Russian Far East

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Abstract

A new species, *Baetis pentaphyllus* **sp. nov**., is described on the basis of larvae from the Far East of Russia (type locality Bolshoi Garmakan River). Larvae of *Baetis pentaphyllus* **sp. nov**. may be distinguished from other *Baetis* species by the presence of only five pairs of tergalii on segments III–VII. The mitochondrial COI sequence obtained from the described species was compared with the data present in GeneBank and BOLD. The DNA barcodes allowed discrimination of *B. pentaphyllus* **sp. nov**. from other species of *Baetis* with available sequence data. The average interspecific K2P distances were 10–15%, which are values well above those associated with intraspecific variation. COI sequences as well as 36 morphological larval characters were analysed using Bayesian inference to relate the described species to the recognized species-groups of the *Baetis* genus. *B. pentaphyllus* **sp. nov** formed a sister clade to *B. vardarensis* + *B. lutheri* which belong to the *Baetis lutheri* species-group.

Key words: Mayflies, larvae, taxonomy, DNA barcoding, *lutheri*-group, Asia

Introduction

The genus *Baetis* (established by Leach in 1815) is currently divided into a number of genera and subgenera. In the Far East of Russia, there are currently 14 species of mayflies belonging to the following five genera: *Baetis* Leach, 1815, *Rhodobaetis* Jacob, 2003, *Nigrobaetis* Novikova & Kluge, 1987, *Labiobaetis* Novikova & Kluge, 1987 and *Tenuibaetis* Kang & Yang, 1994 (Waltz & McCafferty 1997; Fujitani *et al*. 2003; Sroka 2012; Martynov & Godunko 2017). For some authors (Novikova & Kluge 1987; Bauernfeind & Soldán 2012; Kluge & Novikova 2014), on the other hand, these taxa receive subgeneric rank. The genus *Baetis* s. l. includes East-Asian continental and East-Asian Island *B. feles* Kluge, 1980 and *B. thermicus* Ueno, 1931, East-Palaearctic *B. pseudothermicus* Kluge, 1983 and *B. ussuricus* Kluge, 1983, Transpalaearctic *B. fuscatus* (Linnaeus, 1761) and *B. vernus* Curtis, 1834, Circum-Boreal *B. macani* Kimmins, 1957, and Amphipacific *B. bicaudatus* Dodds, 1923. The genus *Nigrobaetis* includes East-Palaearctic species *N. acinaciger* Kluge, 1983 and *N. bacillus* Kluge, 1983. The genus *Labiobaetis* is represented by two Transpalaearctic species *L. atrebatinus* Eaton, 1870 and *L. tricolor* Tshernova, 1928. Additional genera include one East-Palaearctic species *Rhodobaetis silvaticus* Kluge, 1983 and *Tenuibaetis ursinus* Kazlauskas, 1963 (Kluge 1997; Tiunova 2007, 2009). Representatives of all of these genera have simple ovoid tergalii on segments I–VII or II–VII, but recently we collected *Baetis* larvae with five pairs of tergalii located on segments III–VII.

The DNA barcode corresponding to the 650-bp fragment of the mitochondrial gene cytochrome c oxidase I (COI) has proven useful in the subfamily Baetinae (Telfer *et al*., 2015; Hebert *et al.* 2016; Morinière *et al*. 2017; Stauffer-Olsen *et al*. 2017). Despite being of poor utility in discerning deeper phylogenetic relationships, DNA barcodes have nevertheless been shown to be extremely useful for rapid and accurate species delimitation in invertebrates (Wang *et al.* 2018). In recent times, multi-disciplinary approaches to species delimitation, involving both molecular and morphological methods, have gained popularity. This approach provides an acceptable phylogenetic signal to delimit the genus *Baetis*, containing more than 160 described species worldwide to species-groups (Sroka

Accepted by F. Salles: 28 Aug. 2019; published: 2 Oct. 2019

2012). The purpose of this article is to describe the new *Baetis pentaphyllus* **sp. nov**. based on larvae found in two regions of the Far East of Russia and confirm interspecific distances from the nearest species as well as to classify this species to a known *Baetis* species-group.

Material and methods

Total genomic DNA was extracted at the Far Eastern Federal University (Vladivostok) using the Invitrogen PureLink Genomic DNA Mini Kit (Invitrogen corp, Carlsbad, CA 2007) according to the manufacturer's protocol. A 650-bp fragment of the mitochondrial (mtDNA) COI gene was amplified for the studied specimen using the LCO1490 (5'-GGTCAACAAATCATAAAGAT ATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') primers (Folmer *et al*. 1994). Each PCR reaction mixture contained 1 µl of DNA template, 5 µl of Go Taq Green Master Mix (Promega corp, Madison, WI, USA), 0.5 µl of each primer (10 pmol/µl) and molecular grade water for a total volume of 10 µl. Thermal cycling was performed with an initial denaturation for 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at the annealing temperature of 50 °C, 60 s at 72 °C with a final extension of 5 min at 72 °C. All PCR products were verified by electrophoresis on 1.5% TBE agarose gels. Amplicons were purified by incubating 8 µl of PCR products with 0.3 µl of Exonuclease I (20 U/µl) and 0.8 µl of Alkaline Phosphatase (1 U/µl) for 30 min at 37 °C followed by 15 min at 80 °C. Each fragment was purified using ethanol precipitation, air-dried and cycle sequenced on an ABI 3130x (Applied Biosystems) automated sequencer using BigDye terminator v3.1 cycle kit methods. Forward and reverse sequences were aligned and manually edited in MEGA 7 (Kumar *et al*. 2016) and FinchTV and then checked for the presence of stop codons. DNA sequences were uploaded to the GenBank by accession number MK677452. The interspecific distances were estimated using Kimura 2 parameter (K2P) model in MEGA 7.

Bayesian Inference was performed using MrBayes 3.2.7. (Ronquist & Huelsenbeck 2003) with parameters for COI data Nst=6, rates=gamma. In addition to the DNA Barcoding data, a matrix of 36 morphological larval characters was compiled according to Sroka, 2012. The missing data of 11 adult characters were replaced by «?». The final matrix of morphological characters for the larvae of *Baetis pentaphyllus* **sp. nov**. was 0000001110011200000 00001110110101110???????????, and the list of characters can be found in Sroka (2012). The Metropolis-coupled Markov chain Monte Carlo analysis was run for 3 million generations and sampled every 100 generations. A burn-in of 750 000 generations (or 25% of the sampled trees) was used. Moreover, trace files were visually inspected in Tracer 1.7 (Rambaut *et al*. 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.

Baetis pentaphyllus sp. nov.

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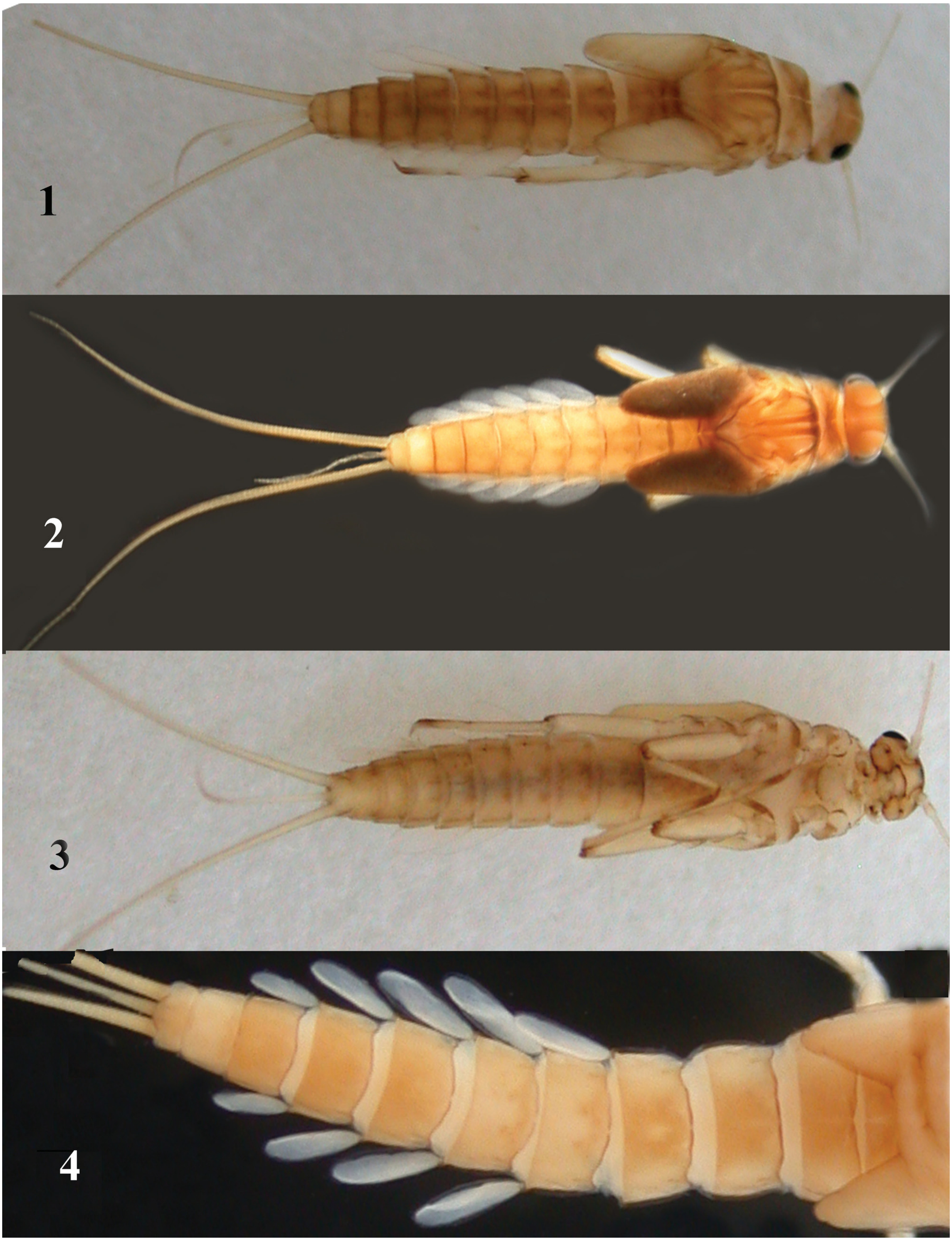
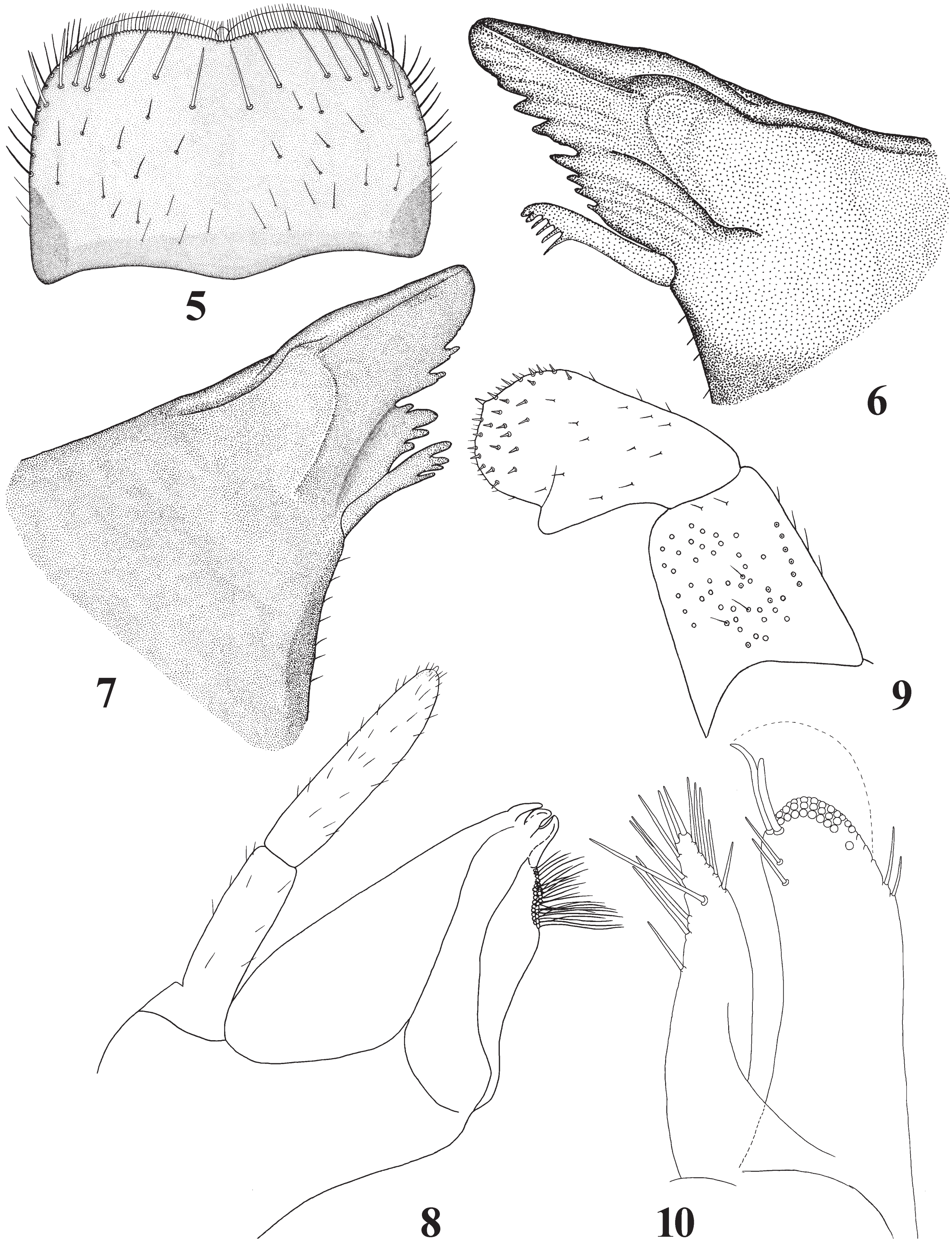
(Figs 1–26)

Material. Holotype larva. **RUSSIAN FEDERATION:** AMURSKAYA OBLAST'**:** Zeyskii Reserve, Zeya Reservoir Basin, Bolshoi Garmakan River, about 300 m above mouth, N 53°53.148 E 127°11.626, 02.VII.2015, T. Tiunova. **Paratypes**: collected the same data and place as holotype: 5 larvae, 1 larva (**BAE-BGAR1_110**); same locality, 08.VII.2015, 4 larvae, T. Tiunova; Zeya Reservoir Basin: Malyi Garmakan River, about 500 m above mouth, N 53°52.295 E 127°10.459, 06.VII.2015, 3 larvae, T. Tiunova; Shirokovka River, about 700 m above mouth, 07.VII.2014, 5 larvae, T. Tiunova. CHUKOTKA AUTONOMOUS DISTRICT, Kolyma River Basin, Malyi Keperweim River, 2 mature larvae, N 68°23.151; E 166°80.348, 03.VIII.2016, A. Semenchenko.

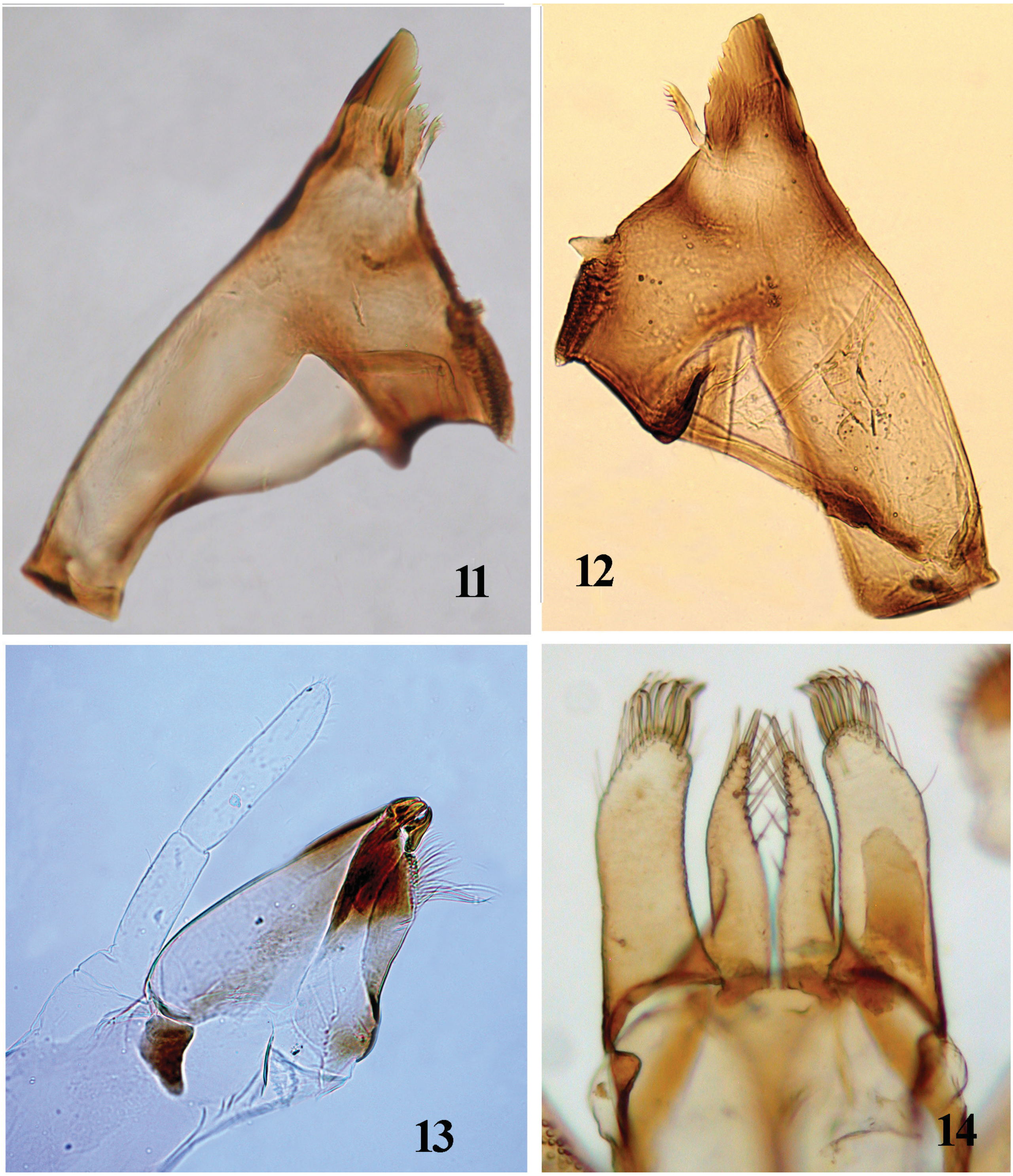
Description. ***Larvae*** (in alcohol). Length (mm): body 4.6–5.7; cerci 2.5–3.1. General body colour brown (Figs 1–4)**. Head:** brown. Antennae brownish, slightly longer than ½ of body length. Scape and pedicel with hair-like setae only. Labrum distinctly wider than long (the width/length ratio 1.69–1.70); dorsal surface with 1 + 6–7 long submarginal setae, arranged in uneven row and row long pointed setae laterally on both margins (Fig. 5). Canines of left mandible with eight teeth divided into two groups (Figs 6, 12). Incisor (outer group) with four teeth; outermost



FIGURES 1–4. *Baetis pentaphyllus* **sp. nov.,** larvae, dorsal view: 1, (material from type locality); 2, larva from Malyi Keperweim River (Chukotka); 3, ventral view; 4, location of tergalii on terga III-VII, dorsal view.

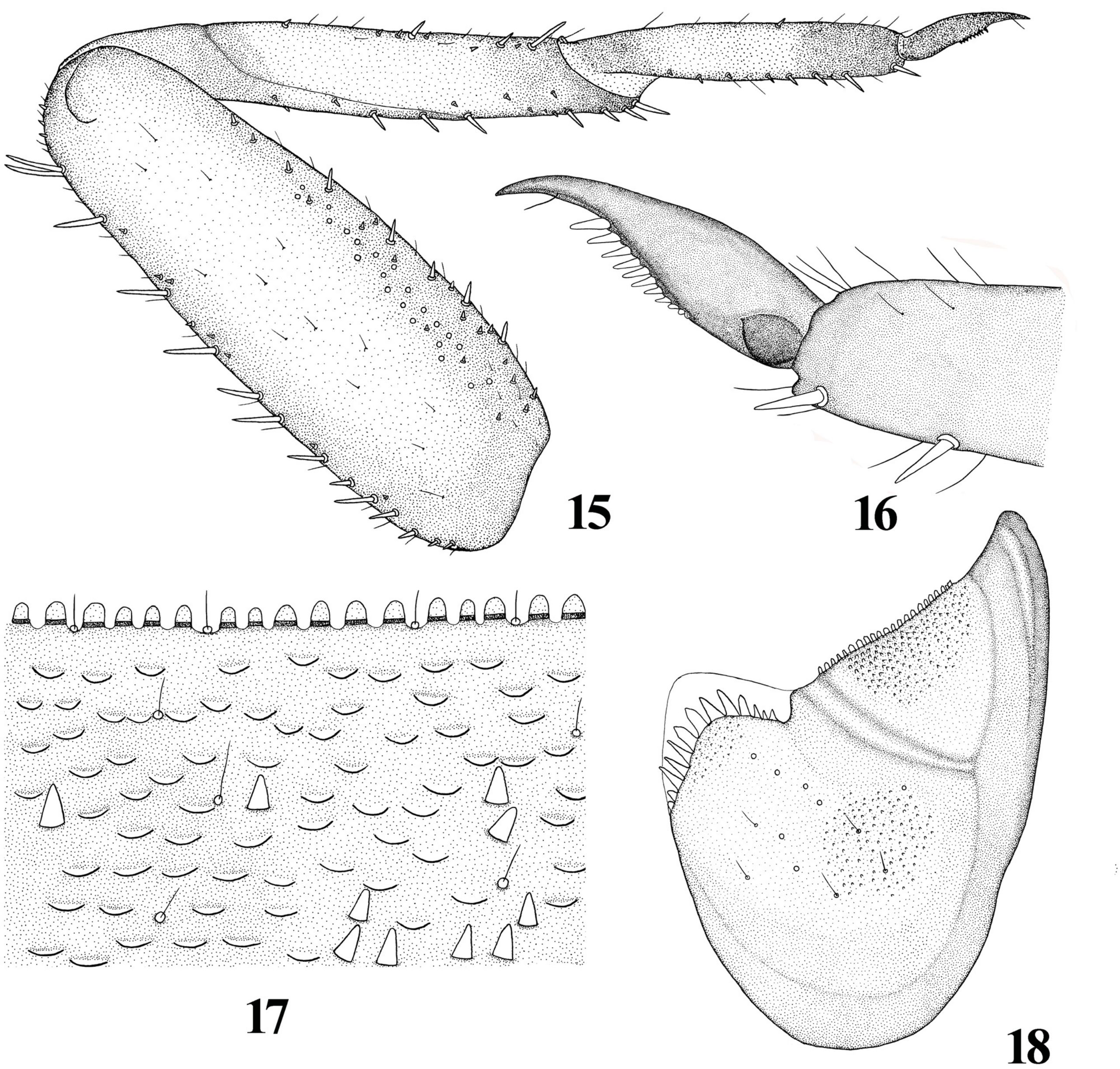


FIGURES 5–10. *Baetis pentaphyllus* **sp. nov.,** larvae, details of mouthparts: 5, labrum, dorsal view; 6, canines and prostheca of right mandible, dorsal view; 7, canines and prostheca of left mandible, dorsal view; 8, partial view of dorsal surface of maxilla; 9, labial palp, ventral view; 10, glossa and paraglossa, ventral view.



FIGURES 11–14. *Baetis pentaphyllus* **sp. nov.,** larvae: 11, right mandible, dorsal view; 12, left mandible, dorsal view; 13, partial view of dorsal surface of maxilla; 14, glossae and paraglossae, ventral view.

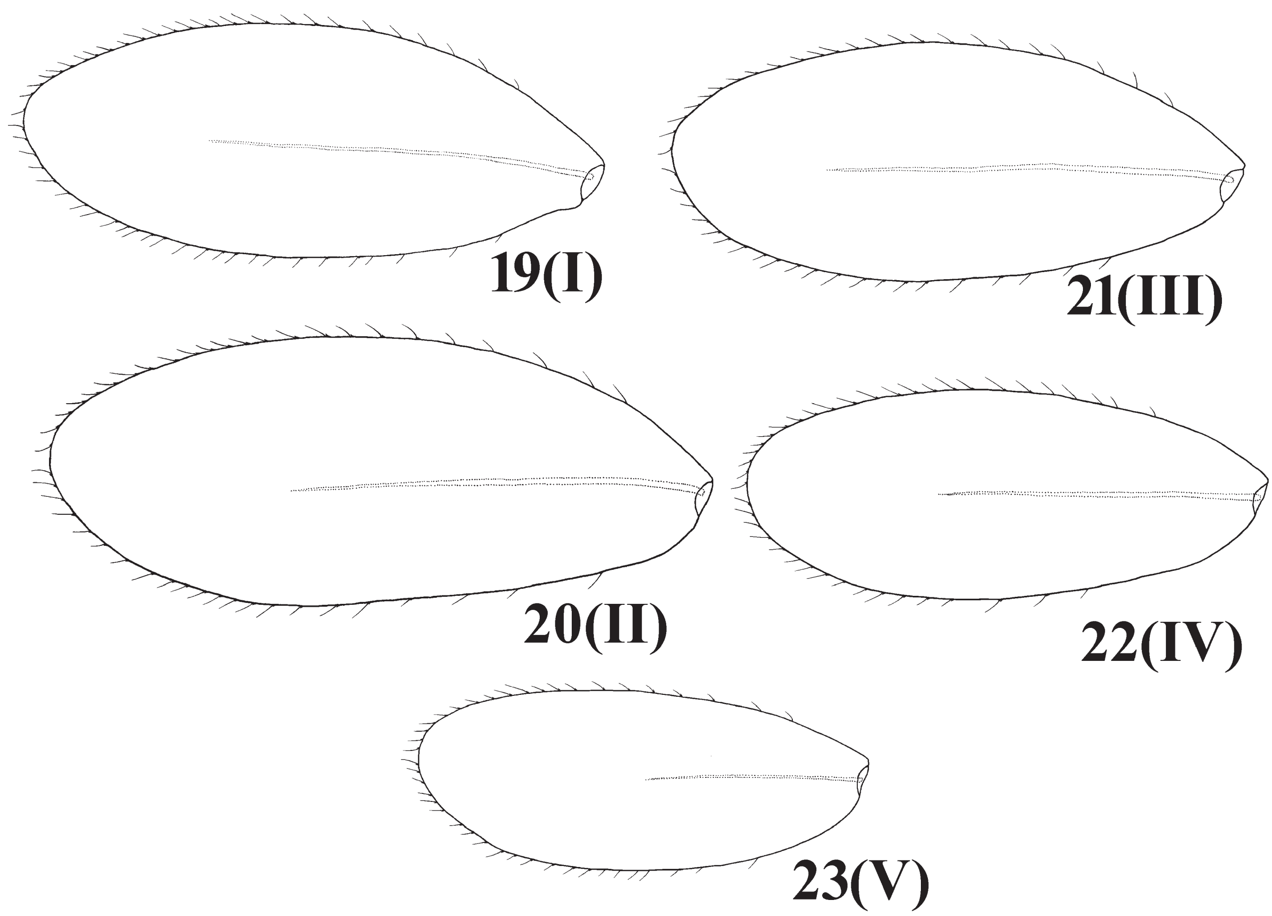
tooth broadened and rounded apically; kinetodontium (inner group) with four small teeth. Both groups separated by a shallow incision. Left prostheca toothbrush-like (Figs 6, 12). The right mandible canines are also separated into two groups of teeth (Figs 7, 11). Incisor with three teeth, first tooth widened and rounded apically; kinetodontium with four teeth, from which second largest. Between the kinetodontium and incisor there is a groove. Right prostheca with four non-pointed teeth (Figs 7, 11). Maxillary palp two-segmented, individual segments approximately equal in length; surface of both segments covered with hair-like seta, which occur more densely on distal part of second segment (Fig. 13). Second segment of labial palpus with rounded apicomedial projection, its width 1.1 times wider than the base of



FIGURES 15-18. *Baetis pentaphyllus* **sp. nov.,** larvae, dorsal view: 15, foreleg; 16, tarsal claw; 17, posterior margin of abdominal tergum VI; 18, paraproct.

third segment; third segment almost symmetrical rounded, relatively wide (Fig. 9); dorsal surface with a row of 4–5 long pointed setae on second segment and long pointed stout setae in apical margin of third segment; ventral surface of third segment covered numerous stout setae accompanied by hair-like seta (Fig. 9). Labium with paraglossae concave in middle, nearly two times wider than glossae; with 7–8 pairs of long stout bristles form three irregular rows on tip and long stout setae near apex; 5–6 long bristles located along outer margin and two stout setae near apex of inner margin (Figs 10, 14). Glossae relatively slender, with row 6–7 of long setae on inner margin and 5–6 bristles on outer margin and by one long stout seta located near apex (Figs 10, 14). **Thorax**: brown with whitish diffuse spots. Anterior margins of pro- and mesonotum darker (Figs 1–2). Legs whitish, joints of leg segments brown (Fig. 15). Femora pale, at edges darker; outer margin with row of long and pointed bristles and short setae located irregular; inner margin with villopore and small pointed setae located irregularly (Fig. 15). Tibia and tarsus brown in apical part. Stout setae arranged in regular row along inner margin of tibia and tarsi and setae of different sizes located along outer margin. Claws brown, with row of 10–11 teeth and a pair of subapical setae (Fig. 16). **Abdomen**: terga

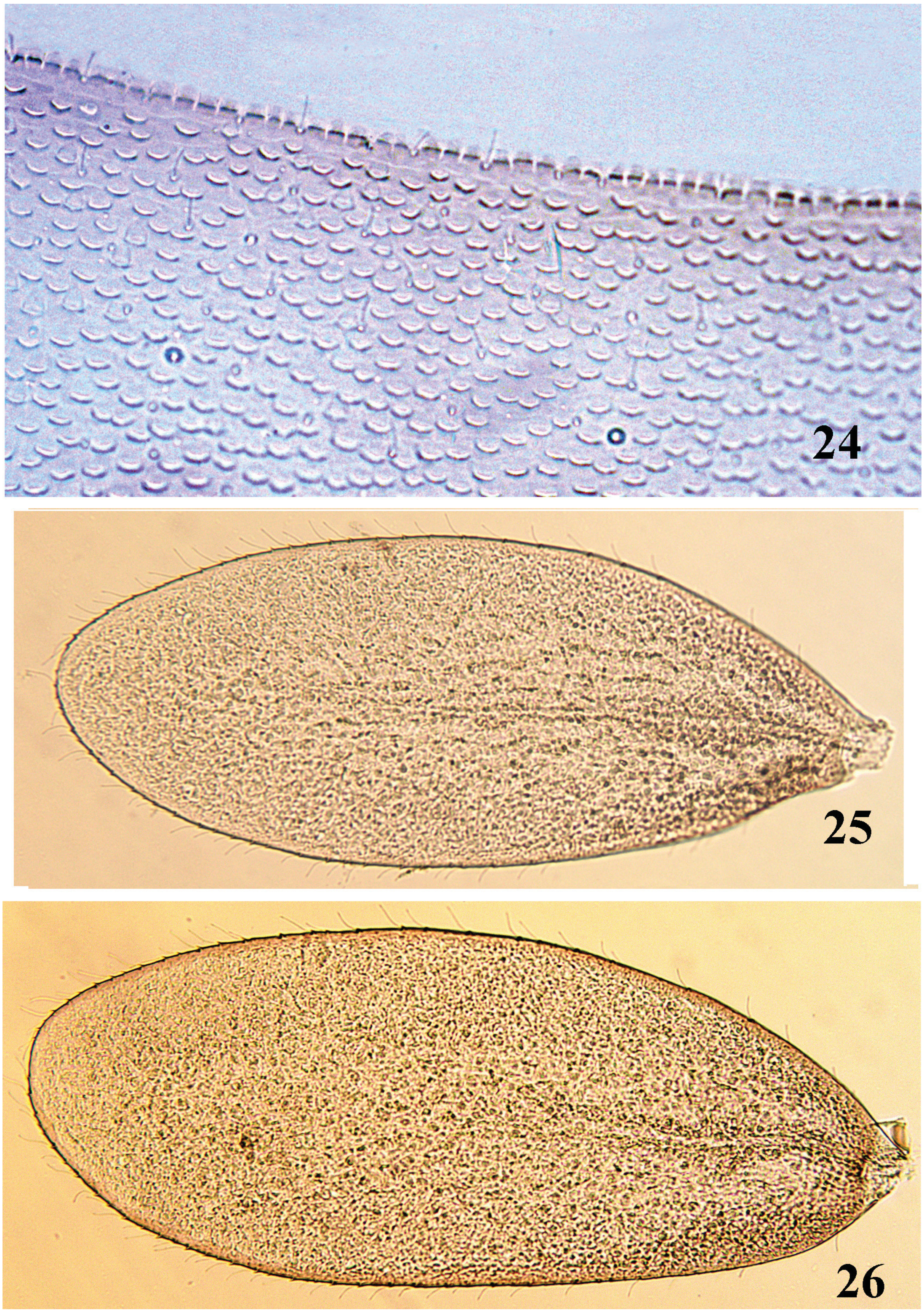
I–VIII brown, lateral area lighter; a pair of dark brown spots located near anterior margin; lateral and posterior margins dark brown (Fig. 1); terga I–VI with median light longitudinal stripe; terga IX–X brown, lighter than other terga; posterior margin of terga with broad, rounded apically teeth (Figs 17, 24). Sterna generally light brown with brown diffused spots near anterior margins (Fig. 3.) Paraproct serrated on posterior part of inner margin (10–13 large pointed teeth of different size) and outer part of posterior part (approximately 19–22 teeth diminishing proximally) (Fig. 18). Five pairs of elongated, oval-shaped tergalii located on III–VII tergites (Fig. 4), approximately 2.5 times longer than broad, rounded (Figs. 19–23), and 1.8–2.0 times longer than corresponding segment (Fig. 4); all tergalii white, tracheation poorly visible; margins hairy; hairs inserted in small teeth bases. Tergalii I and III almost equal length (Figs. 4, 19, 20), tergalii IV slightly shorter than III (Figs. 4, 22, 23). Tergalius II is the largest, 1.2 times longer than the first and 1.6 times longer than V (Figs. 4, 26). Caudal filaments brownish (Figs. 1, 3). Cerci three times longer than paracercus (Fig. 2).



FIGURES 19–23. *Baetis pentaphyllus* **sp. nov.,** larvae, tergalii shape, dorsal view, roman numbers belong to the respective tergalius pairs.

Diagnosis. By the morphological characteristics, the larvae of *Baetis pentaphyllus* **sp. nov.** can be distinguishable from those of other *Baetis* species by the presence of only five pairs of tergalii on segments III–VII. Larvae of *B. pentaphyllus* **sp. nov.** can be distinguished from all Palaearctic representatives of the *Baetis* species-groups as defined by Müller-Liebenau (1969) and Jacob (2003) by the following combination of morphological characters: from the *propingulus*- and *atrebatinus*-groups by absence lateroapical protuberance on scape and incurvation on distal segment of maxillary palp, subapical bristles of claw, medioapical projection paraproct; from *rhodani*-group absence of robust setae on the antennal scape and pedicel and medioapical projection paraproct, subapical bristles of claw; from *vernus*- and *lutheri*- groups by the extent of medioapical protuberance on segment 2 of labial palp, form of femoral setae, which apically rounded, absent subapical bristles of claw, medioapical projection paraproct and thick, spine-like setae on gill margins; from *buceratus*-, *fuscatus*- and *rhodani*-groups absence of dark band on caudal filaments and medioapical projection on paraproct (Ikonomov 1962; Müller-Liebenau 1967, 1969; Morihara

& McCafferty 1979; Jacob 2003; Godunko et al. 2004; Bauernfeind & Soldán 2012; Sroka, 2012; Soldan & Godunko 2012).



FIGURES 24–26. *Baetis pentaphyllus* **sp. nov.,** larvae: 24, posterior margin of abdominal tergum VI; 25, tergalii I; 26, tergalii II.

Distribution. Known from two habitats in the Far East of Russia: Amurskaya oblast' (Figs 27–28) and Chukotka Autonomous District. The larvae of the new species were found in swift riffles with cobble and gravel substrate. Water temperature was 6–9 °C, depth 10–20 cm.

Etymology. The species name is derived from the Latin word *pentaphyllus*, which means five-petalled.

FIGURES 27–28. *Baetis pentaphyllus* **sp. nov.,** localities. 28, Bolshoi Garmakan River, about 300 m above mouth, (Amurskiy Region); 29, Malyi. Garmakan River, about 700 m above mouth, (Amurskiy Region).

Discussion

The final alignment of the COI gene yielded 658 bp for larvae of *Baetis pentaphyllus* **sp. nov**. The closest sequence to *B. pentaphyllus* **sp. nov**. was *Baetis bicaudatus* Dodds, 1923 (in GeneBank, KM530609 and JN291758) with a K2P interspecific distance of 0.161, *Baetiella bispinosa* Gose, 1980 (XJDQD477-18, in BOLD) with a distance of 0.162, *Baetiella foemina* McDunnough, 1936 with a distance of 0.176–0.182, and *Baetis intercalaris* McDunnough, 1921, with a distance of 0.180. The estimated sequence divergence was acceptable for an intraspecific level of mayflies (Ball *et al*. 2005; Sroka 2012; Morinière *et al*. 2017).

However, the high level of interspecific distances and the low phylogenetic signal of COI sequences does not allow one to assign the new species to one of the *Baetis* groups (Müller-Liebenau, 1969; Jacob, 2003; Sroka, 2012). Moreover, molecular data show that both *Baetis bicaudatus* and *Baetiella bispinosa* are closer to *B. pentaphyllus* **sp. nov**. than any other *Baetis* species, despite the multiple morphological characters by which *B. pentaphyllus* **sp. nov**. belongs to the genus *Baetis.* The phylogenetic tree reconstructed using only COI sequences including the genera *Baetis*, *Baetiella, Nigrobaetis,* and *Labiobaetis* (not shown) was unresolved with bush shape.

To improve the phylogenetic signal, as well as the establishment of *Baetis* species-group for *B. pentaphyllus* **sp. nov**. we used Bayesian phylogenetic analysis with morphological and genetic datasets (Fig. 29). Unfortunately, we were limited only to the *Baetis* species used by Sroka (2012). On the basis of the constructed tree, a polytomy node was formed. Specimen BAE-BGAR1_110 formed a monophyletic group with *Baetis lutheri* Müller-Liebenau, 1967 and *Baetis vardarensis* Ikonomov, 1962, which shows its greater similarity with the *lutheri*-group than with other *Baetis* species-group.

Thus, the taxonomic position of *Baetis pentaphyllus* **sp. nov.** remains rather unclear and, most likely, cannot be determined until further collection and study of adults is carried out.

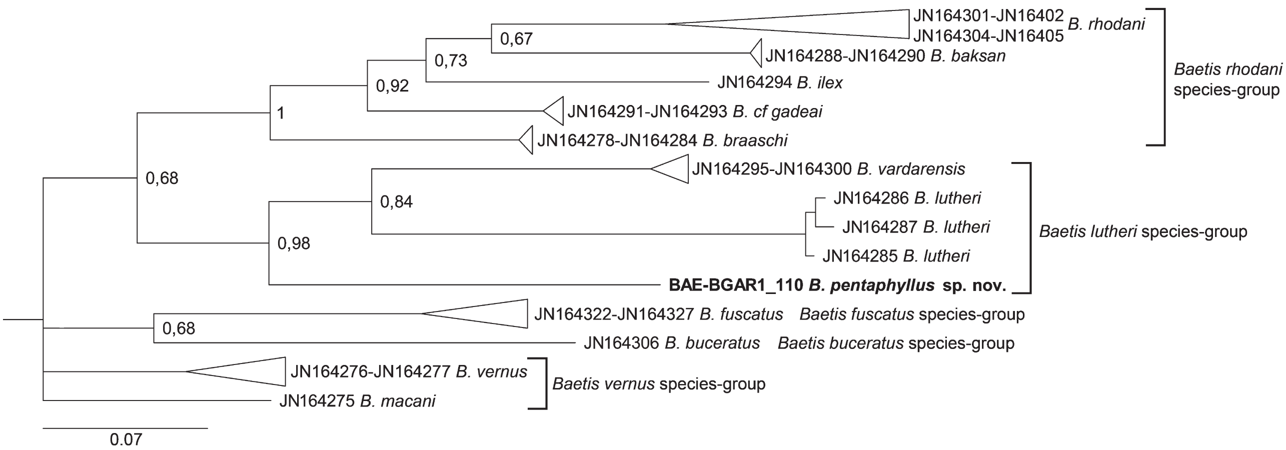


FIGURE 29. Bayesian tree of the genus *Baetis* inferred from the cytochrome c oxidase I (COI) nucleotide sequence data (466 bp) and 47 morphological characters. All data including species-groups are taken from Sroka (2012). Specimen obtained in this study is in bold. Bayesian posterior probabilities (PP) given in tree nodes.

Acknowledgement

The authors are grateful to N. Yu. Kluge (Department of Entomology, Sankt Petersburg State University) for comments when preparing drawings and directorate of the State Nature Reserve Zeysky Ignatenko S. Yu and Ignatenko E.V. for the assistance provided during the work on the territory of the reserve.

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