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DESCRIPTION AND DNA BARCODING OF *BAETIS* (*RHODOBAETIS*) *MONERONENSIS* SP. N., A NEW SPECIES OF MAYFLY (EPHEMEROPTERA: BAETIDAE) FROM MONERON ISLAND IN RUSSIA

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Summary. The larva, subimago, and female imago of a mayfly of the genus *Baetis* are described. The presence of spatulate setae on the tergites, antennal segments, the femora and the paraprocts of the larva determines the affiliation of *B. moneronensis* **sp. n.** with the subgenus *Rhodobaetis*. Bunches of long pine needle-like setae on the pedicel indicate its similarity to *B. thermicus*. Larvae of both species have two apical setae on the claws, which contradicts one of the characteristics of the subgenus *Rhodobaetis*. Bayesian analysis of a DNA barcode of the new species revealed that the *B. moneronensis* **sp. n.** belongs to the *B. thermicus* clade, which is subdivided into five distinct molecular operational taxonomic units (mOTUs) based on ASAP analysis. Interspecific K2P distances between *B. moneronensis* **sp. n.** and lineages within the *B. thermicus* complex averaged 12.1%. The assignment of new species to the *thermicus*-group is considered proven, while its belonging to the subgenus *Rhodobaetis* may be disputed.

Key words: mayflies, larvae, taxonomy, DNA barcoding, *Baetis*, *Rhodobaetis*, Moneron Island, Sea of Japan.

Е. А. Горовая, А. А. Семенченко. Описание и ДНК баркодинг *Baetis* (*Rhodobaetis*) *moneronensis* sp. n., нового вида поденок (Ephemeroptera: Baetidae) с острова Монерон в России // Дальневосточный энтомолог. 2026. N 553. С. 1-16.

Резюме. Приводится описание личинки, субимаго и самки имаго поденки рода *Baetis*. Наличие лопатковидных щетинок на тергитах, основании антенн, бедрах и парапрокте личинки определяет принадлежность *B. moneronensis* **sp. n.** к подроду *Rhodobaetis*. Пучки длинных хвоеподобных щетинок на педицеле указывают на его сходство с *B. thermicus*. Личинки обоих видов имеют по две апикальные щетинки на коготках ног, что идет вразрез с одной из характеристик подрода *Rhodobaetis*. Байесовская филогения показала, что *B. moneronensis* **sp. n.** принадлежит к кладе *B. thermicus*, которая, по данным анализа ASAP, подразделяется на пять отдельных молекулярных операционных таксономических единиц (mOTUs). Межвидовые дистанции K2P между *B. moneronensis* **sp. n.** и линиями внутри комплекса *B. thermicus* составили в среднем 12.1%. Отнесение нового вида к *thermicus*-группе считается доказанным, в то время как его принадлежность к подроду *Rhodobaetis* может быть оспорена.

INTRODUCTION

The subgenus *Rhodobaetis* Jacob, 2003 (genus *Baetis* Leach, 1815) has a complex taxonomic history: from uniting a number of species into the *rhodani*-group (Muller-Liebinau, 1969; Morihara & McCafferty, 1979; Novikova & Kluge, 1987) to assigning a name and designating a type species (Jacob, 2003), identifying combination 17 larval and nine imaginal of structural characters (Godunko *et al.*, 2004). In addition, it is characterized by a complex internal differentiation: the presence of only subtle morphological differences between the larvae identified as *B. thermicus* Ueno, 1931, led to the identification of the *thermicus*-group (Imanishi, 1940), and between the larvae identified as *B. pseudothermicus* Kluge, 1983 – the *pseudothermicus*-group (Godunko *et al.*, 2004). Even the type species *B. rhodani* (Pictet, 1843) has been proposed, based on the results of molecular studies, to be considered as a cryptic species complex (Williams *et al.* 2006; Lucentini *et al.*, 2011).

Larvae of the described species were first collected on Moneron Island by N. Minakawa (23.08.2001) and E.A. Makarchenko (19.07.2004) and identified as *B. thermicus* Ueno, 1931 (Tiunova, 2006; Gorovaya, 2025). This identification was contested based on the results of DNA barcoding perfomed on collections by M.V. Astakhov (2–4.06. 2024). Thus, the list of representatives of the genus *Rhodobaetis* inhabiting the Russian Far East has been expanded to five species. It includes *B. pseudothermicus* and *B. silvaticus* Kluge, 1983, which have been described from the Primorye Territory; *B. molecularis* Tiunova et Semenchenko, 2020, previously recorded for the region as *B. bicaudatus* Dodds, 1923 (Tiunova & Semenchenko, 2020); and *B. thermicus* known from Japan, Korea and Russia (Tiunova, 2007, 2009; Soldan & Godunko, 2008).

For molecular studies, we used all sequences of *B. thermicus* available in the GenBank database, as well as, following Pengxu Mu & Xiaolei Huang (2025), sequences of six of the *Rhodobaetis* species with East Palaearctic distribution (*B. bicaudatus*, *B. braaschi* Zimmermann, 1980, *B. molecularis*, *B. pseudothermicus*, *B. qianlei* Mu et Huang, 2025, *B. silvaticus*) and the type species *B. rhodani*, taken from their type localities or localities close to them.

DNA barcoding, based on the 658-bp fragment of the mitochondrial *cytochrome c oxidase subunit I* (*COI*) gene, is a standard tool for animal species delimitation (Hebert *et al*., 2003; Ratnasingham & Hebert, 2013). This method has proven highly effective for the genus *Baetis* (Morinière *et al*., 2017; Stauffer *et al*., 2017; Tiunova & Semenchenko, 2019, 2020; Tiunova *et al*., 2021; Semenchenko & Tiunova, 2025; Mu & Huang, 2025), and further enables the association of mayflies collected at different life cycle stages. While using *COI* barcodes to reconstruct phylogenetic relationships across distant species groups often results in low support at basal nodes due to mutational saturation at the third codon position and limited phylogenetic signal, but it consistently yields high support among closely related species (Tiunova & Semenchenko, 2019, 2020; Tiunova *et al*., 2021; Semenchenko & Tiunova, 2025). The new species is morphologically and molecularly closely related to *B. thermicus*, which, however, is divided into many operational molecular taxonomic units.

In this paper, we: 1) provide a morphological description of the larvae, subimago, female imago of the new species; 2) construct a Bayesian inference tree including *B. moneronensis* **sp. n.**, all available *B. thermicus* sequences, and some representative taxa of the subgenus *Rhodobaetis*; and 3) perform species delimitation using ASAP analysis and K2P distance calculations to confirm the taxonomic independence of *B. moneronensis* **sp. n.**

MATERIAL AND METHODS

Specimen collection and morphological identification. This paper is based on the material collected by M.V. Astakhov, N. Minakawa and E.A. Makarchenko from the watercourses of Moneron Island (Russia, Sea of Japan). Fresh material for DNA barcoding was fixed in 96% ethanol, while the voucher specimens deposited at the Federal Scientific Center of the East Asia Terrestrial Biodiversity (FSCB) and the material used for morphological study were preserved in 70% ethanol.

Morphological characters were observed under an Olympus CH light microscope. Photographs of specimens were taken with a stereomicroscope Olympus SZX16 equipped with an Olympus DP74 digital camera, and then stacked using with Helicon Focus software. Microstructures were examined with a MERLIN field-emission scanning electron microscope (Carl Zeiss, Germany) at the Center for Collective Use Biotechnology and Genetic Engineering of the FSC Biodiversity. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software.

Molecular methods. Total DNA was extracted from the thorax of larval specimens using the Evrogen Extract DNA Blood & Cells kit, following the manufacturer's protocol, with a final elution volume of 50 µL. Detailed protocols for PCR amplification, thermal cycling conditions, product purification, and sequencing are provided in Semenchenko &. Tiunova (2025).

Interspecific genetic distances based on the Kimura 2-parameter (K2P) model were calculated using MEGA7 (Kumar *et al*., 2016). For species delimitation, Assemble Species by Automatic Partitioning (ASAP) analysis (Puillandre *et al*., 2021) was performed using K2P distances.

To reconstruct phylogenetic relationships, we compiled a dataset including all available *B. thermicus* sequences, along with the type species of the subgenus *Rhodobaetis* and representative individuals of its East Palaearctic species. *Labiobaetis acei* Kaltenbach, Garces et Gattolliat, 2020 was selected as the outgroup.

Phylogenetic analyses were performed using Bayesian inference (BI) in MrBayes 3.2.7 (Ronquist *et al*., 2012). The optimal nucleotide substitution models for the three codon positions were selected using PartitionFinder 2.1.1 (Lanfear *et al*., 2012): F81+I (Felsenstein, 1981), HKY+G (Hasegawa *et al*., 1985), and GTR+G (Tavare, 1986), respectively. The BI analysis consisted of two independent runs of Metropolis-coupled Markov chain Monte Carlo (MCMCMC), each comprising one cold and three heated chains. The chains were run for 1 million generations, with sampling every 500 generations. Convergence and stationary distributions were visually inspected in Tracer 1.7 (Rambaut *et al*., 2018). The resulting phylogenetic tree was visualized using FigTree v. 1.4.4. The new sequence has been deposited in GenBank under accession number PZ319905.

DESCRIPTION OF NEW SPECIES

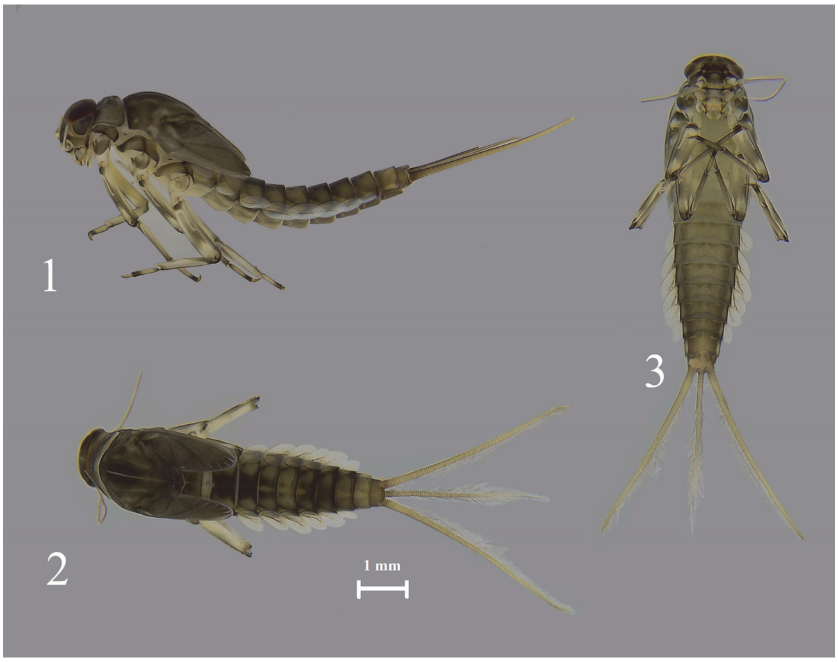
Baetis (Rhodobaetis) moneronensis **Gorovaya et Semenchenko, sp. n.**

https://zoobank.org/NomenclaturalActs/20E798C8-D2FB-424A-AFA5-3AB380B35044
Figs 1–42

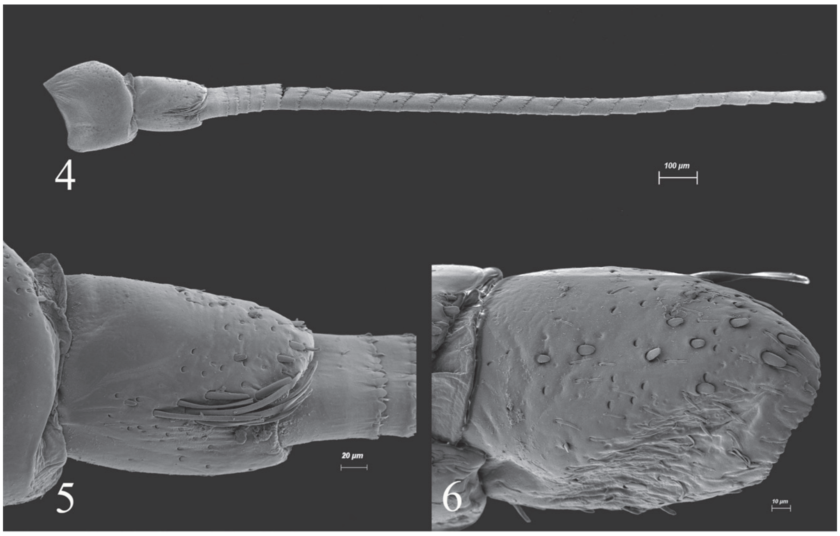
TYPE MATERIAL. Holotype – mature larva, **Russia**: Sea of Japan, Moneron Island, Vodopadnyi Brook, 02.VI 2024, coll. M.V. Astakhov (FSCB). Paratypes (31 specimens): **Russia**: Moneron Island, Vodopadnyi Brook, 02.VI 2024, 7 larvae, 1 larva (GEA1117), coll. M.V. Astakhov; Moneron River, 02.VI 2024, 9 larvae, coll. M.V. Astakhov; the same place, 04.VI 2024, 2 ♀ imago, 2 ♀ and 2 ♂ subimago, coll. M.V. Astakhov; Bolshoi Brook, 04.VI 2024, 2 larvae, coll. M.V. Astakhov; Usova River, 23.VIII 2001, 5 larvae, coll. N. Minakawa; Vodopadnyi Brook, 19.VII 2004, 1 larva, coll. E.A. Makarchenko. (All in FSCB).

DESCRIPTION. **Larvae** (in alcohol). Length (mm): body 4.0–9.5; cerci > 4. General body color dark brown with sand-colored spots (Figs 1–3).

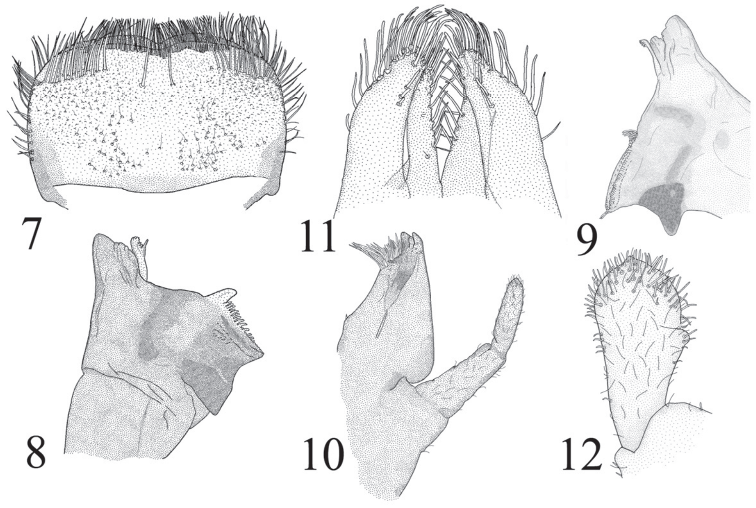
Head: reddish brown. Antennae sand-colored, slightly shorter than 1/2 of body length; with flat-tipped sensilla. Bases wide apart. Basal segment of antenna (Figs 4–6) with smooth outer margin and some chaotically arranged short roundish spatulate



Figs 1–3. *Baetis moneronensis* **sp. n.**, larvae, habitus: 1 – lateral view; 2 – dorsal view; 3 – ventral view.



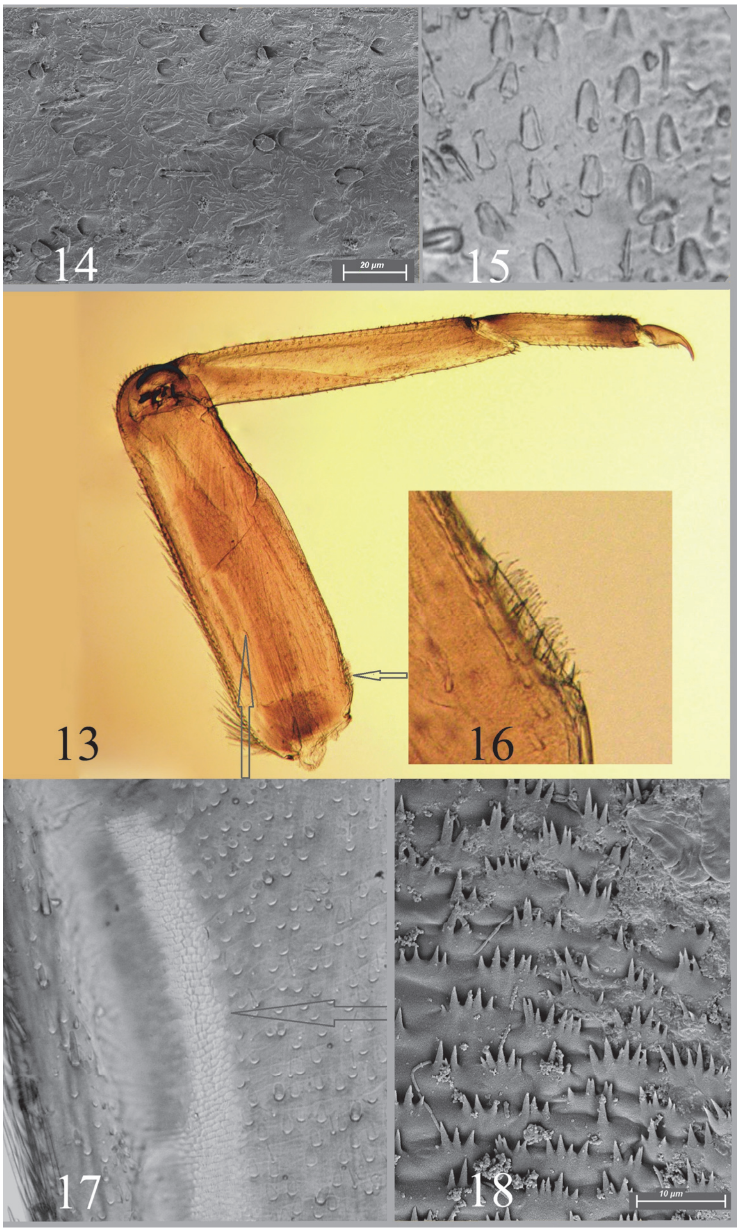
Figs 4–6. *Baetis moneronensis* **sp. n.**, larvae: 4 – antenna; 5, 6 – pedicel: 5 – externo-dorsal view; 6 – intero-ventral view.



Figs 7–12. *Baetis moneronensis* **sp. n.**, larvae, details of mouthparts, dorsal view: 7 – labrum; 8 – left mandible; 9 – right mandible; 10 – right maxilla; 11 – glossae, paraglossae; 12 – left labial palpus.

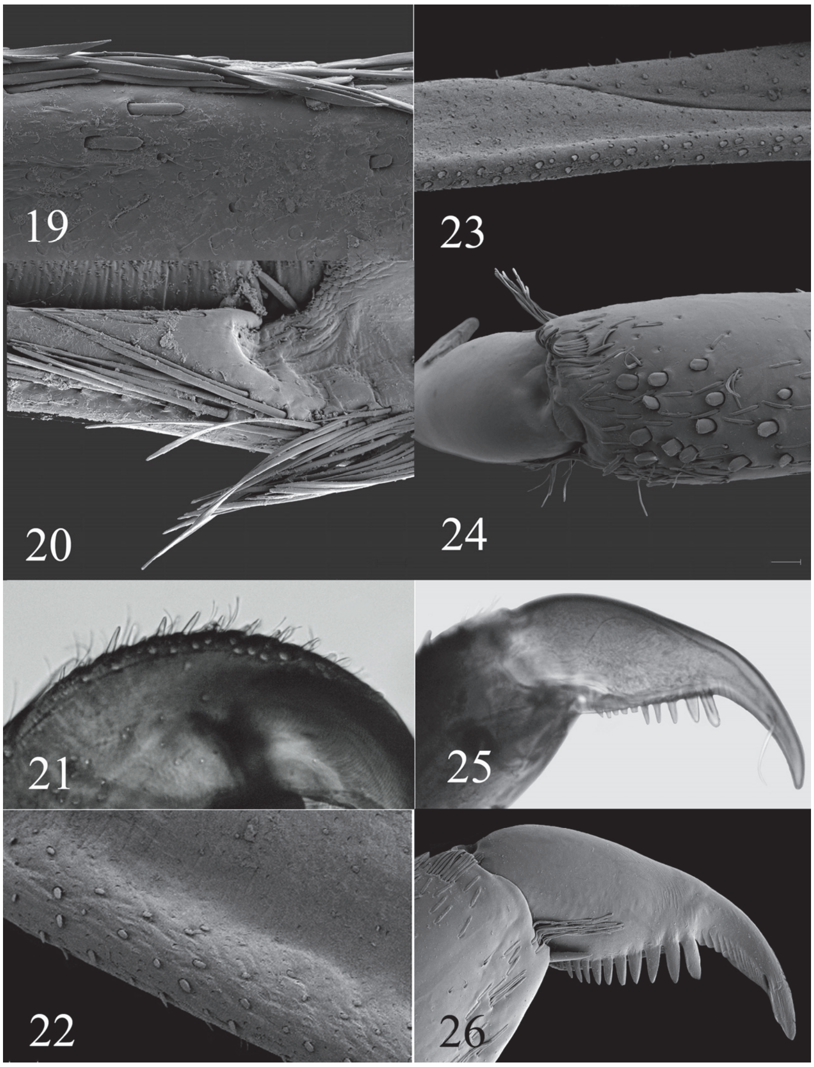
scales on the entire surface. Pedicel with similar scales and, on the externo-dorsal side, with longitudinal bunch of long pine needle-like setae, easily shed, variable in number (*ca.*9–11). (Fig. 5). Labrum with long pointed setae, forming dense row on anterior and both lateral margins, and submarginal groups approximately of 20–24 setae arranged in two closely-spaced irregular rows (Fig. 7). Ratio labrum width to length 1.7. Mandible (Figs 8, 9) broad, without a row of spines. Right mandible: prostheca hand-shaped; apical tooth of incisor clearly expressed, amplate, rounded apically; kinetodontium with four teeth, the largest – second (Fig. 8). Teeth of left mandible poorly separated; incisor amplate, convex apically; kinetodontium with five teeth, prostheca toothbrush- like (Fig. 9). Maxillary palpus rounded with a projection at apex, two-segmented (Fig. 10). Segments equal length, covered with medium-length hairs. Labium with long stout setae on apex and inner margin of narrow, long glossae and on apex and external margin of slightly bent paraglossae (Fig. 11). Glossae without subapical setal tuft. Labial palpus three-segmented: wide-rounded with a projection at apex; third segment densely covered with hairs over entire surface and long stout setae near apex; second segment with small rounded lobe on inner margin (Fig. 12).

Thorax: brown with vague light brown spots and dark brown spot on central part of mesonotum (Fig. 2). Legs sand-colored with brown spots and dark brown joints of leg segments (Figs 1, 13). Dorsal surface of legs densely covered with thin conical scales (Figs 14, 15). Femora sand-colored with bright brown spots: one large oblongated spot and one small cylindrical spot on central part, one big roundish spot at base (Fig. 13). Proximal part of femora of all legs with small ventral patch

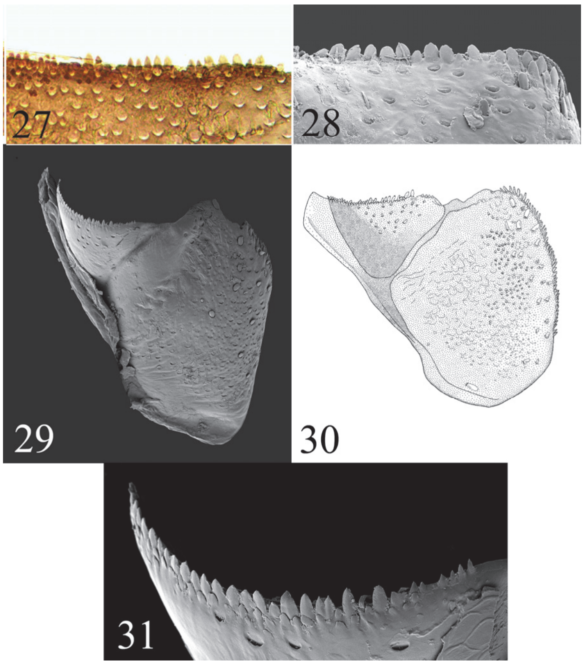


Figs 13–18. *Baetis moneronensis* **sp. n.**, larvae, details of legs (allows showing location of structure): 13 – mid leg, dorsal view; 14, 15 – conical scales of dorsal surface; 16 – ventral patch of closely spaced pointed hairs; 17 – part of submarginal dorsal band with serrated scales; 18 – serrated scales.

(villapore) of closely spaced pointed hairs surrounded by short pointed setae (Fig. 16) and long submarginal dorsal band of serrated scales (Figs 17, 18). Outer margin with several closely spaced rows of long, flat, pointed setae on proximal and medial part (Figs 19, 20) and closely spaced short, round in cross-section, pointed setae and numerous short hairs on distal apex (Fig. 21). Inner margin with irregular rows

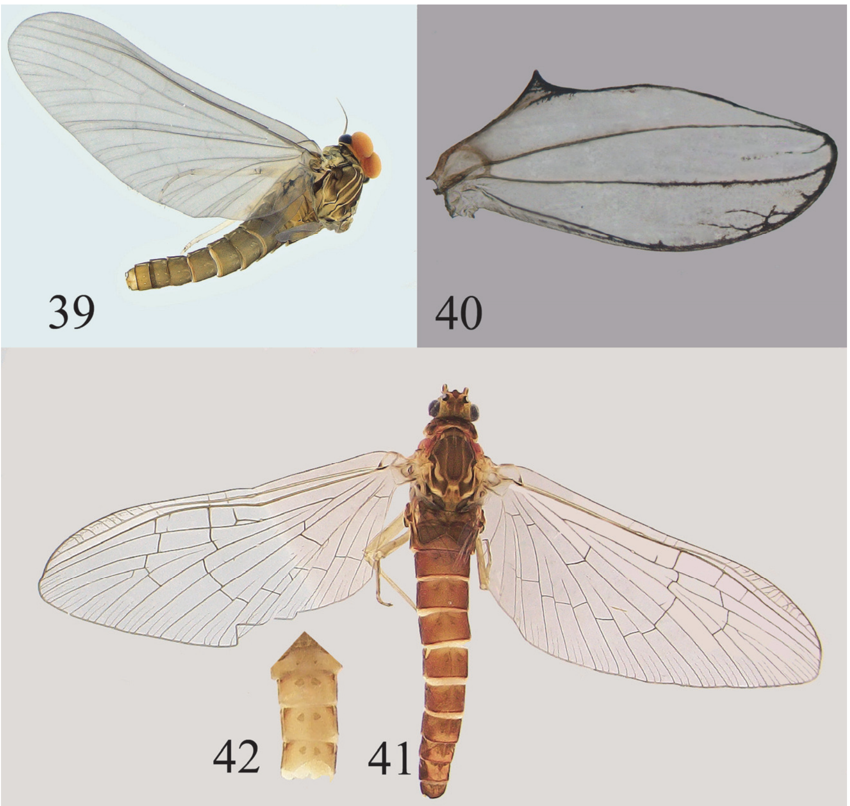


Figs 19–26. *Baetis moneronensis* **sp. n.**, larvae, details of legs. 19, 20 – setae of outer margin of femora; 21 – setae of distal apex of femora; 22 – setae of inner margin of femora; 23 – fragment of tibia; 24 – distal part of tarsus; 25, 26 – claw.



Figs 27–31. *Baetis moneronensis* **sp. nov.,** larvae, details of body: 27 – teeth on caudal margin of VI tergite; 28 – teeth on caudal margin of X tergite; 29,30 – paraproct; 31– teeth on caudal margin of paraproct.

Figs 32–38. *Baetis moneronensis* **sp. n.**, larvae, tergalii shape: 32 – I pair; 33 – II pair; 34 – III pair; 35 – IV pair; 36 – V pair; 37 – VI pair; 38 – VII pair.



Figs 39 – 42. *Baetis moneronensis* **sp. n.**, winged stages: 39–41 dorsal view: 39 – male subimago; 40 – female imago; 41 – hind wing of female imago. 42 – sternites I–IV.

of short, strong, flat, blunted setae (Fig. 22). Tibiae brown with the most brightly colored vestige of patella and with elongated light spot along inner margin of telotibia (Fig. 1). Outer margin of tibia with two uneven rows of sparse short flat blunt setae and thin short hairs between them; same bristles cover dorsal surface of wedge-shaped distal part of tibia; inner margin with two uneven rows of elongated and pointed setae far apart (Fig. 23). Distal one third of tarsus dark brown; marginal rows same as on tibia, distal apex with long thick hairs (Fig. 24). Claws light, with 10–11 teeth and pair of subapical setae (Figs 25, 26).

Abdomen: tergites with pair of dark brown spots located near anterior margin; margins dark brown; tergite I light brown; tergites II–IV, VI–VIII brown, lateral area lighter; tergites V, IX lighter than others (Fig. 2). Caudal margin of tergite VI with broadly conical, slightly rounded teeth (Fig. 27); caudal margin tergite X with oval teeth (Fig. 28). Paraproct with chaotically arranged short, roundish spatulate scales, with flat scales with a wide base and margines distinctly convergent towards a pointed apex and with numerous teeth on caudal and inner margins (Figs 29, 30).

Teeth plane, broad, conical, slightly pointed. On caudal margin situated in two rows (Fig. 31). Postmedial projection absent. All tergites with strong spatulate setae. Sternites sand-colored with light brown dim (Fig. 3.) Tergaliae on tergites I–VII (Fig. 1–3) oval, II–V asymmetrical, VII – elongated (Figs. 32 –38). All tergaliae whitish with dark brown costal margin; tracheation visible on tergaliae II–VII; margins hairy, saw-toothed: hairs inserted in small teeth-like bases; lamella covered thin hairs, along proximal margins sparse short, apically rounded tapering setae. Caudal filaments sand-colored; cerci about two times longer than paracercus (Figs 2, 3).

Male imago. Unknown.

Male subimago (in alcohol). Length (mm): body 7.0–7.2; fore wings 7.8–8.0; hind wings 1.6–1.7. General body color brown (Fig. 39). *Head*: Upper portion of compound eyes orange-brown; lower portion black. Ocelli whitish apically and black basally. *Thorax*: General color dark brown. Anterior part of anteronotal protuberance, median longitudinal suture, medioparapsidal suture, scutellum and parascutellum sand-colored. Wings opaque, grey, margin slightly pubescent. Legs whitish. *Abdomen*: Tergites brown with khaki tinge, with light oblique lateral strokes. Tergites II–VI with a light pattern along middle line: small paired oblique strokes near anterior margin and small paired rounded dots closer to middle. Tergites VII–IX with pair of small rounded dots in middle part. Tergite X light. Sternites light brown with khaki tinge, with two small light oblique strokes near anterior margin. Cerci unknown.

Female imago (in alcohol). Length (mm): body 8.5–9.3; fore wings 8.6–9.0; hind wings 1.5–1.6. General body color orange-brown (Fig. 40). Eyes dark grey. Fore wing transparent, pterostigma opaque white, veins brown. Hind wing with erected costal spur at ¼ of the length of wing, transparent, and approximately 2.4 times longer than wide; three longitudinal veins reaching margin, incomplete veinlets between main longitudinal veins. (Fig. 41). Sternites lighter than tergites, with two small brown roundish dots near anterior margin (Fig. 42).

Female subimago (in alcohol). Length (mm): body 8.6–8.9; fore wings 9.4–9.7; hind wings 1.2–1.6; cerci –11.2. General body color light brown. Pattern on terga similar to male. Sterna and cerci sandy-colored. Cerci with thin brown annulations at segment joints.

DISTRIBUTION AND BIOLOGY. Russian Far East. *Baetis (Rhodobaetis) moneronensis* **sp. n.** is known only from water streams of Moneron island, the Sea of Japan, off the southwestern coast of Sakhalin Island, Russia: Moneron River, Bolshoy Brook and Vodopadnyy Brook the stream valleys are incised into basalts, are largely free of segments represented by weakly silted sand with gravel or a thin layer of gravel. The streams are prone to the occurrence of mudflows, have high flow velocities and numerous waterfalls. The emergence of the species imago occurs from June to August.

ETIMOLOGY. The species is named after Moneron Island, the type locality where the new species was collected.

TAXONOMIC NOTES

The assignment of *B. moneronensi* **sp. n.** to the subgenus *Rhodobaetis* was based on a combination of larval characters: the presence of scales of different structure on the terga, on the antennal segments, on the surface of the femora, and on the paraproct (Godunko *et al.* 2004). These are spatulate scales and scales with a wide base and margins distinctly convergent towards a pointed apex. Furthermore, larvae of the new species possess a character previously known only for *B.* (*R.*) *thermicus*: setae on the externo-dorsal side of the pedicel. I. Jinichi & T. Yasuhiro (2005: p. 53, fig. 23 (1-3)) show the presence of only three subapical hairs arranged in a single horizontal row, while Shin-ichi Ishiwata *et al.* (2013: 182, fig. 10) provide an image of the pedicel with a bunch of four long pine needle-like setae. In *B. moneronensis* **sp. n.** the cluster of pine needle-like setae is elongated longitudinally, and their number is significantly higher but, due to brittleness and easy shedding, variable. Larvae of both species possess a significant number of submarginal setae on the labrum, but in *B. thermicus* (according to Ishiwata et *al.* 2013, referencing Gose (1980) and Kobayashi (1987)) there are 12–14, while in *B. moneronensis* **sp. n.** there are approximately 20–24. This morphological character significantly distinguishes the new species from other representatives of the subgenus *Rhodobaetis*. It is also important to note that both *B. thermicus* and *B. moneronensis* **sp. n.** have a pair of apical setae on the tarsal claw. The presence of this character runs counter to the opinion of R. Godunko *et al.* (2004), who list its absence as one of the characteristics of the subgenus *Rhodobaetis*.

RESULTS OF DNA BARCODING

In this study, we obtained a 658-bp COI sequence for *B. moneronensis* sp. nov. Based on this new sequence, we reconstructed a phylogenetic tree and performed species delimitation using ASAP analysis (Fig. 43). *B. moneronensis* was placed within the highly supported *B. thermicus* clade (Bayesian posterior probability = 1). However, the ASAP results indicate that *B. thermicus* is partitioned into five distinct molecular operational taxonomic units (mOTUs), which corresponds to Barcode Index Numbers (BINs) calculated by the BOLD system. All *B. thermicus* specimens analyzed were collected from various locations in Japan; notably, none originated from the type locality of *B. thermicus*, which is a hot spring called "Nakano-yu" about 1,500 m above sea level, at Kamikochi in Shinano Province, Japan (Ueno, 1931.) The average interspecific K2P distance between *B. moneronensis* and the *B. thermicus* mOTUs was 12.1% (maximum 17.4%). Among all *B. thermicus* lineages, *B. moneronensis* was most closely related to BOLD:AGE5477, BOLD:AHE2343, and BOLD:AEM5191, with K2P distances of 9.27%, 9.35%, and 9.49%, respectively (Tabl.1). Other Eastern Palaearctic species differed from *B. moneronensis* by an average of 23.4%, which is significantly higher than the distances to even the most divergent *B. thermicus* lineages (15.43–15.70%, Tabl.1). These genetic distances are consistent with species-level divergence within the genus *Baetis* (Morinière *et al.* 2017; Tiunova & Semenchenko 2019, 2020; Tiunova *et al.* 2021; Semenchenko & Tiunova 2025; Mu & Huang 2025). Thus, the established phylogenetic relationships and relatively low K2P distances indicate that *B. moneronensis* belongs to the *thermicus*-group.

Table 1. Intraspecific and interspecific K2P nucleotide distances (%) of five mOTUs of *Baetis thermicus* and *Baetis moneronensis* sp.n. estimated using *COI* sequences.

Species, mOTU	Intra sp.	Interspecific				
		1	2	3	4	5
1. *Baetis moneronensis* **sp.n.**	n/a					
2. *Baetis thermicus* BOLD:AEM5191	0.24	9.49				
3. *Baetis thermicus* BOLD:AHE2343	0.00	9.35	4.00			
4. *Baetis thermicus* BOLD:AGE5477	1.23	9.27	8.38	7.33		
5. *Baetis thermicus* BOLD:ACQ6214	1.03	15.43	14.95	13.94	16.89	
6. *Baetis thermicus* BOLD:AEM5190	1.24	15.70	16.40	15.89	17.45	7.65

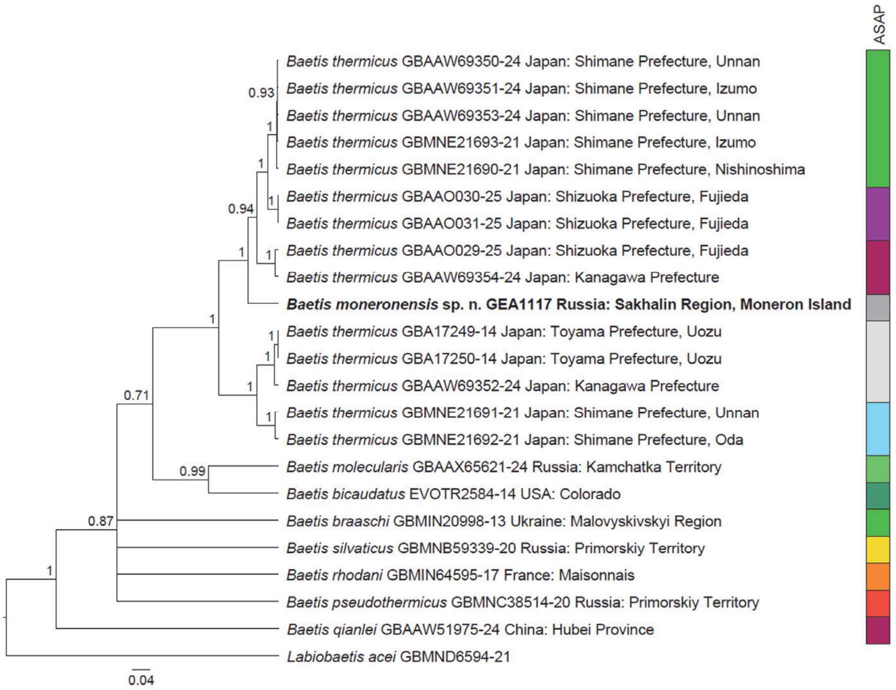


Fig. 43. Ultrametric Bayesian inference (BI) tree based on cytochrome c oxidase subunit I (*COI*) nucleotide sequence data of the genus *Baetis*. Bayesian posterior probabilities are indicated at the nodes. The specimen obtained in this study is shown in bold. *Labiobaetis acei* was used as the outgroup. Vertical colored bars on the right indicate molecular operational taxonomic units (mOTUs) identified by ASAP analysis.

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E.A. designed the study, performed the morphological analysis and wrote the manuscript. A.A. conducted the molecular research (DNA barcoding) and performed the Bayesian inference. All authors reviewed and approved the final version. The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No. 124012400285-7).

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