



## Morphological description and DNA barcoding of *Hydrobaenus golovinensis* sp. nov. (Diptera: Chironomidae: Orthocladiinae) from the Russian Far East

EUGENYI A. MAKARCHENKO<sup>1,3</sup>, MARINA A. MAKARCHENKO<sup>1</sup>,  
ALEXANDER A. SEMENCHENKO<sup>2</sup> & OLEG A. VELIAEV<sup>2</sup>

<sup>1</sup>Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, 100 let Vladivostoku 159, 690022 Vladivostok, Russia. E-mail: [makarchenko@biosoil.ru](mailto:makarchenko@biosoil.ru)

<sup>2</sup>Far Eastern Federal University, Suhanova St. 8, 690950 Vladivostok, Russia. E-mail: [semenchenko\\_alexander@mail.ru](mailto:semenchenko_alexander@mail.ru)

<sup>3</sup>Corresponding author

### Abstract

Illustrated descriptions of adult male, pupa and fourth instar larva, as well as DNA barcoding results of *Hydrobaenus golovinensis* sp. nov. in comparison with closely related species *H. majus* Makarchenko et Makarchenko and *H. sikhotealinensis* Makarchenko et Makarchenko from the Russian Far East are provided. Partial mitochondrial COI gene (DNA barcoding) of the new species has been sequenced and uploaded to GenBank. Comparisons with corresponding regions of COI between *H. golovinensis* and other species in the genus produce K2P genetic distances of 10.3–14.3%, the values well above those associated with intraspecific variation. In contrast, genetic distances among 18 specimens are all within the range of 0–3.5%. The ML tree is also constructed using DNA barcodes obtained in the present study and those of other species of *Hydrobaenus* Fries from GenBank.

**Key words:** Diptera, Chironomidae, *Hydrobaenus*, new species, taxonomy, DNA barcoding, Russian Far East

### Introduction

In the spring of 2016 in the Amur River basin, a new species of the genus *Hydrobaenus* Fries was collected. Morphological studies revealed very slight differences between adult males of this species and those of *H. sikhotealinensis* Makarchenko et Makarchenko and *H. majus* Makarchenko et Makarchenko, while the pupae and larvae are well distinguishable.

The morphological description and results of DNA barcoding of the new species, here named *Hydrobaenus golovinensis*, are thus presented in comparison with those taken from its closest relatives. As a result, we provide an updated part of a key to species of *Hydrobaenus* from the Russian Far East (Makarchenko & Makarchenko 2014). A partial mitochondrial COI gene (DNA barcoding) of the new species has been sequenced, as those used recently for identification of non-biting midges (Montagna et al. 2016; Ekrem et al. 2010; Carew et al. 2005), including Orthocladiinae (Silva & Wiedenbrug 2014, Makarchenko et al. 2015), and uploaded to GenBank. K2P genetic distances and ML tree has also been used to establish specific independence of the new species.

### Materials and methods

The larva, pupa and adult specimens of *Hydrobaenus golovinensis* were associated by using of DNA barcoding as well as for comparing of a new species with its closely related species. The terminology follows Sæther (1980). The material was preserved in 96% ethanol for DNA-analysis and in 70% ethanol for further study of morphology, and slide-mounted following the methods by Makarchenko (1985). Genomic DNA was extracted with using the Qiagen DNeasy® Blood and Tissue kit, following the extraction protocol. Polymerase chain reaction was

performed to amplify the COI barcode fragment using the DreamTaq DNA Polymerase (ThermoFisher scientific) and the primers COIF-ALT (5'-ACAAATCAYAARGAYATYGG-3') and COIR-ALT (5'-TTCAGGRTGNCCRAARAAYCA-3') (Mikkelsen *et al.* 2006). PCR was carried out in a total volume of 10 µl and contained 0.1 µL of DreamTaq DNA Polymerase, 1 µL of DreamTaq DNA Polymerase buffer, 0.25 µL dNTPs, 7.4 µL nuclease-free water, 0.375 µL of each primer (100 ng/µl), and 0.5 µL of extracted DNA. The PCR thermal conditions included an initial denaturation at 94 °C (2.5 min), followed by 35 cycles at 95 °C (denaturation, 30 s), 50 °C (annealing, 30 s), 72 °C (extension, 70 s), and a final extension step at 72 °C (10 min). All PCR products were verified using electrophoresis on a 1.5% TBE agarose by visualizing on GelDoc XR+ imaging systems (Bio-Rad), only 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 BrightDye™ Terminator Cycle sequencing kit by Nimagen in accordance with the protocol. Each sequencing reaction was purified by ethanol precipitation described in the protocol. Finally, all samples were analyzed in an ABI 3130XL® (Applied Biosystems) automated capillary sequencer. We used MEGA7 (Kumar *et al.* 2016) to edit and assemble double stranded sequences. Based on the Kimura-2-Parameter (K2P) model were calculated inter- and intraspecific genetic distances and constructed ML-tree (GTR+G) using MEGA7. The reliability of the internal branches in the ML-tree was assessed by 1000 bootstrap replicates.

Holotype and paratypes of the new species are deposited in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (FSCEATB FEB RAS). DNA sequences of *Hydrobaenus golovinensis* are available in GenBank (NCBI) by numbers KY399212–KY399229.

## Descriptions

### *Hydrobaenus golovinensis* Makarchenko *et* Makarchenko, sp. nov.

(Figs. 1–20)

**Material.** Holotype: adult male, Russian Far East, Khabarovsk Territory, Khabarovsk district, Bolshekhokhtsirsky Nature Reserve, Ussuri River basin, Golovina Stream, N 48°11'131", E 134°41'039", 13.V. 2016, leg. N. Yavorskaya. Paratypes: 4 adult males, 6 mature pupae (males), 1 larva, the same data as holotype; 1 larva, the same data as holotype, except 6.V. 2016; 3 adult males, Primorye Territory, Nadezhdinsky District, unnamed stream not far from railway station "32<sup>nd</sup> kilometer", N 43°26'29", E 131°59'10", leg. O. Orel.

**Etymology.** The specific epithet *golovinensis* is derived from the name of the Golovin Stream, where the type material was collected.

**Adult male** (n=5). Total length 2.5–3.1 mm. Wing length 1.8–2.0 mm. Total length/wing length 1.50–1.61.

Head. Eyes slightly pubescent, with short dorsomedian prolongations Temporal setae: 5–6 outer verticals, 2–3 inner verticals and 4–5 postorbitals. Clypeus with 5–8 setae. Antenna with 13 flagellomeres and well developed plume; 13<sup>th</sup> flagellomere 480–528 µm long, with apex pointed. AR 1.07–1.17. Length of palpomeres 2–5 (in µm): 44–52, 80–96, 72–92, 120–128.

Thorax. Dark brown. Antepnotum with 4–9 lateral setae. Ac 12–16 (short and scalpellate, situated in middle of mesonotum), Dc 5–7, Pa 3–5, Scts 9–10 in one row on yellow background.

Wing. R with 5–8 setae, R<sub>1</sub> without setae, R<sub>4+5</sub> with 1–2 setae apically. R<sub>4+5</sub> ending distally of apex M<sub>3+4</sub>. Costa extension 32–40 µm long. Cu<sub>1</sub> curved in apical part. Anal lobe well developed, rounded. Squama with 6–21 setae.

Legs. BR<sub>1</sub> 1.7–2.6, BR<sub>2</sub> 1.7–2.0, BR<sub>3</sub> 2.0–4.2. Spur of fore tibia 48–52 µm long. Spurs of mid tibia 16–20 µm and 20–22 µm long. Spurs of hind tibia 48–60 µm and 12–20 µm long. Hind tibial comb with 12–14 setae. Basitarsus of hind leg with 6–9 sensilla chaetica in basal part. Lengths and proportions of legs as in Table 1.

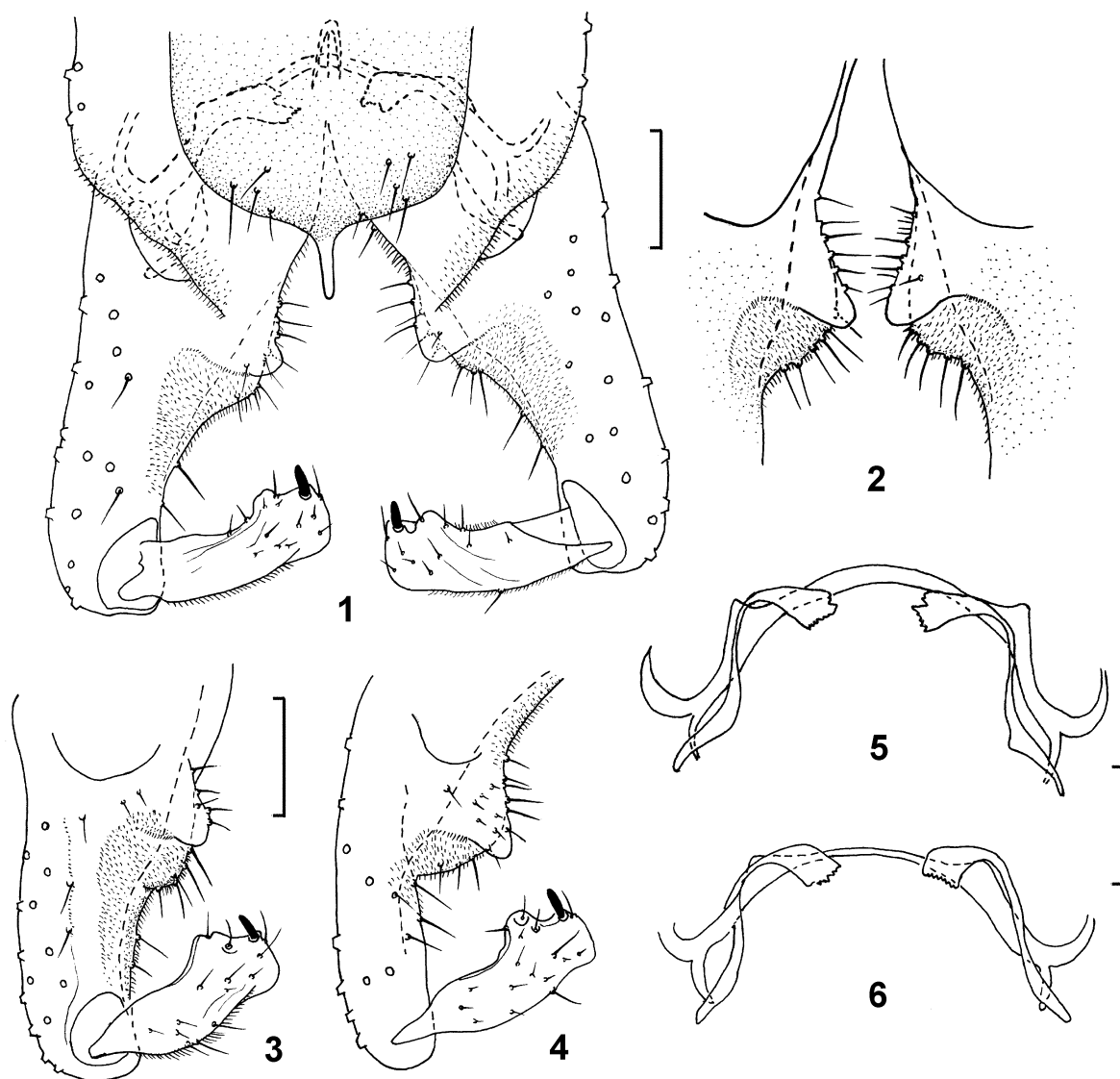
Hypopygium (Figs. 1–6). Tergite IX roundish, with 7–12 setae; anal point bare, 20–28 µm long, with rounded apex. Laterosternite IX with 5–7 setae on each side. Transverse sternapodeme 96–108 µm long, with slightly triangular projections (Fig. 5). Virga 32–36 µm long, consisted of 3–4 setae. Phallapodeme dark in distal part, wide and tape-like, slightly serrated apically (Figs. 5–6). Gonocoxite 200–236 µm long; inferior volsella as shown in Figs. 1–4; dorsal part of inferior volsella nose-like, without microtrichia but with setae; ventral part of inferior

volsella covered with microtrichia and setae. Gonostylus 84–100  $\mu\text{m}$  long, with preapical rounded crista dorsalis (Figs. 3–4); megaseta 8–12  $\mu\text{m}$  long.

**Pupa** (n=4). Total length 2.90–3.5 mm. Cephalothorax brownish, abdominal tergites light brownish or yellowish. Exuviae transparent, only dorsal part of mesonotum darker. Tergites II–VIII and sternites II–VIII with brown or dark brown apophyses.

**TABLE 1.** Lengths (in  $\mu\text{m}$ ) and proportions of leg segments of *Hydrobaenus golovinensis* sp. nov., male (n=4)

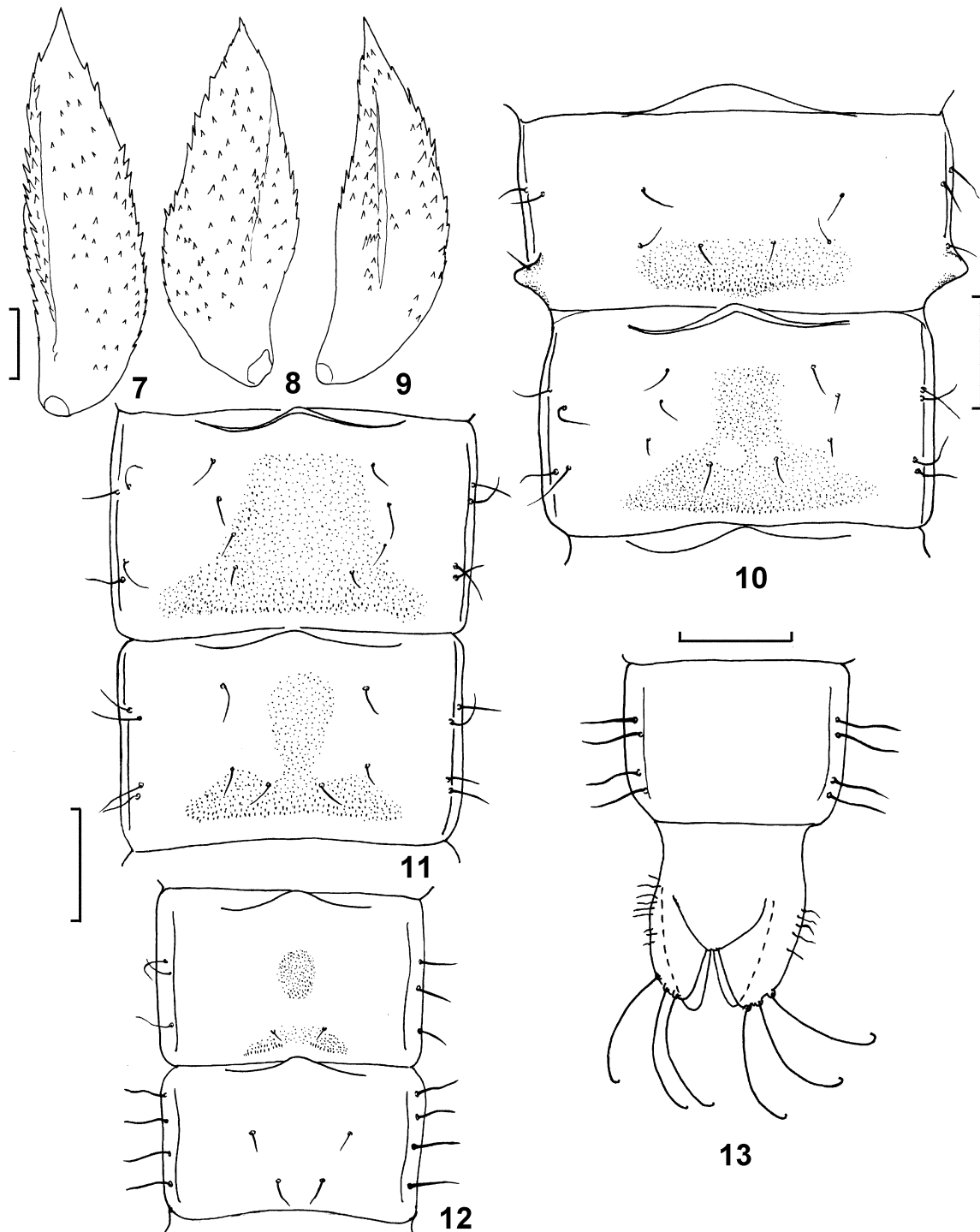
	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	560–672	736–864	464–544	288–368	208–240	144–160	112–120	0.60–0.65	2.22–2.40	2.70–2.93
P <sub>2</sub>	624–720	656–752	320–352	192–224	136–160	112	96–112	0.47–0.49	2.97–3.0	3.90–4.18
P <sub>3</sub>	656–752	800–896	416–480	240–288	176–208	112	104–112	0.51–0.54	2.92–2.96	3.43–3.54



**FIGURES 1–6.** Adult male of *Hydrobaenus golovinensis* sp. nov. **1**, hypopygium in dorsal view; **2**, inferior volsellae; **3–4**, gonocoxite and gonostylus; **5–6**, TSA and phallapodemes. Scale bars 50  $\mu\text{m}$ .

Cephalothorax. Frontal apotome with 2 setae 68–80  $\mu\text{m}$  long and with well developed warts. Anteprepronotum with two median and two lateral anteprepronotal setae 36–88  $\mu\text{m}$  long; both lateral anteprepronotals thin and hair-like. Thoracic horn 244–288  $\mu\text{m}$  long, up to 60–84  $\mu\text{m}$  wide, leaf-shaped with strongly pointed apex, covered by spinules (Figs. 7–9). Precorneal setae lengths (in  $\mu\text{m}$ ): Pc<sub>1</sub> 52–80, Pc<sub>2</sub> 92–120, Pc<sub>3</sub> 32–44. Bases of setae arranged

in form of triangle. Surface of mesonotum covered with small spines along suture. Dorsocentrals thin and hair-like;  $Dc_{1-4}$  40–80  $\mu\text{m}$  long. Distance between  $Dc_1$  and  $Dc_2$  36–52  $\mu\text{m}$ ; between  $Dc_2$  and  $Dc_3$  44–64  $\mu\text{m}$ ; between  $Dc_3$  and  $Dc_4$  28–40  $\mu\text{m}$ .



**FIGURES 7–13.** Pupa of *Hydrobaenus golovinensis* sp. nov. 7–9, thoracic horn; 10, tergites II–III; 11, tergites IV–V; 12, tergites VI–VII; 13, tergite VIII and anal segment. Scale bars: Figs. 7–9 – 50  $\mu\text{m}$ ; Figs. 10–13 – 200  $\mu\text{m}$ .

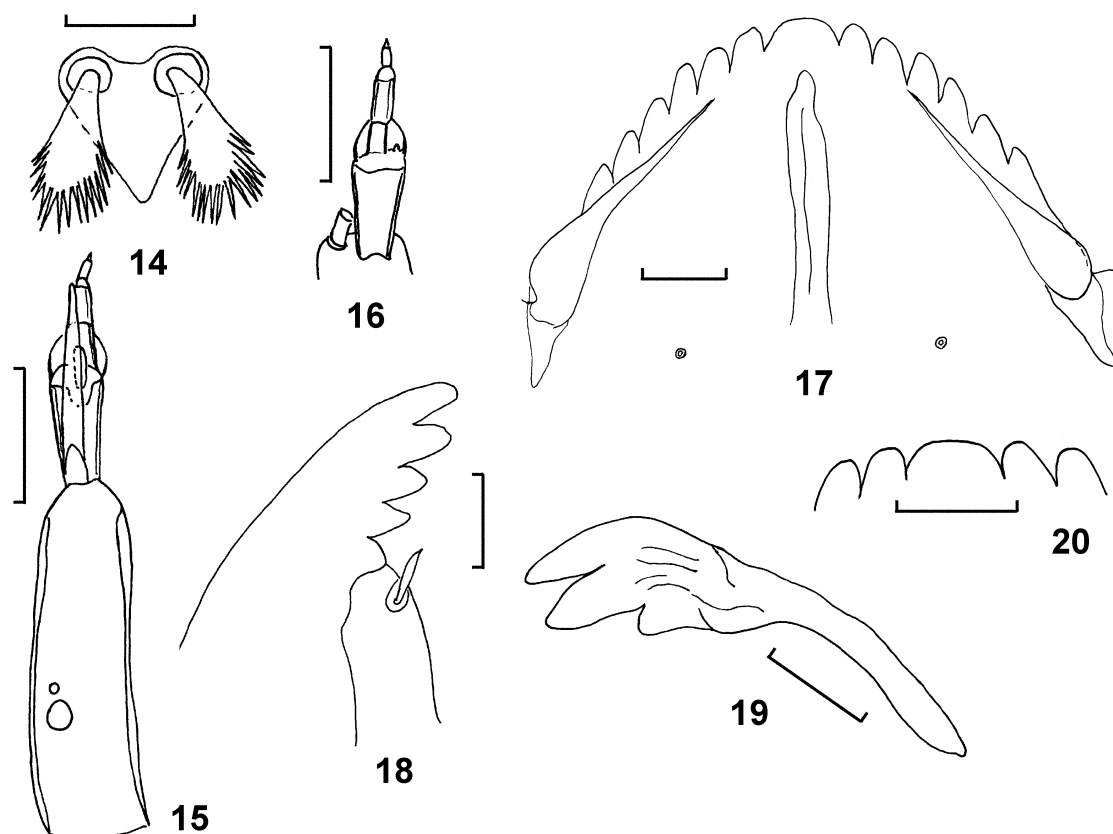
Abdomen. Tergite I without shagreen. Tergite II with shagreen of spinules in middle of posterior half; shagreen at posterior edge with relatively large spines, their apex pointed orally (Fig. 10). Shagreenation of tergites III–VI as shown in Figs 10–12, spinules at posterior edge larger than in middle part. Tergites VII–IX almost bare (Figs. 12–13). Sternites I–IV, VII–IX without shagreen. Sternites V–VI with weakly shagreen along posterior edge. Segment II with PSB. Sternites IV–VII with PSA. Segment I with 0–1 pairs of lateral setae. Segment II with 3–4

pairs of hair-like lateral setae. Segments III–VI with 4 pairs of simple lateral seta. Segments VII mostly with 4 pairs of taeniate lateral setae, and one specimen with 3 pairs of taeniate lateral setae (Fig. 12). The number of setae on segment VIII variable: from 3 to 5, exceptionally segment VIII with 3 taeniate lateral setae from one side and 4 taeniate lateral setae from another side ( $n = 1$ ) or with 5 taeniate lateral setae from one side and 4 taeniate and 1 simple lateral setae from another side ( $n = 1$ ) or 4 taeniate lateral setae from one side and 4 taeniate and 1 simple lateral setae from another side ( $n = 2$ ). Anal lobe 240–288  $\mu\text{m}$  long, with fringe of 4–12 setae which 8–76  $\mu\text{m}$  long, occupy middle part of anal lobe edge (Fig.13). Male genital sac overreaching anal lobe, 20–76  $\mu\text{m}$ . Anal macrosetae 180–200  $\mu\text{m}$  long.

**Fourth instar larva** ( $n=2$ ). Total length 4.7–5.7 mm.

Head. Brown, with postoccipital margin, teeth of mentum, mandible and premandible almost black. Labral setae  $S_I$  plumose, labral lamella abutting sockets of  $S_I$  triangular (Fig. 14);  $S_{II}$  strong,  $S_{III}$  weak and hair-like,  $S_{IV}$  short, tubercle-like. Pecten epipharyngis consisted of 3 long and narrow scales. Premandible distally with 2 long apical teeth and 1 short tooth nearest (Fig. 19). Antenna with 6 segments; AR 1.22–1.50; apex of segment 2 with stylus and lauterborn organs ending at apex of 3<sup>rd</sup> segment; antennal blade ending near base of 5<sup>th</sup> segment; one large and one small ring organs in proximal 1/3 of basal segment (Figs.15–16). Mandible with apical tooth, which shorter of combined width of inner teeth and the same length as 3<sup>rd</sup> inner tooth; seta interna with 6 bare branches, seta subdentalis long, with beak-shaped apex (Fig. 18). Mentum with 1 median tooth and 6 pairs of dark brown or black lateral teeth; median tooth 1.75–2.3 time wider than first lateral tooth. Setae submenti at level of posterior edge of ventromental plates. Ventromental plates extending beyond last lateral tooth (Figs. 17, 20). Maxilla with pecten galearis.

Abdomen. Procercus length/width ratio 1.4, with 7 anal setae 270–380  $\mu\text{m}$  long and 2 thin lateral setae. Supraanal setae 120  $\mu\text{m}$  long. Anal tubules elongated, egg-shaped, shorter than posterior parapods. Posterior parapods with simple hooks in apex.



**FIGURES 14–20.** Larva of fourth instar of *Hydrobaenus golovinensis* sp. nov. **14**,  $S_I$  and labral lamella; **15**, antenna; **16**, antennal segments 2–6; **17**, mentum; **18**, distal part of mandible; **19**, premandible; **20**, middle teeth of mentum. Scale bars 20  $\mu\text{m}$ .

**Diagnostic characters and taxonomic notes.** The new species is closely related to the Far Eastern species *Hydrobaenus majus* and *H. sikhotealinensis*. Adult males of these species are weakly separable from the *H. golovinensis* by morphological features but only the new species has the hypopygium with expanding and dark phallapodemes in distal part, wide and tape-like, slightly serrated apically (Figs. 5–6). The phallapodemes of other species not expanding and straight apically, turned to the middle and without the serrated edge. Data for all other features overlap. In such cases, a comparative study of the morphology of the pupae and larvae, as well as DNA barcoding can provide characters for identification of the species. Pupae and larvae of *H. sikhotealinensis*, *H. majus* and *H. golovinensis* are easily separable by several features. *H. sikhotealinensis* has a pupa with the thoracic horn 310–320 µm long, tapering towards the tip (Makarchenko *et al* 2009: Fig. 48); the abdominal segment VI with 3 pairs of simple lateral setae and the segment VIII with 5 pairs of taeniate lateral setae (Makarchenko *et al.* 2009: Fig. 49); the mentum of larva with one light-brown or brown median tooth and six pairs of lateral teeth of the same colour, the median tooth 4.0–4.5 times wider than the first lateral tooth and the head yellow. *H. majus* has a pupa with the thoracic horn 368–400 µm long, with the extension in distal third (Makarchenko *et al.* 2015: Figs. 9–10); the abdominal segment VI with 1–2 pairs of short hair-like and 2 pairs long taeniate lateral setae, segment VIII with 4 pairs of taeniate lateral setae (Makarchenko *et al.* 2015: Fig. 12); the mentum of larva with 2 yellow or yellowish median teeth and 6 pairs of dark brown lateral teeth (Makarchenko *et al.* 2015: Figs. 15, 17). *H. golovinensis* has the leaf-shaped, 244–288 µm long thoracic horn, with strongly pointed apex (Figs. 7–9), the abdominal segment VI with 4 pairs of hair-like lateral setae, and the segment VIII with 3–5 pairs of taeniate lateral setae, sometimes with 4 taeniate and 1 hair-like lateral seta; the mentum of larva has 1 median tooth and 6 pairs of dark brown or black lateral teeth; median teeth 1.75–2.3 times wider than the first lateral tooth, and the head brown or brownish (Figs. 17, 20).

A comparative characteristic of adults, pupae and larvae of closely related species is also given in our updated keys based on that by Makarchenko & Makarchenko (2014: 431), as well as the results of DNA barcoding.

## Updated keys to species of *Hydrobaenus* Fries from the Russian Far East (part)

### Adult males

11. Dorsal part of inferior volsella with finger-like projection, ventral part short and rounded . . . . . *H. monodentatus* Makarchenko *et* Makarchenko
- Dorsal part of inferior volsella rounded-triangular or triangular, ventral part slightly protruding . . . . . 12
12. Tergite IX narrow; transverse sternapodeme with massive and rounded oral projections; crista dorsalis of gonostylus low and transparent . . . . . *H. maiorovi* Makarchenko *et* Makarchenko
- Tergite IX wide; oral projections of transverse sternapodeme never as above of different shape; crista dorsalis of gonostylus rounded-triangular, protruding, not transparent . . . . . 13
13. Phallapodemes expanding and serrated along edge apically (Figs. 5–6) . . . . . *H. golovinensis* **sp. nov.**
- Phallapodemes not expanding and straight apically, turned to the middle and without serrated edge . . . . . 14
14. Shape of posterolateral section angle of tergite IX angular . . . . . *H. sikhotealinensis* Makarchenko *et* Makarchenko
- Shape of posterolateral angle of tergite IX is rounded . . . . . *H. majus* Makarchenko *et* Makarchenko

### Pupae

4. Anal lobe fringe located around lateral edge to base of megasetae . . . . . 5
- Anal lobe fringe located in anterior two-thirds of anal lobe and not reaching base of megasetae . . . . . 8
5. Thoracic horn with rounded apex. Anal lobe fringe with 15–23 setae . . . . . *H. maladistinctus* Makarchenko *et* Makarchenko
- Thoracic horn with pointed apex. Anal lobe fringe with various number of setae . . . . . 6
6. Tergite VI with 3 pairs of hair-like lateral setae. Tergite II with posterior hooklets in 6–7 rows . . . . .
- . . . . . *H. sikhotealinensis* Makarchenko *et* Makarchenko
- Tergite VI 3–4 pairs of hair-like and taeniate lateral setae. Tergite II with simple spinules (not hooklets) in 3–4 rows . . . . . 7
7. Thoracic horn 368–400 µm long, with extension in distal third, covered by spinules (Makarchenko *et al* 2015: Figs. 9–10) . . . . .
- . . . . . *H. majus* Makarchenko *et* Makarchenko
- Thoracic horn 260–320 µm long, almost parallel-sided, slightly narrowing subapically (Makarchenko *et al.* 2009: Fig. 13) . . . . .
- . . . . . *H. kisosecundus* Sasa *et* Kondo
8. Anal lobe fringe located in anterior one-third of anal lobe. Segment V with 2 pairs of hair-like lateral setae and 2 pairs of taeniate lateral setae . . . . . *H. conformis* (Holmgren)
- Anal lobe fringe located in anterior two-thirds of anal lobe. Segment V with 4 pairs of hair-like lateral setae, sometimes setae of one pair taeniate . . . . . 9
9. Thoracic horn leaf-shaped. Anal lobe fringe with 4–12 setae 8–76 µm long . . . . . *H. golovinensis* **sp. nov.**
- Thoracic horn never leaf-shaped. Anal lobe fringe with 16–25 setae 100–130 µm long . . . . . 10

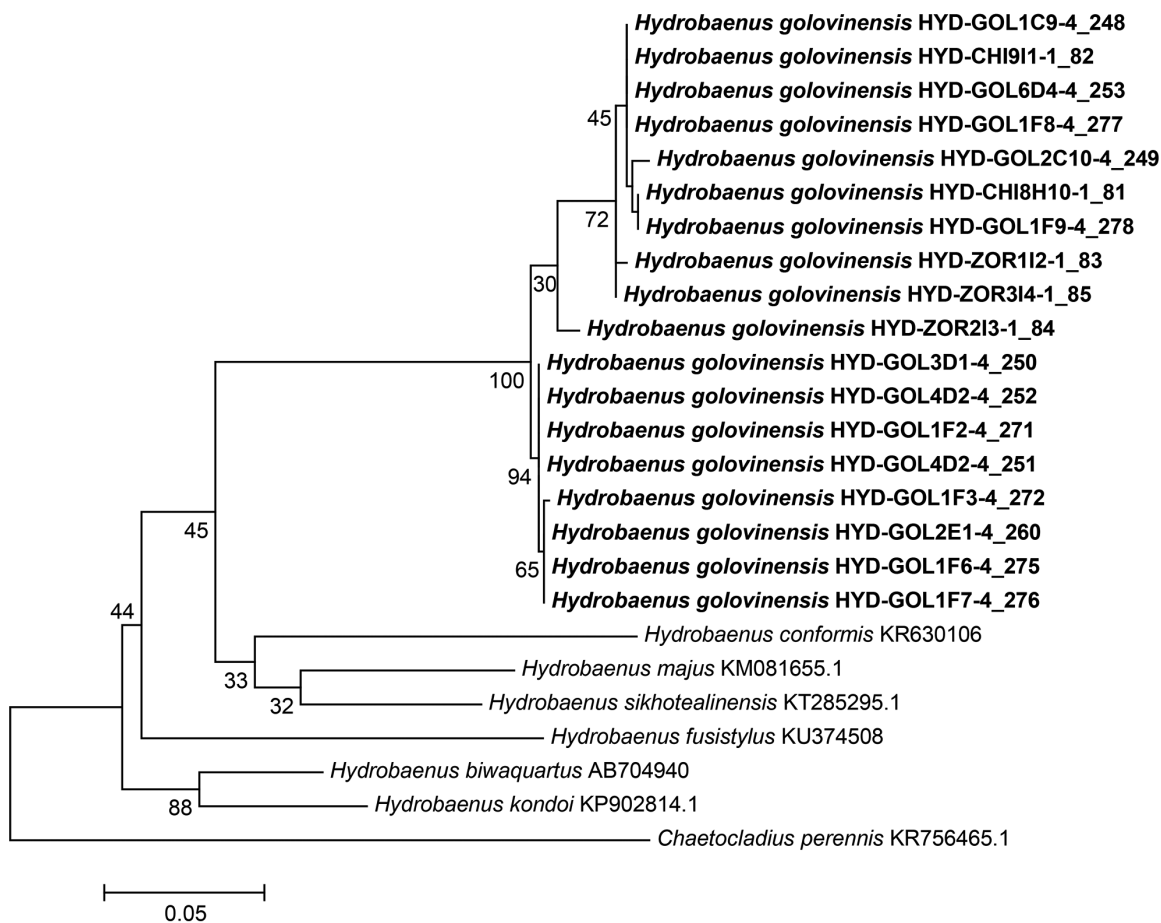
10. Segment VI with 2 pairs of hair-like and 2 pairs of taeniate lateral setae. Segment VIII with 5 pairs of taeniate lateral setae. Genital sheath of male with dark papilla. Tip of anal lobe smooth. . . . . *H. fusistylus* (Goetghebuer)
- Segment VI with 1 pair of hair-like and 3 pairs of taeniate lateral setae. Segment VIII with 4 pairs of taeniate lateral setae. Genital sheath of male without papilla. Tip of anal lobe rugulose . . . . . *H. biwaquartus* Sasa et Kawai

Larvae of fourth instar

7. Median tooth of mentum brown, as all lateral teeth, with rounded tip . . . . . 8
- Median tooth of mentum lighter than lateral teeth, with convex or straight tip . . . . . 9
8. Median tooth of mentum with rounded tip, 4–4.5 times wider than first lateral tooth. Head yellowish. Apical tooth of mandible lighter than other teeth. . . . . *H. sikhotealinensis* Makarchenko et Makarchenko
- Median tooth of mentum with slightly convex tip, 1.75–2.3 times wider than first lateral tooth. Head brownish. Apical tooth of mandible brown as other teeth . . . . . *H. golovinensis* sp. nov.
9. Median tooth of mentum with straight tip, 4–4.5 times wider than first lateral tooth. AR 2.7–2.8. Body length 4.8–5.2 mm . . . . . *H. kisosecundus* Sasa et Kondo
- Median tooth of mentum with convex tip, 2–2.3 times wider than first lateral tooth. AR 1.1–1.3. Body length 3.1–3.3 mm . . . . . *H. monodentatus* Makarchenko et Makarchenko

**Results of DNA barcoding**

Overall, we have sequenced fragments of the cytochrome oxidase I (658 bp) of 18 specimens of *Hydrobaenus golovinensis*. The nucleotide composition of the studied sequences has following values: 40,6 % of T, 16,8 % of C, 25,9 % of A, 16,7% of G. Totally, we have determined 31 variable sites, and only one of them contains a transversion. Our analysis of the amino-acid structure has shown that two substitutions are non-synonymous and have methionine or isoleucine in protein sequence (positions 155 and 165).



**FIGURE 21.** A phylogenetic tree of the 7 species of the genus *Hydrobaenus* and *Chaetocladus perennis* (Meigen) (outgroup) based on the mitochondrial cytochrome c oxidase I (COI) barcode gene sequences (658 bp). The phylogenetic reconstruction was performed with the Maximum Likelihood analysis (GTR+G distance model), implemented in MEGA version 7.0 (Kumar et al. 2016). Numbers are support values for 1000 bootstraps. Specimens obtained in this study are in bold.

Pairwise sequence divergence (K2P) within *H. golovinensis* ranges from 0% to 3.5%. Studied sequences have been compared with sequences of other species of the genus *Hydrobaenus*. Intraspecific pairwise distances (K2P) between *H. golovinensis* and six other species range from 10.3% to 14.3% (the average value is 12%), which is acceptable for an intraspecific level (Montagna *et al.* 2016). The Maximum-likelihood (GTR+G) analysis has shown that *H. golovinensis* individuals analysed form a monophyletic group, with a maximum support (100% bootstrap value) (Fig. 21), thus the molecular analyses pointed to the independence of this new species.

## Acknowledgments

We are grateful to Dr. N.M. Yavorskaya from the Institute of Water and Ecological Problems FEB RAS (Khabarovsk), O.V. Orel from the Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS for making material available to us and to Dr. R.S. Andronova from the Joint Directorate of State Natural Reserves and National Parks of Khabarovsk Territory “Zapovednoye Priamurye” for support of investigations in Bolshekhkhtsirsky Nature Reserve. We wish also to acknowledge Dr. Martin Spies (Munich, Germany) for sending us copies of hard-to-reach taxonomical articles and Dr. Wojciech Gilka (Gdańsk, Poland) for editing the manuscript. This work partly was supported by the Russian Science Foundation (project No. 14-50-00034).

## References

- Carew, M.E., Pettigrove, V. & Hoffmann, A.A. (2005) The utility of DNA markers in classical taxonomy: using cytochrome oxidase I markers to differentiate Australian *Cladopelma* (Diptera: Chironomidae) midges. *Annals of the Entomological Society of America*, 98, 587–594.  
[https://doi.org/10.1603/0013-8746\(2005\)098\[0587:TUODMI\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2005)098[0587:TUODMI]2.0.CO;2)
- Ekrem, T., Stur, E. & Hebert, P.D.N. (2010) Females do count: Documenting Chironomidae (Diptera) species diversity using DNA barcoding. *Organisms Diversity & Evolution*, 10, 397–408.  
<https://doi.org/10.1007/s13127-010-0034-y>
- Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33 (7), 1870–1874.  
<https://doi.org/10.1093/molbev/msw054>
- Makarchenko, E.A. (1985) *Chironomids of the Soviet Far East. Subfamilies Podonominae, Diamesinae and Prodiamesinae (Diptera, Chironomidae)*. DVNC AN SSSR Press, Vladivostok, 208 pp. [in Russian]
- Makarchenko, E.A., Makarchenko, M.A. & Yavorskaya, N.M. (2009) New records of chironomids (Diptera, Chironomidae, Orthoclaadiinae) in Far East and bordering territories. VI. *Hydrobaenus* Fries. *Euroasian Entomological Journal*, 8 (supplement 1), 33–50. [in Russian]
- Makarchenko, E.A. & Makarchenko, M.A. (2014) On taxonomy of *Hydrobaenus* Fries, 1830 (Diptera: Chironomidae: Orthoclaadiinae) from the Russian Far East, with a key to species. *Zootaxa*, 3760 (3), 429–438.  
<https://doi.org/10.11646/zootaxa.3760.3.9>
- Makarchenko, E.A., Makarchenko, M.A. & Semenchko, A.A. (2015) Morphological description and DNA barcoding of *Hydrobaenus majus* sp. nov. (Diptera: Chironomidae: Orthoclaadiinae) from the Russian Far East. *Zootaxa*, 4000 (2), 287–293.  
<https://doi.org/10.11646/zootaxa.4000.2.7>
- Mikkelsen, P.M., Bieler, R., Kappner, I. & Rawlings, T.A. (2006) Phylogeny of Veneroidea (Mollusca: Bivalvia) based on morphology and molecules. *Zoological Journal of the Linnean Society*, 148 (3), 439–521.  
<https://doi.org/10.1111/j.1096-3642.2006.00262.x>
- Montagna, M., Mereghetti, V., Lencioni, V. & Rossaro, B. (2016) Integrated Taxonomy and DNA Barcoding of Alpine Midges (Diptera: Chironomidae). *PLoS ONE*, 11 (3), e0149673.  
<https://doi.org/10.1371/journal.pone.0149673>
- Sæther, O.A. (1980) Glossary of chironomid morphology terminology (Chironomidae, Diptera). *Entomologica Scandinavica*, 14 (Supplement), 1–51.
- Silva, F.L. & Wiedenbrug, S. (2014) Integrating DNA barcodes and morphology for species delimitation in the *Corynoneura* group (Diptera: Chironomidae: Orthoclaadiinae). *Bulletin of Entomological Research*, 104 (1), 65–78.  
<https://doi.org/10.1017/S0007485313000515>