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Baetis (*Rhodobaetis*) *molecularis* sp. nov., a new mayfly species (Ephemeroptera: Baetidae) from the Russian Far East

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Abstract

A new species *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. is described and illustrated based on larvae and reared adults from the Far East of Russia. The differential diagnosis of this species is provided with regard to other representatives of the subgenus *Rhodobaetis* Jacob, 2003 from East Palaearctic and Nearctic Regions. A dataset including novel and publicly available COI mtDNA sequences of 16 species of *Rhodobaetis* has been assembled to provide a reference dataset for DNA barcoding. The comparison between *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. and other species produced K2P genetic distances of 0.201 in average, values well above those associated with intraspecific variation. The closest species was *Baetis foemina* McDonough with a K2P distance value 0.114. A Bayesian phylogeny of available *Rhodobaetis* is also provided.

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

Introduction

The subgenus *Rhodobaetis* Jacob, 2003 (genus *Baetis* established by Leach in 1815) is widely distributed throughout the Palaearctic, Nearctic, Oriental and other regions.

According to recent studies by several authors (Jacob 2003; Godunko *et al*. 2004; Gattolliat & Sartori 2008) the following combination of characters makes possible to distinguish species of *Rhodobaetis* from other species of *Baetis* s. l.: surface of terga with scales of different structure; antennal segments, surface of femora and paraproct plate with different kind of scales; unistyliger approximately square or slightly elongated; inner margin of basal segment of forceps sometimes with small subapical protuberance; segment 1 of forceps with subparallel margins basally and medially; inner margin of segment 1 of forceps with subapical protuberance; segment 3 of forceps of various shapes (oval and slightly asymmetric, almost square with truncate inner margin, almost spherical, elongated).

The greatest diversity of representatives of this subgenus is noted in the West Palaearctic region (22 species) (Müller-Liebenau, 1969; Müller-Liebenau & Hubbard 1985; Soldán & Godunko 2008; Sroka *et al*. 2012a; Gattolliat *et al*. 2018; Yanai *et al*. 2018). In the East Palaearctic region, this subgenus is currently represented by 12 species: Holarctic *Baetis bicaudatus* Dodds; Palaearctic *B. braaschi* Zimmermann; Central Asian *B. issyksuvensis* Brodsky, *B. heptapotamicus* Brodsky, *B. praemontanus* Braasch, *B. oreophilus* Kluge, and *B. taldybulaki* Sroka *et al*.; *B. khakassicus* Beketov & Godunko known only from the Republic of Khakassia, Russian Federation, and *B. noshaqensis* Ueno from Pamir; East-Asian Island *B. shinanonis* Ueno and *B. thermicus* Ueno; and East-Palaearctic *B. silvaticus* Kluge (Braasch 1999; Soldán & Godunko 2008; Tiunova 1995, 2007, 2009; Kluge 1997; Sroka *et al*. 2012a; Sroka *et al*. 2012b).

Baetis bicaudatus is one of the few known representatives of the subgenus *Rhodobaetis* distributed in more than one biogeographical area. In Nearctic it is distributed from British Columbia and Alberta to New Mexico (specify

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here where is the type-locality); in the Palaearctic, in northern part of Asia, all over Siberia with the area extending to northern Ural (Kluge 1997) and all Far East of Russia (Tshernova *et al.* 1986; Kluge 1997; Ishiwata *et al*. 2000; Tiunova 2009), Japan (Ishiwata 2001) and Mongolia (Enkhtaivan & Soldán 2008).

Baetis bicaudatus is one of the most common and most abundant species in the rivers and streams of the Russian Far East (Ishiwata *et al*. 2000; Tiunova 2007, 2009; Tiunova & Gorovaya 2015; Khamenkova *et al*. 2017). However, in a detailed study of the Far Eastern larvae of *B. bicaudatus*, we found a number of contradictions. In the description of the larvae, Morihara and McCafferty (1979) indicated the presence on the pronotum of two submedian bilobed dark marks. In the work of Kluge (1997), in the keys for determining the larvae of *B. bicaudatus*, the absence of apical hairs on the claw is indicated. These characteristics are not apparent in our specimens, which have an inconspicuous pattern on the pronotum and apical hairs on the claw. Analysis of the mitochondrial gene *cytochrome c oxidase subunit I* showed that larvae, previously defined as *B. bicaudatus* for a number of regions of the Russian Far East are different from GeneBank *B. bicaudatus* sequences and specimens from all three BIN BOLD numbers of *B. bicaudatus* at species level. Using the list of larval and imaginal characters to distinguish the West Palaearctic species of the subgenus *Rhodobaetis* (Godunko *et al*. 2015) and based on a more detailed study of larval and adult morphology, we have identified a new species *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. from the Russian Far East, which is described below.

Material and methods

The material was collected in almost 40 watercourses of five regions of the Russian Far East: Chukotka Autonomous District, Kamchatka Peninsula, and Magadanskaya Oblast', Khabarovskiy Kray and Jewish Autonomous Oblast' during 2014–2019. Practically from each watercourse, instances of larvae or adults were taken for DNA analysis. 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.

Total DNA was extracted from thorax or legs using Qiagen DNeasy Blood and Tissue Kit in compliance with the manufacturer's protocols. Approximately 650 base pairs of the COI were amplified from diluted genomic DNA by polymerase chain reaction (PCR) in a total volume of 10 µl with 5 µl of Go Taq Green Master Mix (Promega Corp, Madison, WI, USA), 0.5 µl of each primer (10 mmol), 3 µl of nuclease-free water and 1 µl of total DNA. COI was sequenced using the primers LCO1490 (5'-ggtcaacaaatcataaagatattgg-3') and HCO2198 (5'-taaacttcagggtgaccaaaaaatca-3') (Folmer *et al.* 1994). PCR was carried out on a ProFlex PCR System (Applied Biosystems, Foster City, USA), with the following cycling parameters: a 3 min denaturing step at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 50°C,and 60 s at 72°C, and a subsequent 10-min final extension at 72°C. Amplification of PCR products was checked by electrophoresis using a 1.5% agarose gel in TBE buffer stained with Ethidium bromide and visualized on GelDoc XR+ imaging systems (BioRad). Positive PCR products were purified for cycle sequencing using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) by ThermoFisher Scientific. DNA sequencing was performed on both strands for each positive PCR product using BigDye™ Terminator v3.1 Cycle kit and run on an ABI 3130xl (Applied Biosystems™). Sequences were manually assembled and edited using Finch TV and MEGA 7 (Kumar *et al.* 2016). To estimation the Kimura 2 parameter (K2P) intra- and interspecific distances we also use MEGA 7.

For reconstruction of phylogenetic relationships we added all available sequences of *Rhodobaetis* from GeneBank. Of them *Baetis adonis* Traver, *B. tricaudatus* Dodds, *B. magnus* McCafferty & Waltz, *B. canariensis* Müller-Liebenau, *B. rhodani* Pictet had several distinct sequences from different BIN BOLD numbers, therefore we used only specimens collected close to the type locality. We also added sequences of three specimens from each BIN BOLD numbers of *B. bicaudatus* Dodds. *Baetis pentaphyllus* (*B. lutheri* species group) was used as outgroup to root the tree.

Bayesian phylogenetic analyses were conducted with MrBayes v. 3.2.7 (Ronquist & Huelsenbeck 2003). PartitionFinder 2.1.1 (Lanfear *et al*. 2012) was used to select the best-fit partitioning scheme and models separately for each codon position of COI gene. The best fit models for three codon positions were GTR+I (Tavare 1986), F81+I (Felsenstein 1981) and HKY+G (Hasegawa *et al*. 1985) respectively. Bayesian Inference was performed with two independent runs of Metropolis-coupled Markov chain Monte Carlo analyses, with each run comprising one cold chain and three heated chains. The chains were run for 5 million generations and sampled every 100 generations. The 1 250 000 generations (or 25 % of the sampled trees) were discarded using a burn-in. Moreover, trace files were visually inspected in Tracer 1.7 (Rambaut *et al.* 2018). To clarify the bootstrap supports in the branch nodes we conducted a Maximum likelihood tree using RAxML v. 8.2.4 (Stamatakis 2006). FigTree v. 1.4.4 was used to visualize phylogenetic tree after analysis.

All *B. molecularis* **sp. nov**. sequences have been deposited in GeneBank (MT027014–MT027030). To obtain a more representative tree we included in phylogenetic tree three additional sequences of *B. silvaticus* (MT231287–MT231289).

Results

***Baetis* (*Rhodobaetis*) *molecularis* sp. nov.**
urn:lsid:zoobank.org:act:FAB4B007-959E-41E3-AB7C-07B5AA1D15C3
(Figs 1–44)

Baetis bicaudatus Tshernova *et al*. 1986:133, figs 3–4.
Baetis bicaudatus: Ishiwata *et al*. 2000:71, fig. 4A–H.
Baetis (*Baetis*) *bicaudatus*: Tiunova 2007:185.
Baetis (*Baetis*) *bicaudatus*: Tiunova 2009:678.
Baetis bicaudatus: Tiunova & Gorovaya 2015:230.
Baetis bicaudatus: Khamenkova *et al*. 2017: http://biosoil.ru/doclib/article_188.doc

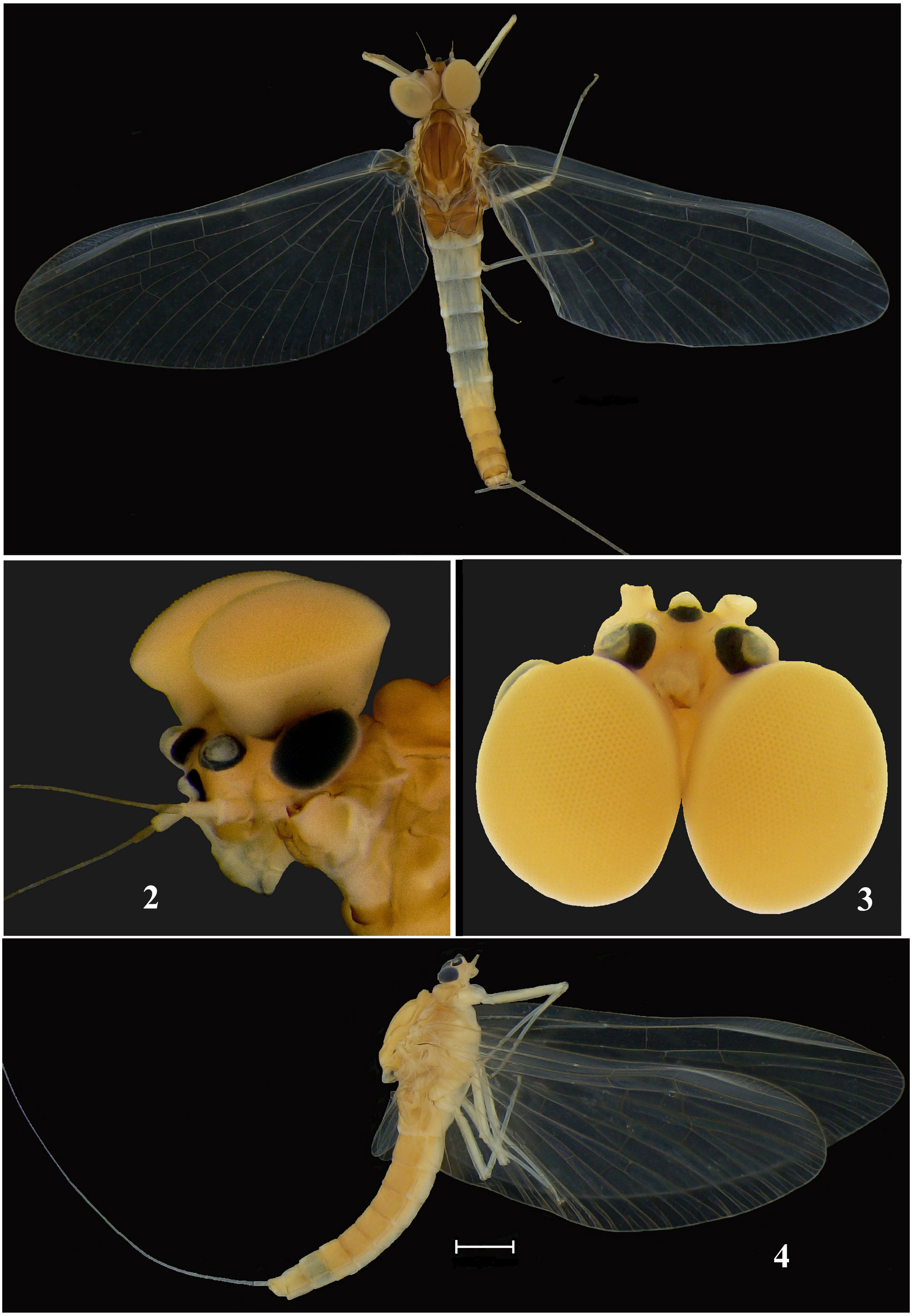
Material. Holotype male imago (reared from larva), **RUSSIAN FEDERATION:** KAMCHATSKAYA OBLAST': Kamchatka Peninsula: Paratunka River Basin: Bistraya River, a turn on the Paratunka village, N 53°58.019 E 157°44.495, 04.IX.2018, I. Tiunov. **Paratypes**: collected the same date and place as holotype: 17 larvae, 1 male, 2 female, female (**MT027023)** imagines (reared from larva); same place, 30.VII. 2015, 3 male, 4 female imagines, I. Tiunov; Trezubez River, at the Paratunka Fish hatchery, 26.VII.2015, 13 larvae, larva (**MT027021),** I. Tiunov; Avacha River Basin: Topolovaya River, tributary of Koryakskaya River, N 53°07. 037 E 156°51.474, 28.VII.2015, 1 larvae; same place, 30.VII.2015, 9 larva; same place, 04.IX.2018, 7 larvae, I. Tiunov; Koryakskaya River, mouth, duct Svetlaya, 28.VII.2015, 8 larvae, I. Tiunov; Ozernaya River, tributary of Koryakskaya River, 28.VII.2015, 6 larvae, I. Tiunov; Poperechnaya River, tributary of Koryakskaya River, 31.VII.2015, 40 larvae, I. Tiunov; Karymshina River Basin, Serebryannyi Stream, N 52°54. 372; E 158°12.034, 29.VII.2018, 19 larvae, larva (**MT027028**), I. Tiunov; Bistraya River Basin: highway to the Ust'-Kamchatsk city: Vatkan Malkinskiy River, bridge, N 53°29.176; E 157°35.219, 04.IX.2018, 8 larvae, larva (**MT027027),** larvae (**MT027019**), I. Tiunov; Tumkhan River, bridge, N 53°36.019; E 157°38.217, 04.IX.2018, 11 larvae, larva (**MT027021),** T. Tiunov; Watkan Ganalsky River, N 53°31.599; E 157°36.311, 04.IX.2018, 14 larvae, larva (**MT027025),** I. Tiunov; Poperechnaya River, N 53°25. 499 E 157°32.230, 04.IX.2018, 22 larvae, larva (**MT027022**), I. Tiunov; Kizhichonok River, bridge, N 53°48.11.0 E 157°40.205, 04.IX.2018, 4 larvae, I. Tiunov; Milkovskiy district, Malaya Klukvennaya River, N 54°19.560 E 158°15.387, 04.IX.2018, 5 larvae, I. Tiunov; Denohonok River, bridge, N 54°15.564 E 158°07.206, 04.IX.2018, 12 larvae, I. Tiunov; Kashkan River, N 54°10.162 E 157°58.101, 04.IX.2018, 18 larvae, 3 female, female imagines (**MT027026**), I. Tiunov; Kamchatka River Basin: highway to the Ust'-Kamchatsk city: Pravaya Kamchatka River, N 54°01.240 E 157°51.130, 04.IX.2018, 11 larvae, larva (**MT027020)**, I. Tiunov; Bersh River Basin: Greshnaya River, N 54°15.046 E 158°06.348, 04.IX.2018, 31 larvae, larva (**MT027025**), I. Tiunov; Kirgurop River, N 53°45.355 E 157°39.249; 05.IX.2018, 11 larvae, I. Tiunov; Mumoch River, bridge, N 53°15.354 E 157°27.115; 05.IX.2018, 7 larvae, I. Tiunov; Bacostits Stream, N 53°43.052 E 157°38.153, 05.IX.2018; Krutaya River, tributary of the Tumkhan River, N 53°35.122 E 157°38.073, 05.IX.2018, 13 larvae, I. Tiunov; stream between Kirgurop River and Bacostits Stream, N 53°43.526 E 157°39.007, 05.IX.2018, 9 larvae, I. Tiunov; Elezovskiy district, Plotnikova River Basin, Nachilova River, N 53°07. 038 E 156°51.474, 31.VII.2018; 11 larvae, larva (**MT027029**), I. Tiunov; CHUKOTKA AUTONOMOUS DISTRICT, Elgygytgyn Lake Basin, nameless stream, N 67°43.565 E 172°08.972, 13.VIII.2017, 7 larvae, larva (**MT027018**), A. Semenchenko; MAGADANSKAYA OBLAST', Khasynskiy urban district, Ola River, below the bridge, 130-137 km, N 60°28.522 E 151°26.531, 29.IV.2014, 3 larvae, larva (**MT027015**), E. Khamenkova; KHABAROVSKIY KRAY, Ulban Bay, stream in the southeastern part of the bay, N

53°37.565 E 138°02.549, 20.VIII.2016, 5 larvae, larva (**MT027017**), I. Tiunov; AMURSKAYA OBLAST'**:** Zeyskii Reserve, Zeya Reservoir Basin, Bolshoi Garmakan River, about 300 m above mouth, N 53°53.148 E 127°11.626, 07.VII.2015, 9 larvae, larva (**MT027014**), T. Tiunova; JEWISH AUTONOMOUS OBLAST', Bastak Nature Reserve, Ikura River, Ryabinovii Cordon, 4.VIII.2019, 3 larvae, larva (**MT027030**), T. Vshivkova.

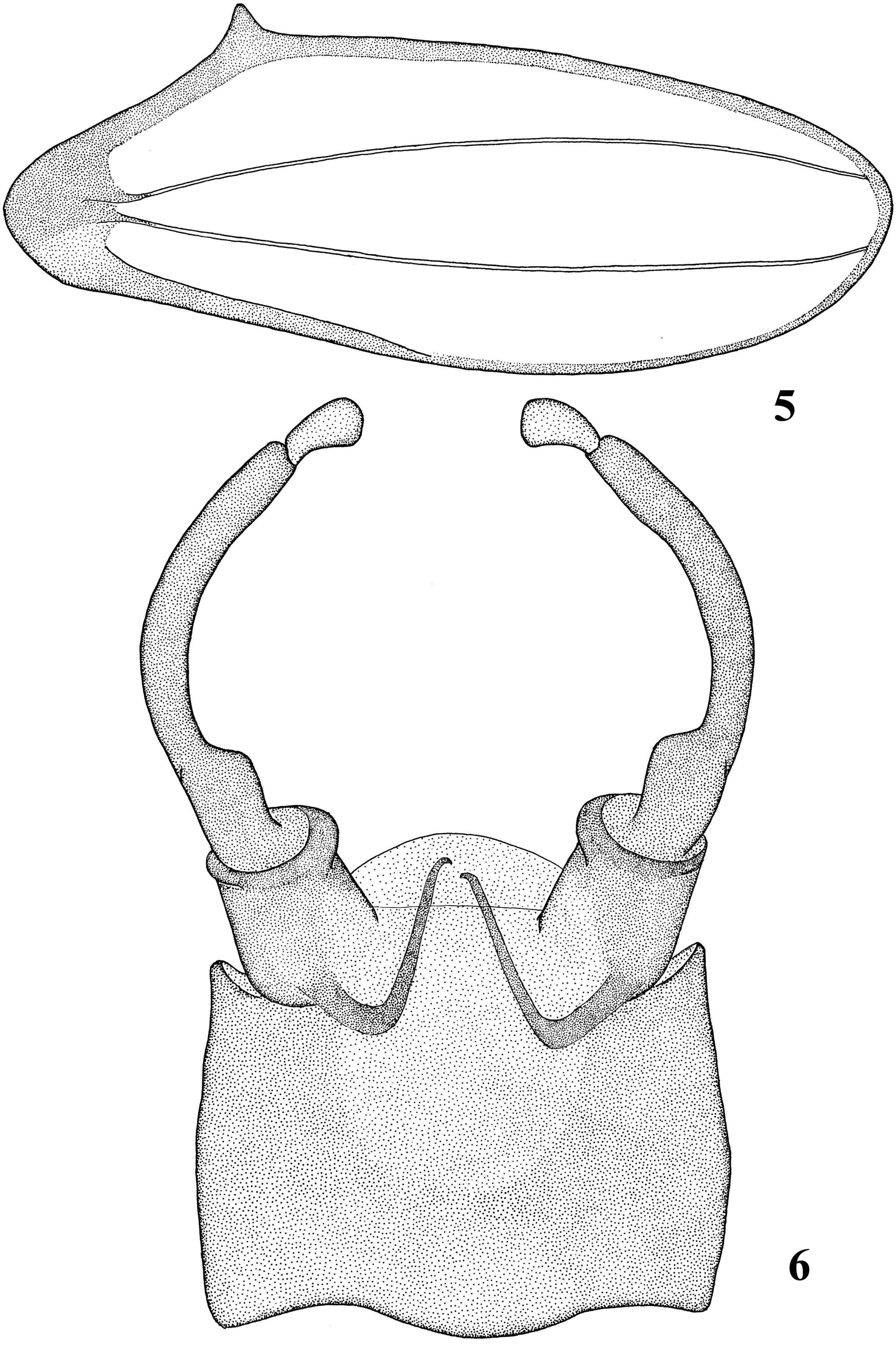
Description. Male imago (in alcohol) (Fig. 1). Length (mm): body 7.6–8.0; forewings 7.8–8.0; cerci 16.3. **Head:** brown or brownish; antennae brownish. Turbinate eyes moderately high (Fig. 2); faceted surface oval in dorsal view, approximately 1.4 times longer than wide (Fig. 3); faceted surface brownish or yellowish; the shaft lighter, grayish or dirty yellow without rings. **Thorax:** Anterior phragma, anteronotal protuberance, medioscutum and submedioscutum brown or dark brown; median longitudinal and medioparapsidal sutures blackish; scuto-scutellar impression and scutellum pale; lateroparapsidal suture light brown (Fig. 1). Forelegs brownish or yellowish, middle and hind legs whitish with brownish distal spot on femora. Lengths ratio of individual foreleg segments: 1.6:2.5:1.1:1.0:0.6:0.25. Forewing transparent, all veins brownish; pterostigma milky on a dark background (Fig. 1). Hind wing hyaline, transparent, and approximately 2.7 times longer than wide, rounded apex and three simple longitudinal veins; third vein ending at approximately half of wing length; cross veins absent; costal projection well developed (Fig. 5). **Abdomen:** Terga I–V pale with brownish lateral sides; terga VI–X light brown, posterior margins darker. Sternum I brownish; sterna VII–IX light brown. Styliger with white middle area and brown anterior and lateral sides (Fig. 6). Unistyliger brown with whitish inner lateral area; nearly as long as wide with rounded bulge on the inside apex of the corner. Segment I of forceps brown with subparallel margins; segments II and III pale; segment II elongated and relatively narrow; inner margin of segment II noticeably concave; segment III widened in the distal part and truncated. Caudal filaments brownish or whitish.

Female imago. Length (mm): body 5.8–7.8; forewings 7.1–9.0; cerci 11.3–13.2. General color of body yellow to yellow-brown (Fig. 4). Head yellowish, antennae with brownish flagellum, darker than head. Eyes and base of ocelli black; apical part of ocelli whitish. Thorax yellow-brown, with brown median longitudinal and medioparapsidal sutures; submedioscutum darker than medioscutum. Wings transparent, all veins brownish; hind wing approximately 2.6 times longer than wide. Legs whitish, tarsal joints brownish. Abdominal terga yellow to yellow-brown. Terga VIII–X whitish in the middle area. Sterna from yellowish to whitish, lighter than terga. Cerci whitish.

Mature larvae (in alcohol). Length (mm): body 4.2–7.7; cerci 3.4–6.0. General body color brown or light brown (Figs 7–8). **Head:** brown, light between compound eyes and ocelli (Fig. 7). Antennae brownish, slightly shorter than ½ of body length; antennal pedicel no more than with three robust and small setae and fine hairs (Figs 10–11). Scape with long narrow distinctly pointed robust setae located on the side at the base (Figs 12–13). Labrum distinctly wider than long (width/length ratio of 1.74); dorsal surface with 1+9–10 long submarginal setae, arranged in one irregular row, and row of long pointed setae laterally on both margins; posterior area with thick long hair-like setae (Fig. 16). Canines of right mandible with 8 teeth divided into two groups. Incisor (outer group) with three teeth; outermost tooth broadened and almost straight apically; kinetodontium (inner group) with five teeth; the second largest; inner margin with row of short thin setae; prostheca elongated and slender with few not sharp teeth (Figs 14, 17). Left mandible canines with 8 teeth. Incisor with three teeth, first tooth widened and almost straight apically; kinetodontium with five teeth, from which first smallest and second largest; prostheca toothbrush-like (Fig. 18). Nymphs often with worn canines and not divided into groups; teeth are practically not expressed; left and right prostheca with short and rounded teeth (Figs 19–20). Maxillary palp two-segmented; tip of second segment rounded, with a single small spine situated at apex (Fig. 15); surface of both segments covered with hair-like setae; second segment longer than first segment (1.3 times) (Fig. 23). Labium with paraglossae concave in middle, approximately two times wider than glossae; apical part of paraglossae with two regular rows of long setae; 5–6 long bristles located along outer margin and one rounded stout subapical seta near top (Fig. 21). Glossae triangular with broad base, with row of 11–12 of long stout setae located near apex (Fig. 21). Second segment of labial palp with rounded apicomedial projection, its width 1.2 times wider than the base of third segment; third segment symmetrically rounded; ventral surface covered with numerous stout setae accompanied by hair-like setae; surface of second segment covered with hair-like setae only (Fig. 22). **Thorax:** brown with light brown diffuse spots (Fig. 7). Anterior margins of pro- and mesonotum darker, sometimes pronotum slightly darker than mesonotum. Mesonotum with pair of light spots near base of protoptera and between them. Legs brownish, joints of leg segments dark brown (Fig. 24). Femora with brownish medial area and diffuse light spot near basal and distal margin; outer edge with dense row of long pointed bristles, which are more densely located at the base; inner margin with a regular row of small pointed setae; dorsal surface of femora covered with hairs and small short bluntly pointed setae (Figs 24, 31–32);

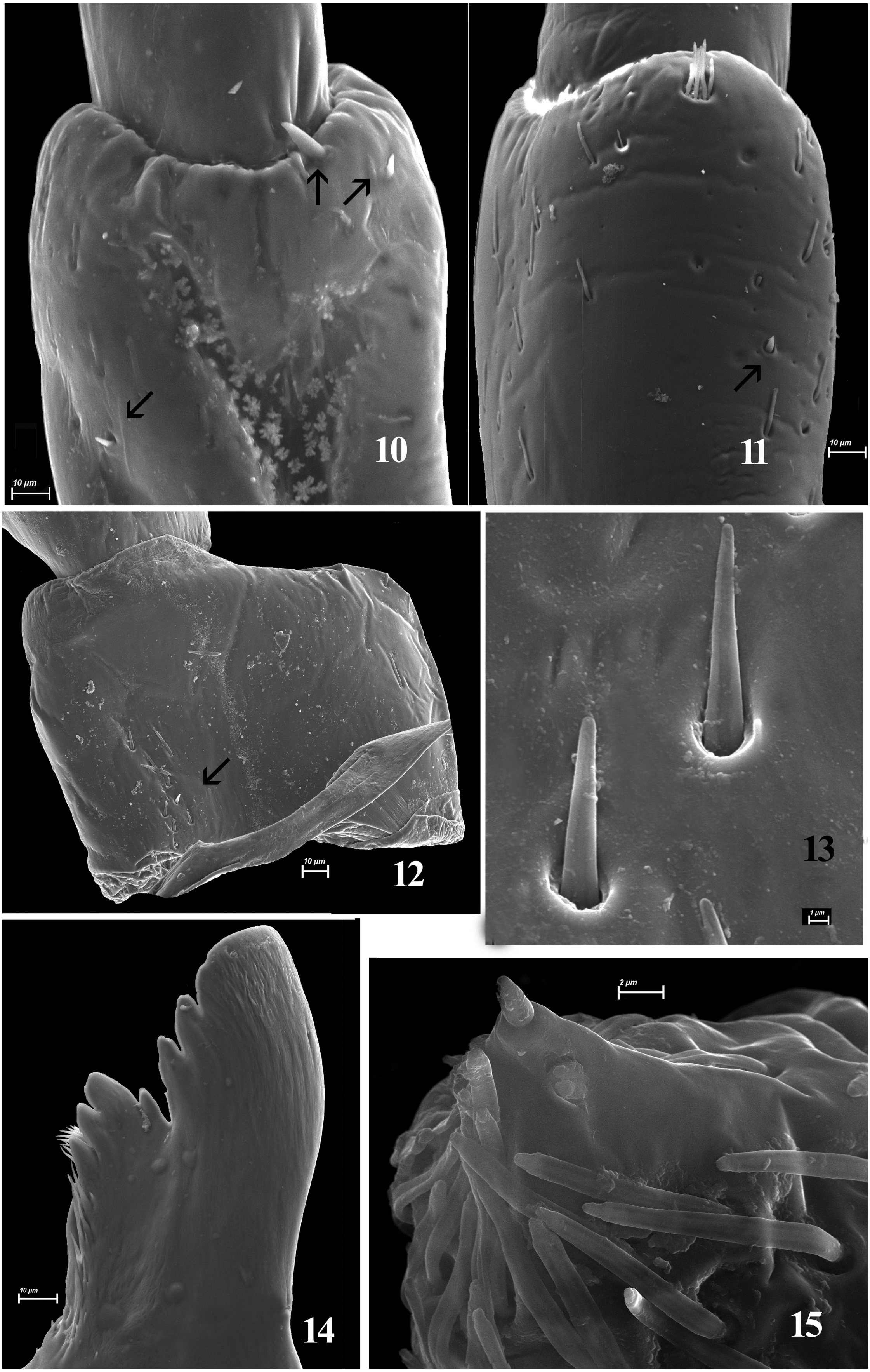


FIGURES 1–4. *Baetis (Rhodobaetis) molecularis* **sp. nov.**, male imago:1, dorsal view; 2, head, lateral view. 3, head, dorsal view; 4, female imago, lateral view. Scale bar: 1 mm.

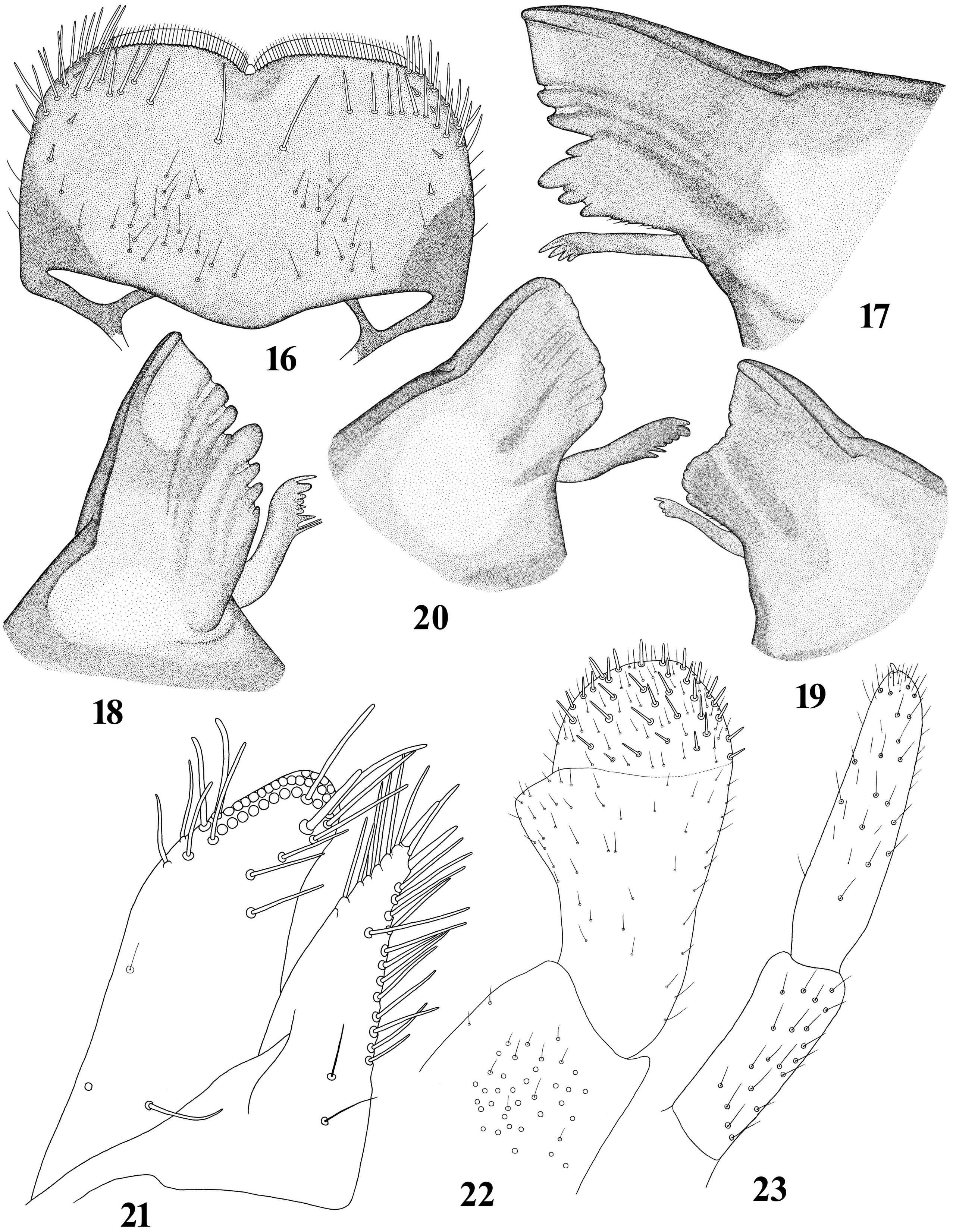


FIGURES 5–6. *Baetis (Rhodobaetis) molecularis* **sp. nov.**, male imago: 5, hind wing; 6, styliger and gonostyli.

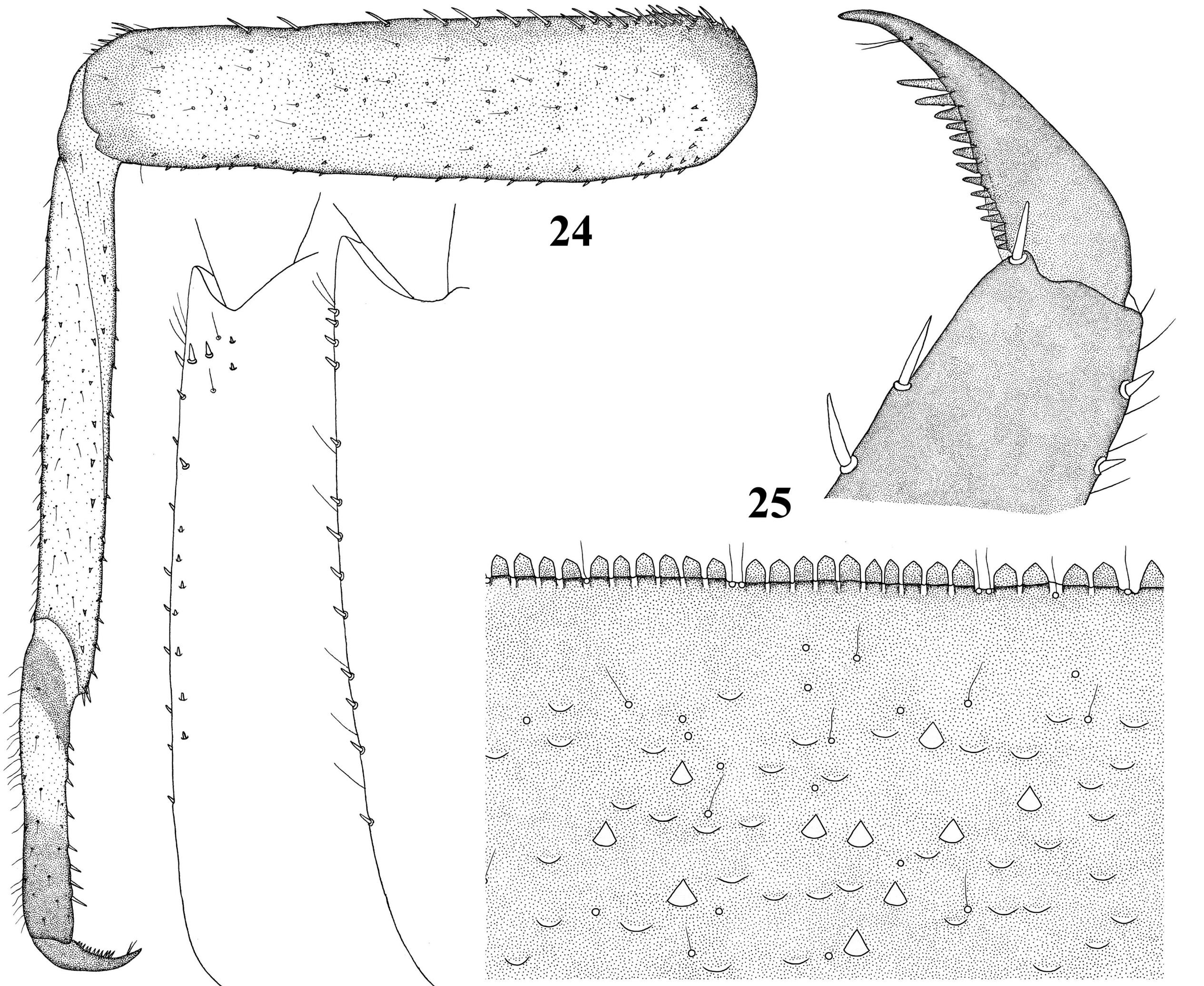
FIGURES 7–9. Habitus of *Baetis (Rhodobaetis) molecularis* **sp. nov.**, larvae, paratypes (material from type locality): 7–8, dorsal view; 9, ventral view. Scale bar: 1 mm.



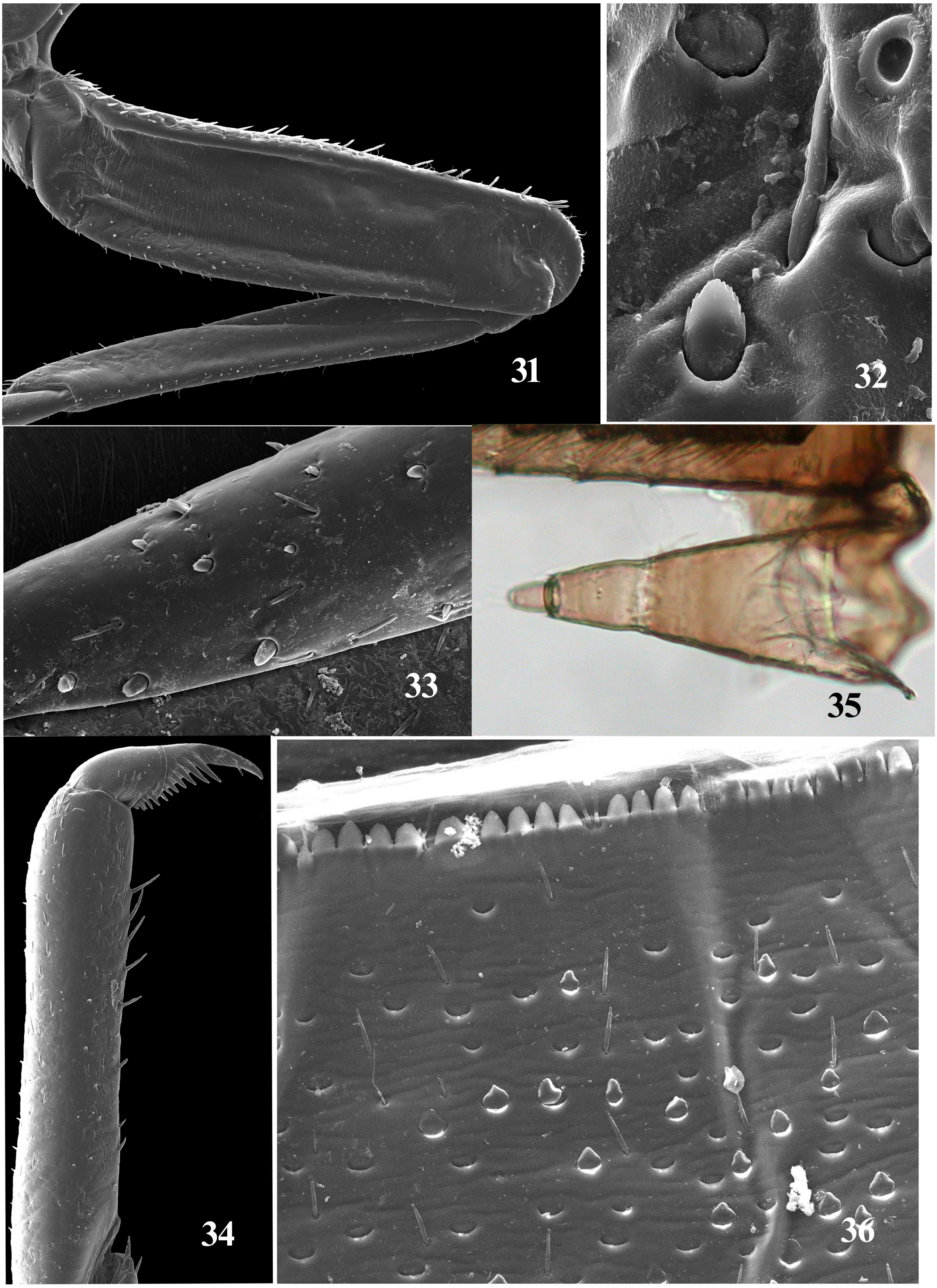
FIGURES 10–15. *Baetis (Rhodobaetis) molecularis* **sp. nov.**, larvae, paratypes: 10–11, setae on pedicel (shown with an arrow); 12–13, setae on scape (shown with an arrow); 14, canine of right mandible; 15, apical part of maxillary palp.



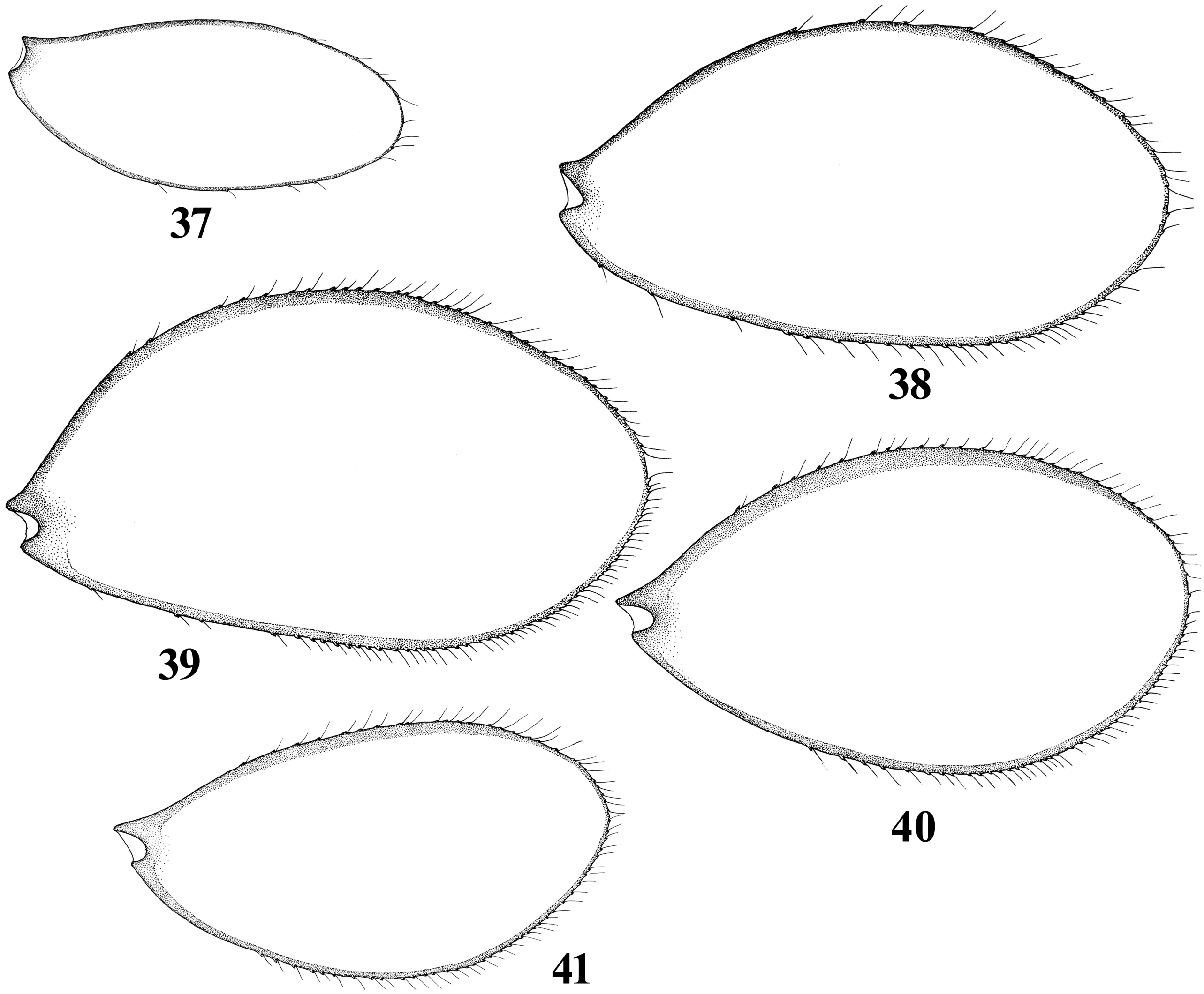
FIGURES 16–23. *Baetis (Rhodobaetis) molecularis* **sp. nov.**, larvae, details of mouthparts, paratypes: 16, labrum, dorsal view; 17,canines and prostheca of right mandible, dorsal view; 18, canines and prostheca of left mandible, dorsal view; 19, worned canines and prostheca of right mandible, dorsal view; 20, worned canines and prostheca of left mandible, dorsal view; 21, glossa and paraglossa, ventral view; 22, labial palp, ventral view; 23, partial view of dorsal surface of maxilla.



FIGURES 24–30. *Baetis (Rhodobaetis) molecularis* **sp. nov.**, larvae, paratypes, dorsal view: 24, foreleg; 25, claw; 26, lateral margin of tergum VI, ventral view; 27, lateral margin of tergum VI, dorsal view; 28, posterior margin of abdominal tergum VI; 29, abdominal tergum X; 30, paraproct.



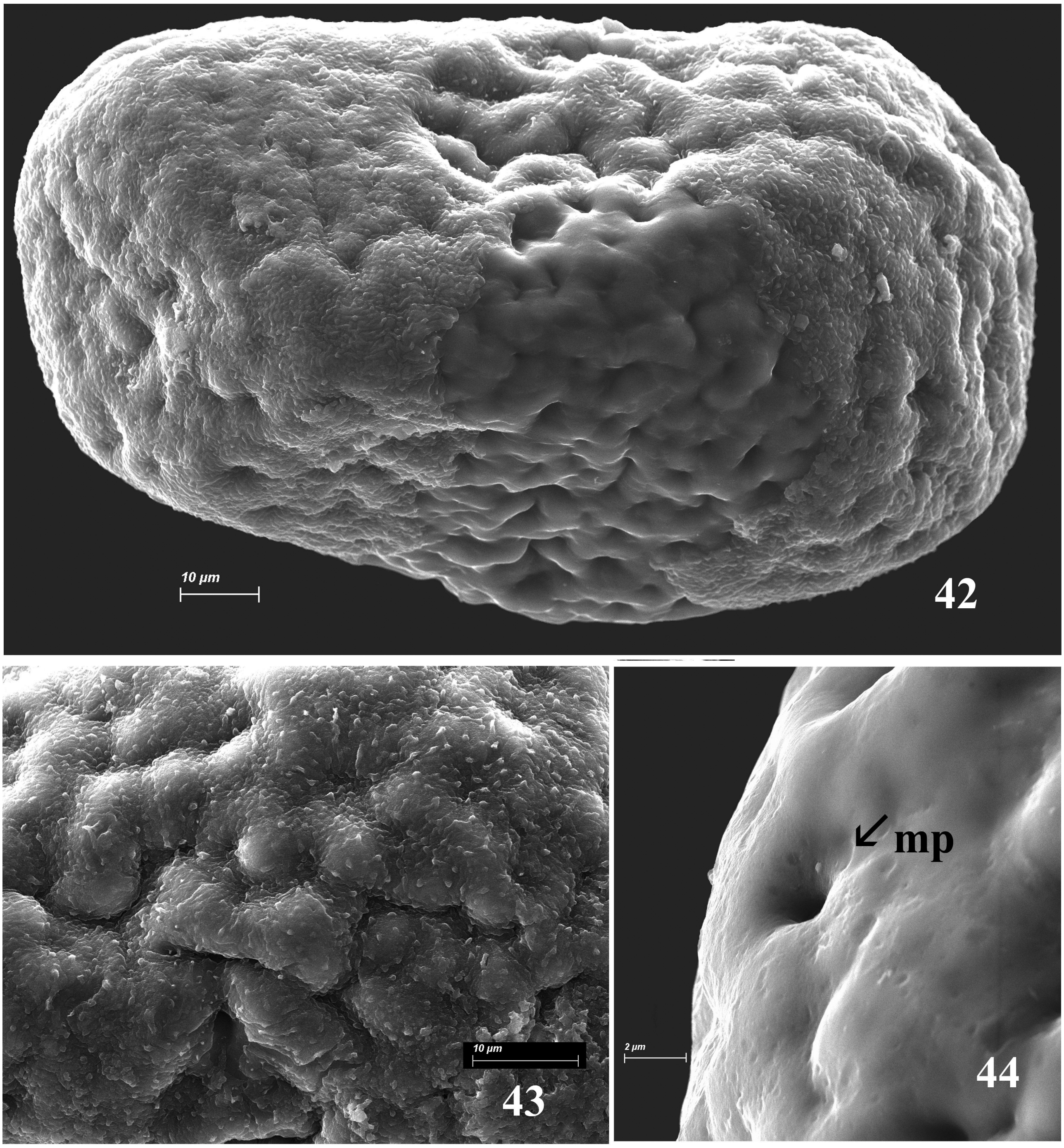
FIGURES 31–36. *Baetis (Rhodobaetis) molecularis* **sp. nov.**, larvae, paratypes: 31, femur and tibia of foreleg; 32, setae on the surface of femur; 33, outer margin of tibia; 34, tarsus and claw; 35, paracercus; 36, posterior margin and surface of abdominal tergum VI.



FIGURES 37–41. *Baetis (Rhodobaetis) molecularis* **sp. nov.**, larvae, tergalii shape, dorsal view: 37, tergalius I; 38, tergalius II; 39, tergalius III; 40, tergalius VI; 41, tergalius VII.

femoral villopore present. Tibiae brownish or yellowish; small stout setae rare, located evenly along the inner and outer margins (Figs 31, 33); surface covered with bristles and hairs similar in size and shape. Patella-tibial present. Tarsus brownish, distal third and base dark brown; inner margin with small setae and hairs; outer margin with a row of pointed setae (Figs 24, 34); claws brown, with row of 13–14 teeth increasing in length toward the apex and a pair of subapical setae (Figs 25, 34). Lengths (mm) of the leg segments as follows: Foreleg: femur 0.9–1.3; tibia 0.7–1.1; and tarsus 0.5–0.7. Middle leg: femur 1.1–1.2; tibia 0.8–1.0; and tarsus 0.5–0.6. Hind leg: femur 1.1–1.2; tibia 0.8–1.1; and tarsus 0.5. **Abdomen:** Terga II–III brown, terga VI–VIII darker, lateral area light brown; anterior and posterior margins darker; terga I and IX brownish, posterior margin darker; tergum X brown (Fig. 7); posterior margin of tergum VI with almost regular row of pentagonal bluntly pointed teeth (Figs 28, 36); lateral margins of segments with a row of small pointed setae and sparse hairs (Fig. 27); surface of terga densely covered with numerous semilunar impressions, hairs and rare conical scales (Figs 28, 36). Sterna brownish; sterna V–VII darker; sterna I–VII with pair of small diffuse brown spots near anterior area; sternum IX pale (Fig. 9); along lateral margins with pointed setae and posteriorly more density near tergalii insertions (Fig. 26). Tergalii (abdominal gills) almost oval-shaped; all tergalii white with dark brown margins, without apparent tracheation; margins without spines, with numerous hairs inserted at the base of small teeths. Tergalius I smallest and 1/3 times shorter than corresponding segment (Fig. 37); tergalius II longer than tergalius I, and 1.9 times longer than wide (Fig. 38); tergalii III–V almost equal in length, 1.8 times longer than wide (Fig. 39); tergalius VI smaller than V one (Fig. 40); tergalius VII small, slightly more than tergalius I, and two times longer than wide (Fig. 41). Paraproct with 17–19 marginal pointed teeth like spines of different size; surface of paraproct with a few robust pointed scales (Fig. 30). Cerci brownish at the base and lighter distally; paracercus reduced, with two, three or four segments (Fig. 35). Of the 70 specimens studied, 54 individuals had a three-segmented paracercus, 13 had two, and 3 had four-segmented.

Eggs. General form oval 131–142 µm length and 76–81 µm width (Fig. 42). Chorion wrinkled and shagreened, with small fossae (Fig. 43). One or two small round micropyles located on tops (Fig. 44).



FIGURES 42–44. *Baetis (Rhodobaetis) molecularis* **sp. nov.**, egg: 42, general view; 43, structure of chorion; 44, detail of chorion, the arrow shows the micropiles (mp).

Diagnosis. The imagoes of *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. are distinguishable from the other representatives of the subgenus by the following combination of characters: unistyliger nearly as long as wide and with a rounded bulge on the inside apex of the corner; segment III widened in the distal part and truncated (Fig. 6). The larva of *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. can be distinguished by the following combination of characters: the presence of rare robust setae on pedicel (Figs 10–11); the presence of a row of long narrow distinctly pointed

FIGURES 45–50. Localities of *Baetis (Rhodobaetis) molecularis* **sp. nov.**: 45, Bolshoi Garmakan River (Amurskaya oblast', photo M. Tiunov); Kamchatka Peninsula (photos I. Tiunov): 46, Bacostits Stream; 47, Bistraya River; 48, Bistraya River Basin, unnamed stream; 49, Vatkan Malkinskiy River; 50, Kashkan River.

robust setae on scape (Figs 12–13); the absence of spines of external margin of tergalius (Figs 37–41); the presence of a pair of subapical setae on claws (Figs 24, 34); the mean width/length ratio of labrum (1.74); tergalius not elongated, less than twice as long as wide (Fig. 16); canines of both mandibles with outer tooth broadened and almost straight apically (Figs 17–18); posterior margin of terga with almost regular row of pentagonal bluntly pointed teeth (Figs 28, 36); paracercus reduced, with two to four segments.

Distribution. Russian Far East: Chukotka Autonomous District, Kamchatskiy and Khabarovskiy Kray, Magadanskaya, Amurskaya and Jewish Autonomous Oblast'.

Baetis (*Rhodobaetis*) *molecularis* **sp. nov**. is a common species in rivers, streams, and springs of the Kamchatka Peninsula (Figs 45–50). Typical substrate in its habitats is composed mainly of pebbles and rocks of various sizes. Water temperature in the collection periods of larvae and reared imagoes did not exceed 15°C. According to our data, adult emergence period is from late July to mid September.

Etymology. Since the foremost basis for the species delimitation was based on molecular studies, we considered it more correct to name it as *molecularis.*

Results of DNA barcoding. The final alignment of the COI gene yielded 658 bp for 17 specimens of *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. with 7 haplotypes, one of which was detected in 10 specimens. Total pairwise intraspecific sequence divergence ranged from 0.0000 to 0.0123 (avg 0.0036), which is based on eleven synonymous substitutions.

Interspecific pairwise distances (K2P) between *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. and other 12 GeneBank available species of *Rhodobaetis* (Fig. 51) ranged from 0.114 to 0.246 (the average value is 0.201). Genetically the closest species to *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov.** was *Baetis foemina* (K2P distances – 0.114) from northeastern Canada whereas the Palaearctic species showed higher distances.

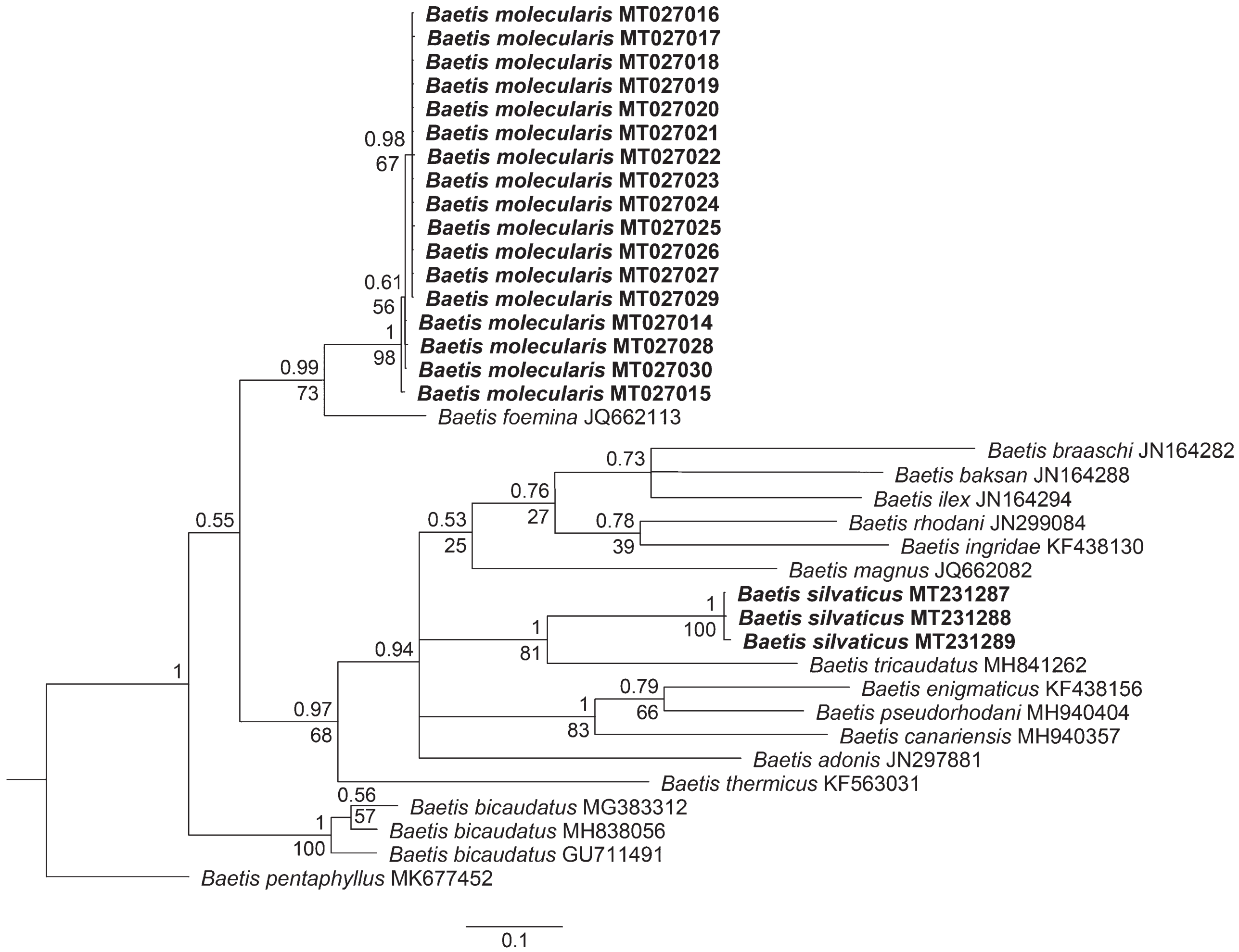


FIGURE 51. Bayesian mitochondrial COI phylogeny of the *Rhodobaetis* species from 16 taxa. Bayesian posterior probabilities (PP) are given above tree nodes and bootstrap support values found in the ML analysis are shown below nodes. Sequences obtained in this study are in bold.

The phylogenetic trees reconstructed using Bayesian Inference and Maximum likelihood had varied topology. We use *Baetis pentaphyllus* Tiunova as outgroup. The BI phylogeny revealed three well-supported clades. The earliest branching clade includes three sequences of *B. bicaudatus* each of which relates to a different BOLD BIN number (PP = 1, Maximum likelihood bootstrap value percent, ML = 100). The second clade includes two sister species, *Baetis foemina* and *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. (PP = 0.99, ML = 73). The remaining species of *Rhodobaetis* were placed to the third clade (PP = 0.97, ML = 68). *Baetis silvaticus* was sister to *Baetis tricaudatus* and also placed to the third clade. In Maximum Likelihood tree (not shown) *B. bicaudatus* was sister to *B. foemina* and *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. but support of this node was low (ML = 49).

Discussion

Baetis (*Rhodobaetis*) *molecularis* **sp. nov**. belongs to the subgenus *Rhodobaetis* and exhibits the group characteristics, such as the presence of stout setae on scape, pedicel and paraproct, setae on the surface of abdominal terga and femora (Müller-Liebenau 1969; Jacob 2003; Godunko *et al*. 2004).

Among the 12 species of the subgenus *Rhodobaetis* occurring in the East Palaearctic, only larvae of *B. bicaudatus* have reduced 2–3 segmented paracercus (described by Morihara & McCafferty 1979). The larvae of *B. bicaudatus* can be separated from *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. by the shape and numbers of robust setae on the pedicel. In the new species the pedicel with small and very rare, no more than three, robust setae (Figs 10–11), but in *B. bicaudatus* they are numerous (Morihara & McCafferty 1979: 168, fig. 3). The posterior margin of the terga in the new species presents pentagonal bluntly pointed teeth (Figs 28, 36), unlike *B. bicaudatus* which has a posterior margin with triangular teeth. In *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. the labrum is distinctly wider than long (the width/length ratio is 1.74) (Fig. 16), in contrast to *B. bicaudatus* in which labrum is nearly quadrate (Morihara & McCafferty 1979). Additionally, the larvae of *B. bicaudatus* can be distinguished by the features of body color (pronotum with two submedian bilobed dark areas: by Morihara & McCafferty 1979: 192, fig. 17c), while in the new species the pronotum is only brown without maculation (Fig. 7).

The male imago of *B. bicaudatus* has never been described in detail, therefore, it is not possible to compare it with *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. Genitalia of the male imago and hind wing which figured in the work of Tshernova *et al*. (1986: 133, figs 3–4), in our opinion, refer to *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**.

Baetis (*Rhodobaetis*) *molecularis* **sp. nov**. differs from other East Palaearctic species of the subgenus *Rhodobaetis* in the imaginal stage by the shape of the gonostyli. Firstly, shape of unistyliger, which have a rounded bulge on the inside apex of the corner (Fig. 6). Secondly, by the form segment III of forceps, which is widened in the distal part and truncated (Fig. 6) in contrast to B. *braaschi, B. heptapotamicus, B. thermicus, B. silvaticus* and *B. taldybulaki* with segment III oval and small, sometimes elongated, and to *B. issyksuvensis* and *B. oreophilus* with long segment III. *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. have hind wings with tree simple longitudinal veins in contrast to *B. noshaqensis* in which only two simple longitudinal veins are present.

Geographically closest to the Russian Far East is the Nearctic region. In this region the subgenus *Rhodobaetis* is represented by the following species: *B. adonis* Traver, *B. foemina*, *B. magnus* McCafferty & Waltz, *B. palisadi* Mayo, *B. parallela* Banks, *B. piscatoris* Traver, and *B. tricaudatus* Dodds.

Among species with available larval descriptions, *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. differs from Nearctic species *B. adonis, B. magnus*, *B. palisadi*, *B. parallela*, *B. piscatoris* and *B. tricaudatus* by the reduced paracercus. The same is known only for *B. foemina.* The larvae of *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. can be separated from the larvae of *B. foemina* by the following features: all tergalii are not elongated, less than twice as long as wide (Figs 37–41) (in contrast to *B. foemina,* in which tergalius is more than twice as long as wide); no more than three robust setae are present on pedicel (Figs 10–11) (in *B. foemina* at least five robust setae are present on pedicel) (Morihara & McCafferty 1979: 193, Fig. 18a).

Male imago of *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. differs from all Nearctic species by the shape of unistyliger, which have a rounded bulge on the inside apex of the corner and by the form segment III of forceps, which widened in the distal part and truncated (Fig. 6). Additionally, the imago of *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. can be distinguished by the features of body color, which has brown or light brown thorax (in *B. adonis* and *B. magnus* reddish-brown or red-brown; in *B. magnus* with dark marks on sterna and ventral surface on the pedicels).

The results of DNA barcoding confirm the validity of the new species described here. The average K2P divergence for COI between species *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. and the closest species, *Baetis foemina,* was 0.114, which is acceptable for an interspecific level (Ball *et al*. 2005, Sroka 2012, Morinière *et al.* 2017). Other species of *Baetis* differed from the described *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. on 0.201 in average which also confirms its species independence. The Bayesian inference and Maximum likelihood bootstrap analysis showed moderate support for monophyly of *Baetis* (*Rhodobaetis*) *molecularis* **sp. nov**. clade. *Baetis foemina* is the sister-taxon of the newly described species, while the remaining species of *Baetis* occur on other clades on the reconstructed tree.

In conclusion, we can assume that under the name ”bicaudatus” there is a group of closely related species. It would be interesting to conduct a series of studies of the Palearctic ”bicaudatus” based on molecular and detailed morphological original data. The history of this study proves the need, in some cases, of the importance of combining evidence from various sources for taxonomic studies, in this case molecular and morphological.

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