



## Review of archaic nymphomyiids (Diptera, Nymphomyiidae) of the Russian Far East and bordering territories, with describing of new taxa and DNA barcoding of known species

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### Abstract

Nymphomyiidae from the Russian Far East and bordering territories are revised, including data on taxonomy, redescriptions, and results of DNA barcoding. *Nymphomyia orientalis* **sp. nov.** with two subspecies *N. orientalis orientalis* **subsp. nov.** and *N. orientalis makcha* **subsp. nov.** are described and information on biology, ecology and distribution are presented. Key of *Nymphomyia* species for adult males and females of the world is provided. Three species delimitation approaches (ASAP, mPTP, GMYC) using the mitochondrial gene cytochrome *c* oxidase I (COI) confirm validity of described taxa as well as available nymphomyiids in GenBank. Morphologically indistinguishable specimens of *N. orientalis* **sp. nov.** from Makcha and Polovinka rivers belongs to separate mOTUs with the lowest distances in the genus 1.99% that is the lower interspecific threshold.

**Key words:** Diptera, Nymphomyiidae, review, new species, DNA barcoding, Russian Far East and bordering territories

### Introduction

The history of the appearance of the family Nymphomyiidae in the system of Diptera began in March 1932, when at sunset the Japanese dipterologist M. Tokunaga, walking in the evening along the Kibune mountain stream in the Botanical garden of Kyoto Imperial University, saw a huge cloud of small white insects swarming over the river and falling like semolina from the sky onto the coastal stones (Fig. 1). And since professional entomologists usually walk with a net, part of this swarm was caught, and upon further study of the contents of the net, it turned out that they had caught Diptera unknown to science and which were difficult to compare with any recent representatives. After conducting fundamental research on the morphology and anatomy of these insects, M. Tokunaga established for them an independent family Nymphomyiidae with the beautiful Japanese name “Haneka”, into which he placed the genus *Nymphomyia* Tokunaga, 1932 with the species *N. alba* Tokunaga, 1932 (Tokunaga 1932, 1935, 1936). The originality of nymphomyiids was later confirmed by other dipterologists, primarily by B.B. Rodendorf, who believed that Nymphomyiidae are a phylogenetic relict of probably the primary group of Diptera, possibly close to forms from the Upper Jura. In his system, he divided the order Diptera into two suborders—Nymphomyiina

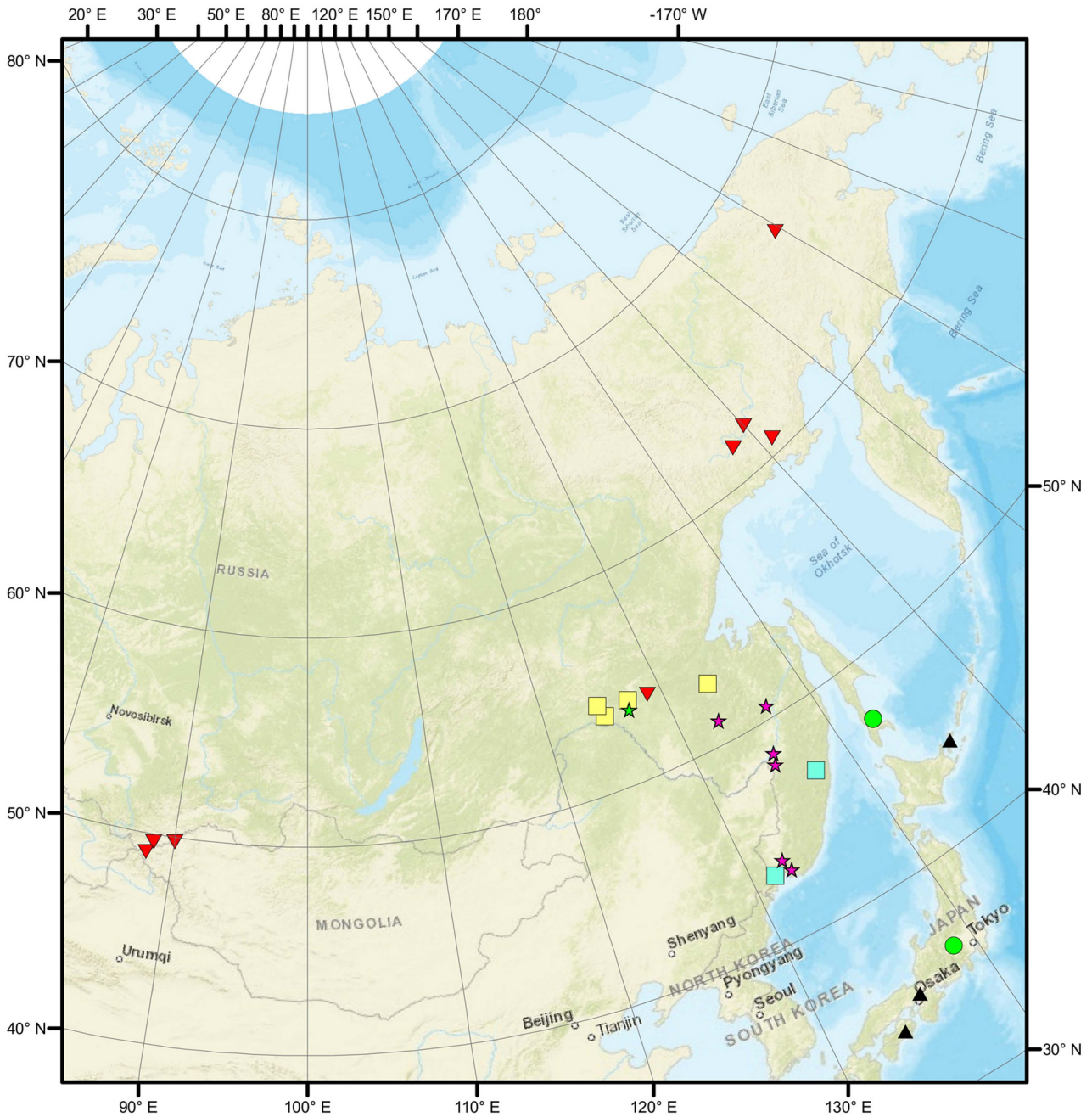
(=Archidiptera) and Muscina (=Eudiptera). The suborder Nymphomyiina included 3 infraorders, of which only one infraorder Nymphomyiomorpha with the family Nymphomyiidae was represented in the modern fauna (Rodendorf 1964, 1977, 1980). Confirmation of the statements of B.B. Rodendorf about the archaic nature of these dipterans appeared quite recently, after the sensational discovery of the adults of *Nymphomyia alissae* Wagner et Müller in Cretaceous Burmese amber (Wagner & Müller 2020). The fossil species fits well into the diagnosis of the genus and differs from modern representatives mostly in the structure of the genitalia, which, however, is characteristic of all living species, the males and females of which differ mostly in this feature.



FIGURE 1. Swarming of adults *Nymphomyia kannasatoi* Makarchenko et Gunderina in Kanna River, Japan (photo by S. Sato).

Currently, ten species of nymphomyiids are known in the world fauna: *Nymphomyia alba* Tokunaga, 1932 (Japan, Russian Far East—Kunashir Island), *N. walkeri* Ide, 1964 (Eastern Canada and USA), *N. brundini* Kevan, 1970 (Eastern Himalayas), *N. levanidovae* Rohdendorf et Kalugina, 1974 (Russian Far East—Southern Primorye and the upper reaches of the Bikin River of Amur River basin), *N. rohdendorfi* Makarchenko, 1979 (Russian Far East—Kolyma River basins, Chukotka, Altai), *N. dolichozepea* Courtney, 1994 (eastern USA), *N. holoptica* Courtney, 1994 (Hong Kong), *N. kaluginae* Makarchenko, 2013 (Russian Far East—Zeya River basin, a tributary of the Amur River), *N. kannasatoi* Makarchenko et Gunderina, 2014 (Japan—Honshu, Russian Far East—Southern Sakhalin), *N. aijuanae* Makarchenko et Tang (China, Fujian Province) (Courtney 1994; Makarchenko 2022; Makarchenko & Tang 2024). In addition, pupae of *Nymphomyia* sp. found in the Northern Altai of Mongolia (Hayford & Bouchard 2012), which, in our opinion, should be attributed to *N. rohdendorfi*. Two fossil species are also known from adults: *N. succina* Wagner *et al.* from the Eocene (Baltic amber) (Wagner *et al.* 2000), *N. alissae* Wagner et Müller from the Cretaceous (Burmese amber) (Wagner & Müller 2020), and one (*Nymphomyia* sp.) from the remains of subfossil larvae from bottom sediments of Oron Lake, Irkutsk Region (Enushenko & Makarchenko 2019).

Six of the known species live in the Russian Far East and bordering territories (Fig. 2). In this review, we present new data on the taxonomy of these species, provide redescriptions and results of DNA barcoding and descriptions of the new species *N. orientalis* **sp. nov.** with two subspecies—*N. orientalis orientalis* **subsp. nov.** and *N. orientalis makcha* **subsp. nov.**, as well as information on biology, ecology and distribution. Finally, we present updated key on adults (to Courtney 1994) for all known species of *Nymphomyia* of the world.



- |   |                         |
|---|-------------------------|
| ▲ <i>Nymphomyia alba</i>                      | ■ <i>N. kaluginae</i>   |
| ● <i>N. kannasatoi</i>                        | ■ <i>N. levanidovae</i> |
| ★ <i>N. orientalis orientalis subsp. nov.</i> | ▼ <i>N. rohdendorfi</i> |
| ★ <i>N. orientalis makcha subsp. nov.</i>     |                         |

FIGURE 2. Map of nymphomyiid localities in the Russian Far East and bordering territories.

Molecular data of nymphomyiids are used to resolve phylogenetic relationships of dipterans (Bertone *et al.* 2008, Wiegmann *et al.* 2011) or to assess the diversity of the genus *Nymphomyia* using DNA barcoding (Makarchenko & Gunderina 2012, Makarchenko *et al.* 2014, Makarchenko & Gunderina 2019). Resolved phylogeny using both

ribosomal (28S rDNA) and protein-coding (CAD, TPI and PGD) genes (Bertone *et al.* 2008) as well as 14 nuclear loci and complete mitochondrial genomes (Wiegmann *et al.* 2011) confirmed the archaic nature of Nymphomyiidae. Rodendorf (1964, 1977, 1980) and Wagner & Müller (2020) showed these insects as the earliest branching lineages after the divergence of Deuterophlebiidae from the basal dipterans branch. The first DNA barcodes of Nymphomyiidae were obtained for *Nymphomyia rohdendorfi* from Polovinka river, Amur River basin (now it is *N. orientalis orientalis* **subsp. nov.**) (Makarchenko & Gunderina 2012), later were added COI sequences of *N. kannasatoi* from Japan (Makarchenko *et al.* 2014), *N. alba* from Kunashir Island and *N. levanidovae* from Primorye Territory (both in Makarchenko & Gunderina 2019). Standardized 658-bp region of the mitochondrial cytochrome c oxidase subunit I (COI) have proven as the successful gene for species delimitation and life stage association in nymphomyiids.

The aim of this study is (a) to obtain and analyze DNA barcodes for nymphomyiids from Makcha, Allenga (both Amur River basin), Churendya River (Kolyma River basin), Kuruk River (Katun' River basin) which presumably belong to *N. rohdendorfi*; (b) to sequence COI-5P gene for *N. alba* from Monobe River (Japan) to clarify its conspecificity with the Kunashir population and for the first time for *N. kaluginae* from Bolshoi Garmakan River (Amur River basin); (c) to give morphological descriptions of *Nymphomyia orientalis* **sp. nov.** with two subspecies *N. orientalis orientalis* **subsp. nov.** and *N. orientalis makcha* **subsp. nov.**

## Materials and methods

Material was preserved in 96% ethanol for DNA-analysis, in 70% ethanol for further morphological studies. The adult males, pupae and larvae were slide-mounted in polyvinyl lactophenol and Fora-Berleze solution. The morphological terminology used below generally follows Courtney (1994) and Makarchenko & Gunderina (2012).

The photographs were taken using an Axio Lab.A1 (Karl Zeiss) microscope with an AxioCam ERc5s digital camera, and then stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software.

Holotype and paratypes of the new species, as well as all other material, 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).

Total genomic DNA was extracted using DNeasy Blood and Tissue kit or QIAamp DNA Investigator Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. We amplified mitochondrial protein coding gene cytochrome c oxidase I using standard primers LCO1490 and HCO2198 primers (Folmer *et al.* 1994). For reaction protocols, cycle programs used for amplification, purification of PCR products, sequences, species delimitation approaches and phylogenetic analysis see Makarchenko *et al.* (2023). The best models for the first and second codon position of COI was HKY+I (Hasegawa *et al.* 1985), while the best model for the third codon position was HKY+G. List of sequenced specimens with GenBank accession numbers is in Table 1.

**TABLE 1.** List of taxa, locations, geographic coordinates and GenBank accessions. Samples from this study are in bold.

Species	No. of specimens	Country, location	Coordinates	Accession
<i>N. alba</i>	6	Russia: Sakhalin Region, Kunashir Island, unnamed stream	44.17 N 145.98E	MK144520–MK144525
<i>N. alba</i>	4	<b>Japan: Kochi Prefecture, Monobe River near Beppukoshikata Shrine</b>	<b>33.76200 N 134.02881 E</b>	<b>PP473544–PP473547</b>
<i>N. kannasatoi</i>	8	Japan: Gunma Prefecture, Kawawa Area, Katsuyama, Ueno Village, Kanna River	36.083 N 138.776 E	KP208867–KP208874
<i>N. kaluginae</i>	3	<b>Russia: Amur Region, Zeysky District, Zeya Nature Reserve, Amur River basin, Bolshoi Garmakan River</b>	<b>53.886185 N 127.193704E</b>	<b>PP473548–PP473550</b>
<i>N. levanidovae</i>	8	Russia: Primorye Territory, Khasansk District, Kedrovaya Pad Nature Reserve, Kedrovaya River	43.097900 N 131.556867E	MK144526–MK144533

...Continued on the next page

**TABLE 1.** (Continued)

Species	No. of specimens	Country, location	Coordinates	Accession
<i>N. orientalis makcha</i>	7	Russia: Amur Region, Zeya District, Amur River basin, Makcha River	53.685022 N 127.064882E	PP473536– PP473542
<i>N. orientalis orientalis</i>	6	Russia: Khabarovsk Territory, Amur River basin, Polovinka River, environs of the of Novotroitskoye Village	48.30732 N 134.83607 E	KP208875, JX196859– JX196862
<i>N. rohdendorfi</i>	3	Russia: Amur Region, Zeya District, Amur River basin, Allenga River,	53.763783 N 127.826967E	PP473551– PP473553
<i>N. rohdendorfi</i>	1	Russia: Magadan Region, Khasynsky District, Kolyma River basin, Khurendya River	60.690241 N 151.244209E	PP473543
<i>N. rohdendorfi</i>	1	Russia: Republic of Altai, Kosh-Agachsky District, Mukhor-Tarkhatinsky rural settlement, Ob River basin, Kuruk River	49.582780 N 88.427670 E	PP528510

## Systematics

### Family: Nymphomyiidae Tokunaga, 1932

### Genus: *Nymphomyia* Tokunaga, 1932

#### *Nymphomyia orientalis* Makarchenko et Semenchenko, sp. nov.

<http://zoobank.org/NomenclaturalActs/BE8D3565-9098-4603-8138-D58DE5F12668>

(Figs. 3–14)

*Nymphomyia rohdendorfi* Makarchenko; Makarchenko & Gunderina, 2012: 20 (misidentification); Yavorskaya & Makarchenko 2015: 524 (misidentification).

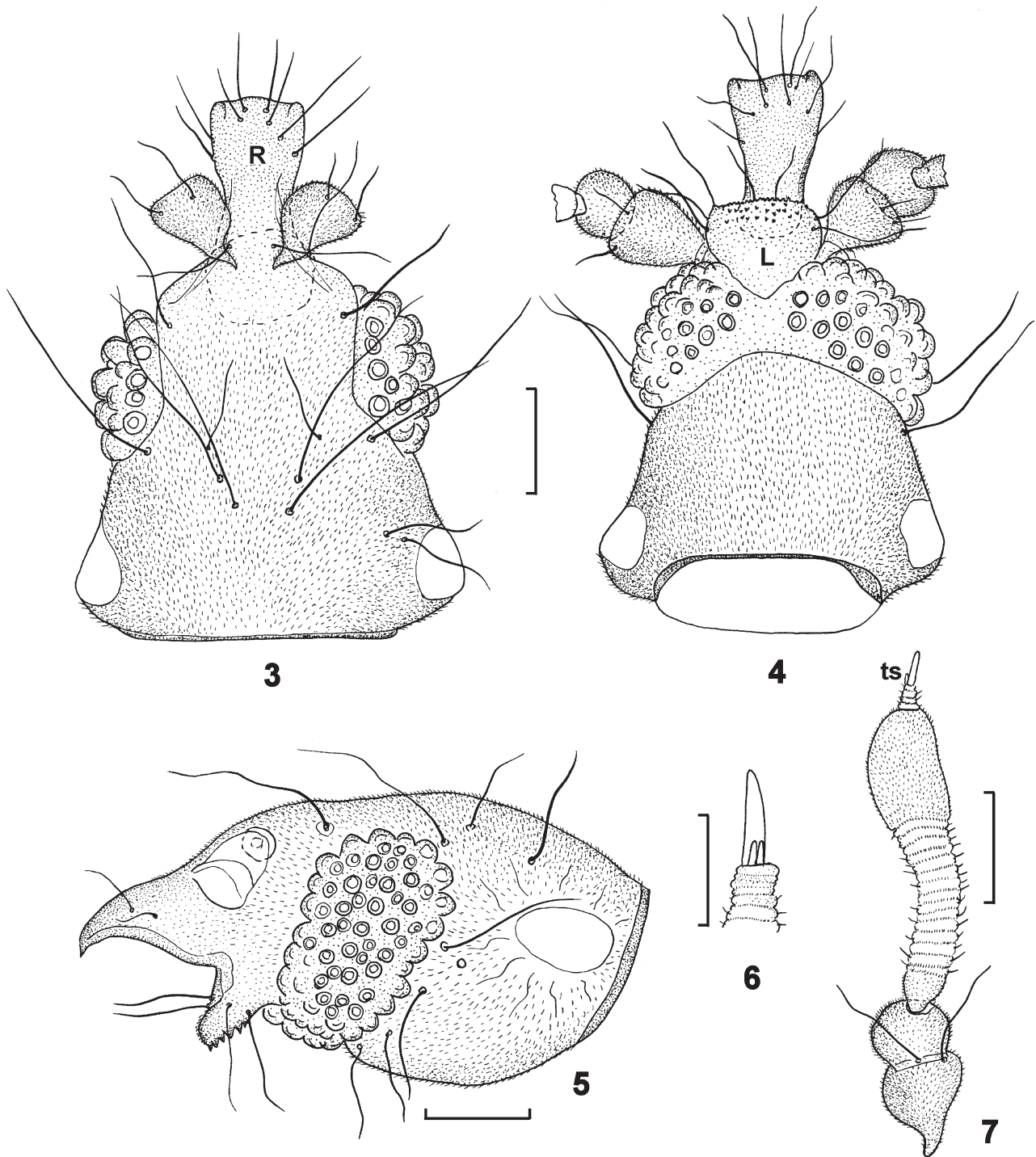
**Remarks.** In earlier investigations (Yavorskaya & Makarchenko 2015) after comparing the adults of males and females of the *N. rohdendorfi* from the Kolyma River basin and Chukotka with those from the Amur River basin we noted differences in the structure of the females and assumed that an independent species lives in the Amur River basin, the identification of which we postponed until obtained DNA barcoding data. After getting such data, now we presenting a description of an independent new species *N. orientalis* sp. nov. from the Amur River basin. Moreover, a comparison of the results of DNA barcoding of *N. orientalis* sp. nov. from the Polovinka and Makcha rivers showed that specimens from the populations of these rivers differ at the species level. However, we did not find any morphological differences between males and females of these populations, but nevertheless decided to distinguish two subspecies—*N. orientalis orientalis* subsp. nov. for specimens from the Polovinka River and *N. orientalis makcha* subsp. nov. for individuals from the Makcha River. Also, according to morphological data, males of *N. orientalis* sp. nov. and *N. rohdendorfi* are almost indistinguishable. See more detail below in “Results of DNA barcoding.”

**Diagnosis.** See identification key below.

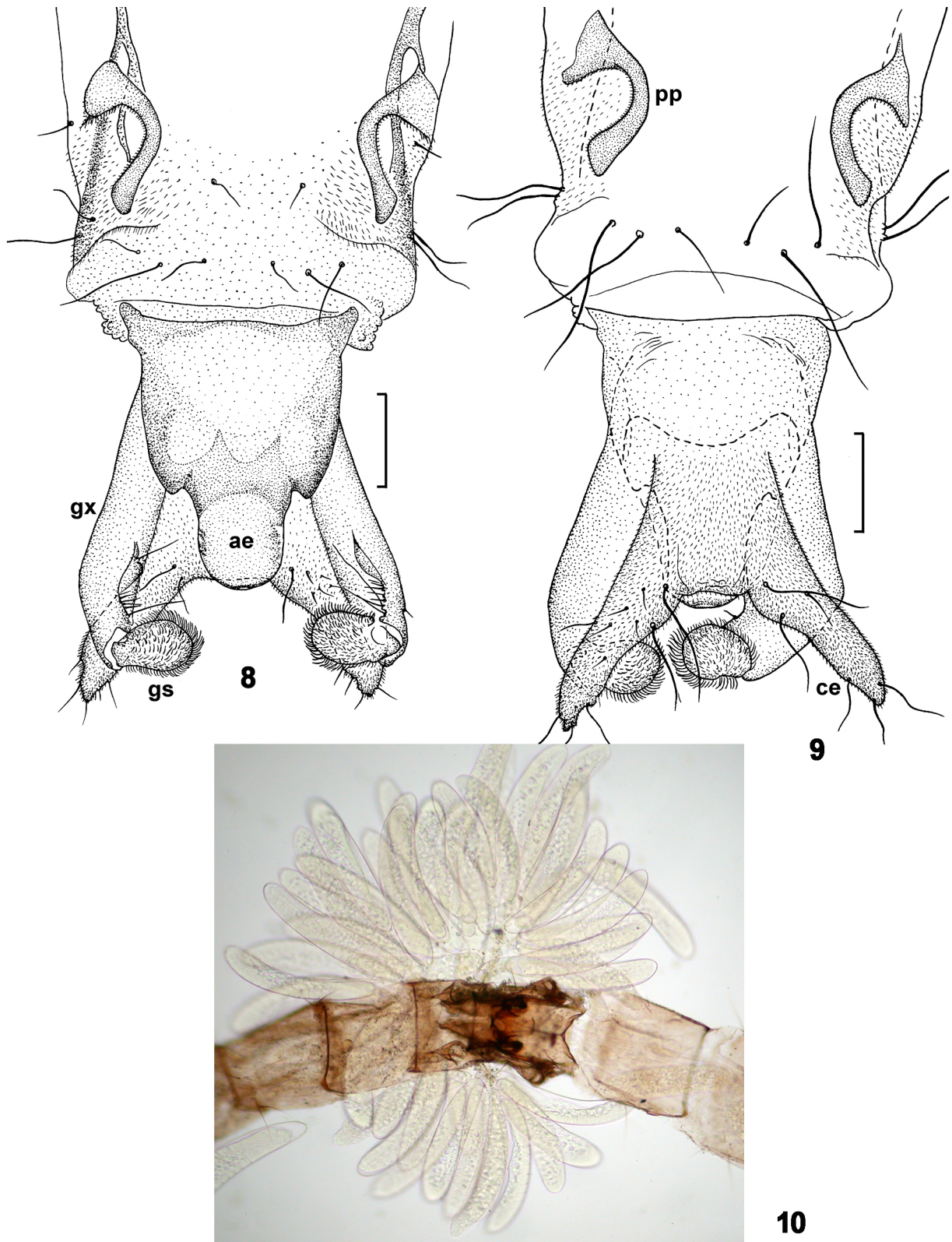
#### *Nymphomyia orientalis orientalis* Makarchenko et Semenchenko, subsp. nov.

(Figs. 3–11, 13)

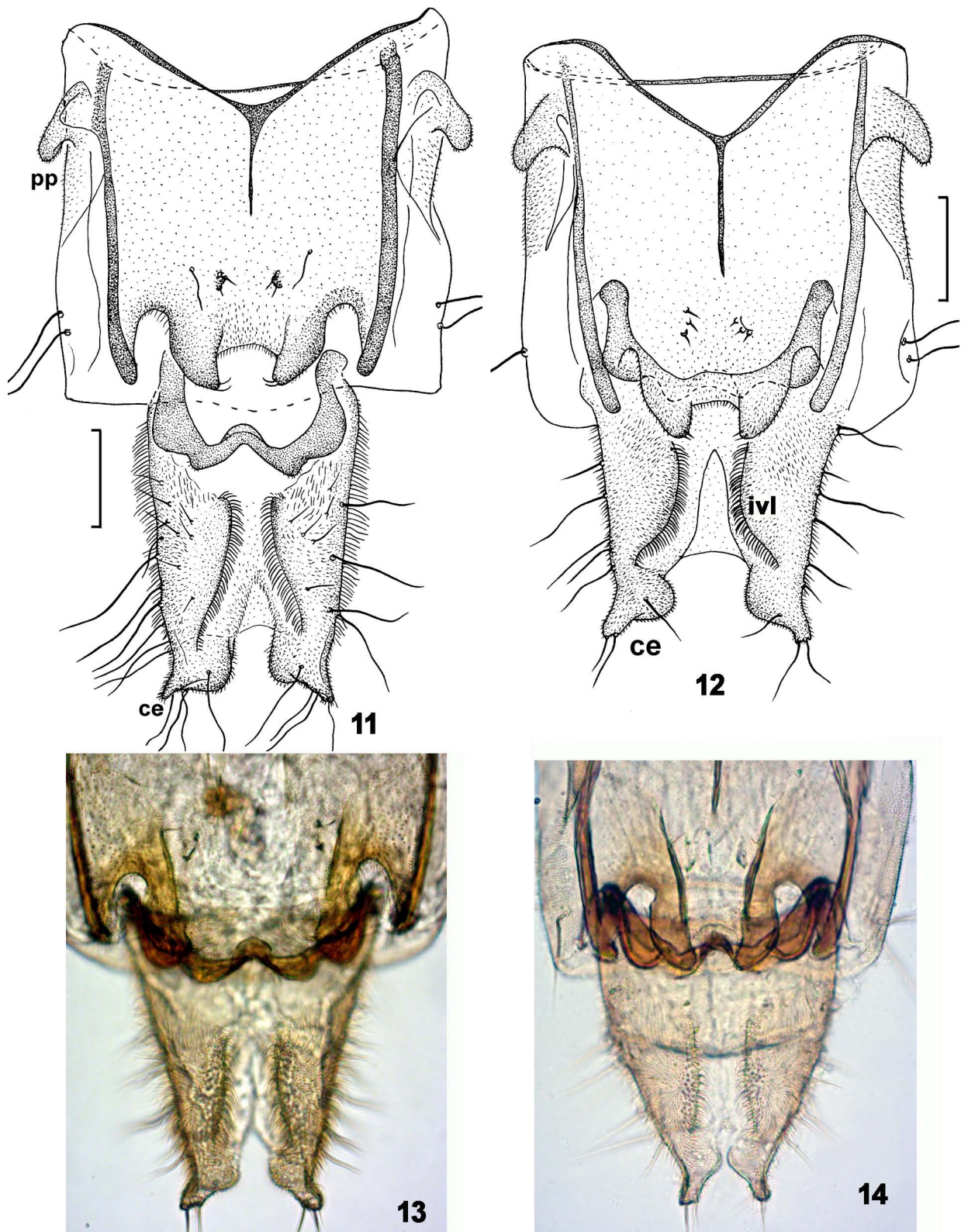
**Type material.** Holotype: adult male, RUSSIA: *Khabarovsk Territory*, Khabarovsk District, Polovinka River (Amur River basin), environs of the of Novotroitskoye Village, 48.30732 N 134.83607 E, 6.VI.2010, leg. N. Yavorskaya. Paratypes: 4 adult males, 3 females, the same data as holotype, 6.VI.2010, leg. N. Yavorskaya; adult males and females in quantities, the same data except 3.VI.2011, 3.VI.2012, 4.VI.2023, leg. N. Yavorskaya.



**FIGURES 3–7.** Adult male of *Nymphomyia orientalis orientalis* **subsp. nov.** **3**, head in dorsal view; **4**, head in ventral view; **5**, head in lateral view; **6**, terminal antennal sensilla; **7**, antenna. R, rostrum; L, labium; ts, terminal antennal sensilla. Scale bar for Figs. 3–5, 7–50  $\mu\text{m}$ ; for Fig. 6–20  $\mu\text{m}$ .



**FIGURES 8–10.** Adult male terminalia of *Nymphomyia orientalis orientalis* **subsp. nov.** in ventral view (8) and in dorsal view (9), eggs on anal segments of male and female in copula (10). Scale bars—50  $\mu$ m.



**FIGURES 11–14.** Terminalia of adult females of *Nymphomyia orientalis orientalis* subsp. nov. (11, 13) and *N. orientalis makcha* subsp. nov. (12, 14) in ventral view. ce, cerci; ivl, inner ventral lobe; pp, paratergal projection. Scale bars—50  $\mu$ m.

**Material examined.** Russia: *Khabarovsk Territory*, 2 adult males, Bolshekhehtsirsky Nature Reserve, Odyr River, 48.110133 N 134.8731 E, 9.VI.2016, leg. N. Yavorskaya; 2 mature pupae, 1 pupal exuvia, 1 larva, the same data except environs of Kiya Village, Fartovyi Spring, tributary of Kiya River (Ussuri River basin), 47.829705

N, 135.715348 E, 10.VI.1982, leg. E. Makarchenko and M. Makarchenko; 10 adult males, 12 females, the same data except Levaya River, environs of Korsakovka-2 Village, 48.334917 N, 134.950167 E, 15.VI.2012, leg. N. Yavorskaya; 1 adult male, the same data except Matryonikha River, 48.339767 N, 135.000800 E, 15.VI.2012, leg. N. Yavorskaya; 1 adult male, 1 female, the same data except Komsomolsky District, Tsurkul River, tributary of Silinka River, 50.663919 N, 136.893314 E, 7.VII.2010, N. Yavorskaya; 1 adult male, the same data except B. Khurba River, 50.390028 N, 136.856625 E, 9.VII.2010, leg. N. Yavorskaya; 1 adult male, the same data except Pokha River, 50.354961 N, 136.859925 E, 12.VII.2010, leg. N. Yavorskaya; 1 adult male, 1 female (in copula), 3 larvae, the same data except Vyazemsky District, Avan River, 47.442095 N, 134.616778 E, 26.V.2011, leg. S. Sirotsky; 2 mature pupae, the same data except Verkhnebureinsky District, Satansky Stream (Bureya River basin), environs of Chegdomyn Village, 51.185414 N, 133.002731 E, 8.VII.1994, leg. E. Makarchenko. *Primorye Territory*, 2 larvae, Ussuryisky District, M. Kamenka River, (Razdolnaya River basin), 43.661082 N, 132.363692 E, 2.V.1982, leg. T. Vshivkova; 3 mature pupae, 24 larvae, the same data except Pravaya Komarovka River, Ussuri Nature Reserve named after V.L. Komarova, 43.649150 N, 132.407251 E, 31.V.1984, leg. T. Vshivkova; 1 adult male, 1 female (in copula), 3 larvae, the same data except environs Artem City, Zybunnyi Stream of Bolotnaya River basin, 43.339444 N, 132.148333 E, 2.VI.2013, leg. A. Skriptsova.

**Derivatio nominis.** The names of the species and nominative subspecies comes from the Latin word *orientalis*, which means eastern.

## Description

*Adult male* (n=10, except when otherwise stated). Pale grey, weak chitinized. Total length 2.50–2.72 mm. Total length/wing length 1.22–1.24.

Head length 232–316  $\mu\text{m}$  long and 156–164  $\mu\text{m}$  wide, gradually narrowed anteriorly and ends rostrum, which is distally expanded, its anterior margin undulating, with a slightly curved ventral side in the beak-shape (Figs. 3, 5); rostrum dorsally with 3–4 pairs of setae. Compound eyes on the dorsal side of the head are wide apart, the distance between them in 3.9–4.1 times the width of the end of rostrum; on the ventral side of the compound eyes are fused, but at the midline of ommatidia do not touch (Fig. 4). Antenna 168–196  $\mu\text{m}$  long, with two rounded basal segments, 1<sup>st</sup> segment 36–44  $\mu\text{m}$  long, 2<sup>nd</sup> segment 24–28  $\mu\text{m}$  long, 3<sup>rd</sup> segment 104–116  $\mu\text{m}$  long, slightly extended to the top, with a terminal sensilla of three rod-like structures, most of which are 12–14 microns in length (Figs. 6–7); antenna 0.80–0.85 times shorter than the head. Labium with almost straight or slightly rounded front edge and with tooth-covered spines, very apparent when viewed from the side (Fig. 5), laterally with 2–3 pairs of long setae, medially with a pair of short setae.

Thorax length 0.64–0.67 mm; carries a pair of boomerang-shape wings, halteres and 3 pairs of legs; wing length 2.12–2.20 mm, maximum width 0.28–0.32 mm, with reduced venation, on the edge with whip-shape setae 0.46–0.49 mm long; halteres 0.14 mm long. Structure of the legs and thorax are typical for genus.

Terminalia (Figs. 8–9). Abdomen 1.65–1.80 mm long. Sternites V and VI simple. Tergite VIII with one pair of large, elongate, posteriorly directed, lateral paratergal projections, 52–72  $\mu\text{m}$  long, with blunt apex; dorsal paratergal projection absent. Last abdominal segment dorsally with cerci, in ventral side with of gonocoxites, gonostylus and aedeagus; cerci 60–76  $\mu\text{m}$  long, distally narrowed, digitiform; gonocoxite length 140  $\mu\text{m}$ , in 2.9–3.8 times as long as gonostyles, distally to the top with a little strength; gonostylus length 36–48  $\mu\text{m}$ , lanceolate or rounded (depending on the position in slide), covered with numerous hair-like setae directed inward; aedeagus length 44–60  $\mu\text{m}$ , width—40  $\mu\text{m}$ , with rounded apex.

*Adult female* (n=10) in general similar to male. Total length 2.50–2.60 mm. Wing length 1.90–1.96 mm. Total length/wing length 1.30–1.33.

Head length 204–228  $\mu\text{m}$  and 152  $\mu\text{m}$  wide. The distance between the compound eyes dorsally 3.5–4.0 times the width of the end of the rostrum. Antenna 170–184  $\mu\text{m}$  long; length of 1<sup>st</sup> segment 32–38  $\mu\text{m}$ , length of 2<sup>nd</sup> segment 24–28  $\mu\text{m}$ ; 3<sup>rd</sup> segment 110–116  $\mu\text{m}$  long; terminal sensilla maximum length 12–16  $\mu\text{m}$ ; antenna 0.71–0.74 times shorter than the head.

Thorax length 0.45–0.48 mm. Wing length 1.92–1.96 mm, maximum width about 0.30 mm.

Terminalia (Figs. 11, 13). Segment VIII with pair small lateral paratergal projections 12–16  $\mu\text{m}$  long. Sternites V–VII simple, without appendages. Sternite VIII consists of broad middle plate; posterior edge with hollow in the

middle, above which is pair of lobes 48–64  $\mu\text{m}$  long, each with 2 short (about 8  $\mu\text{m}$  long) setae at the apex. The last abdominal segment with cerci, tapering apically, with rounded “heel” and their distal part is 12–16  $\mu\text{m}$  long to the outer edge, subapical with 4 pairs of long seta. Internal lobes of gonocoxite approximately same width or slightly widen proximally (Figs. 11–14).

**Ecology and biology.** Larvae and pupae of *N. orientalis orientalis* **subsp. nov.**, like most other members of the family, inhabit fast flowing foothill and mountain rivers, high oxygen content in water, low specific electrical conductivity and a pH value from slightly acidic to slightly alkaline, settling mainly on rocky and gravel-pebble soils, which in May–June are often covered with algae. Larvae and pupae were collected in Polovinka River (Fig. 46) and other watercourses when the water temperature in them reached 6 °C, with a pH value from 6.0 to 7.5. Nymphomyiids were not found in colder water. It can be assumed that in the early larval instars they live in the groundwater of the stream and only in the last instar before pupation do they “rise” to the substrate surface of watercourse. Larvae of IV instar and pupae of *N. orientalis orientalis* **subsp. nov.** were found in the bottom samples in May–June, and adults in June. Total biomass of larvae and pupae during the 2014–2015 surveys in the Polovinka, Levaya and Pravaya rivers it was low (0.20 and 0.64 g/m<sup>2</sup>), respectively. Maximum values of numbers for larvae in the Khabarovsk region reached 912 ind./m<sup>2</sup> (Levaya River) and 2224 ind./m<sup>2</sup> for pupae (Polovinka River) in May. Swarming of adults was observed on the Polovinka River. They fly at 1–6 m above the water surface. The insects stayed almost above the water’s edge, preferring the 1–2 meter coastal zone of the watercourse. Mating dances began after 19 hours and actively continued until sunset (21 hours 30 minutes). In calm, sunny weather, swarming was widespread and active; in windy and slightly cloudy weather, it was sporadic and very weak, with nymphomyiids flying mainly at a height of 0.5–1 m above the water surface. During heavy rain and strong winds, insects did not swarm. With a weak wind, the swarm “scattered” and after about 5–7 minutes re-formed at a height of more than 1 m above the river and quickly rose upward. The maximum size of the swarm, consisting of thousands of males and females, reached about 2 m in diameter and had a spherical shape. The swarming insects made a barely audible rustling sound. Adults of nymphomyiids copulated in the air, then descended onto coastal stones and into the water, after which they lost their wings. In benthic samples in June 20, 2015, adults without wings were found sporadically. That is, copulating pairs still live on stones in the water for some time and the clutch of eggs at this time is attached to the anal end of the body of the female, and sometimes the male (Fig. 10). There are 50–56 eggs in a clutch. Egg length 248–268  $\mu\text{m}$ , width 48–60  $\mu\text{m}$ . The obtained results of benthological studies of the Polovinka River from April to November 2014 and 2015, the Levaya and Matryonikha rivers from May to November 2012 showed that the life cycle of *N. orientalis orientalis* **subsp. nov.** in the Amur River basin is univoltine (Yavorskaya & Makarchenko 2015).

**Distribution.** *N. orientalis orientalis* **subsp. nov.** is known from type locality in Polovinka River of Amur River basin and in our opinion the range of this subspecies covers the Middle Amur basin, with exception of the Makcha River of Zeya River basin, and extends to the Primorye Territory, including the basins of the Razdolnaya and Artemovka rivers (Fig. 2).

### *Nymphomyia orientalis makcha* Makarchenko et Semenchenko, **subsp. nov.**

(Figs. 12, 14)

**Type material.** Holotype: adult male, RUSSIA: *Amur Region*, Zeiskyi District, environs of Zeya Town, Makcha River, 53.685022 N 127.064882 E, 29.VI.2016, leg. N. Yavorskaya and E. Makarchenko. Paratypes: 18 adult males, 21 females, the same data as holotype, 29.VI.2016, leg. N. Yavorskaya and E. Makarchenko.

**Derivatio nominis.** The subspecies is named after the Makcha River, from which the type material was collected. The name is a noun in apposition.

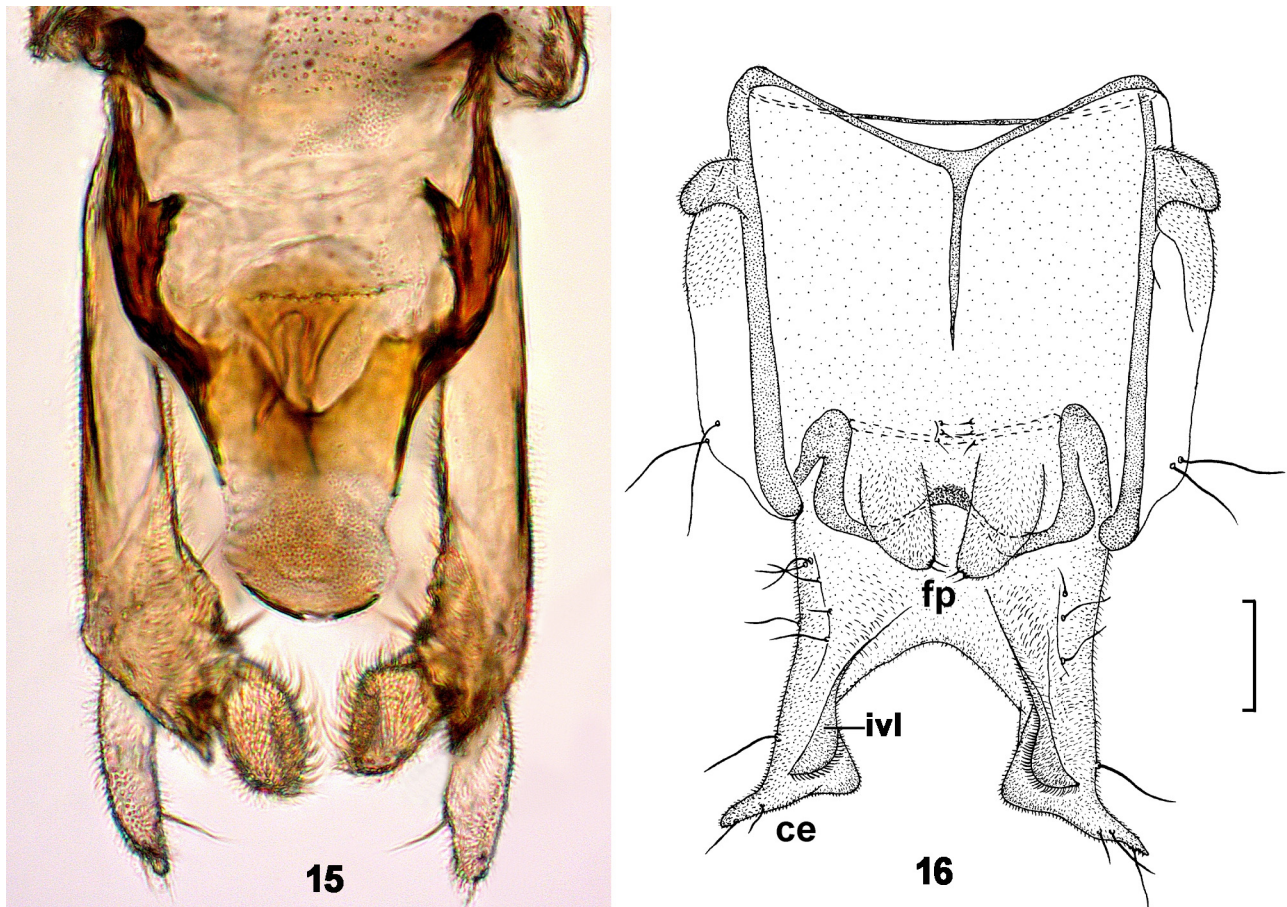
**Ecology and biology.** Larvae and pupae of *N. orientalis makcha* **subsp. nov.** were collected in the Makcha River (Fig. 47) on the gravel-pebble soil of a riffle, at a depth of 10–20 cm, at a water temperature of 12.9 °C and pH 7.5–7.7. Numbers of larvae reached 48 ind./m<sup>2</sup> in July 1, 2016, and 144 ind./m<sup>2</sup> for pupae. Along with pupae, wingless adults were found in the samples, which confirms the emergence of nymphomyiids already in June when the water in the river warms up to 12 °C. Adult swarming on this river was observed on June 29, 2016 (Yavorskaya & Makarchenko 2015).

**Distribution.** *N. orientalis makcha* **subsp. nov.** is known from type locality in Makcha River of Zeya River basin only (Fig. 2).

*Nymphomyia rohdendorfi* Makarchenko

(Figs. 15–20)

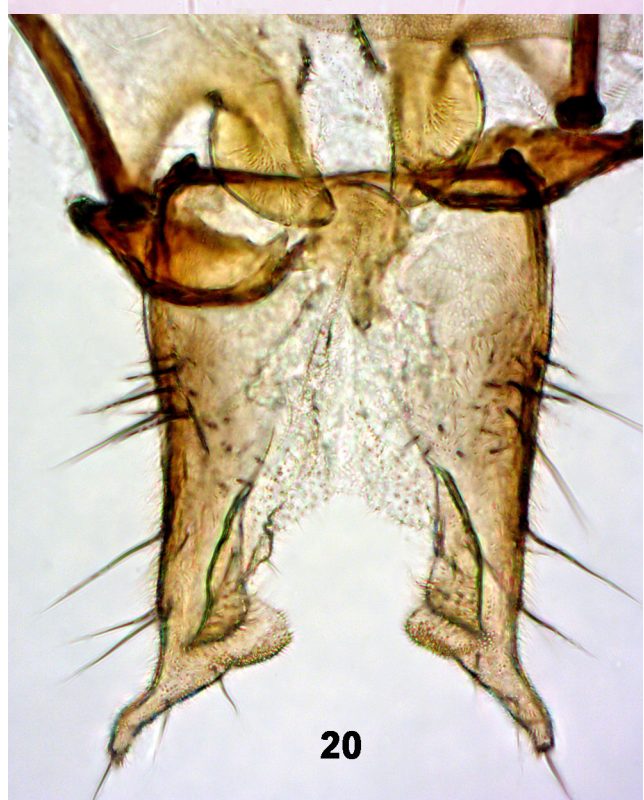
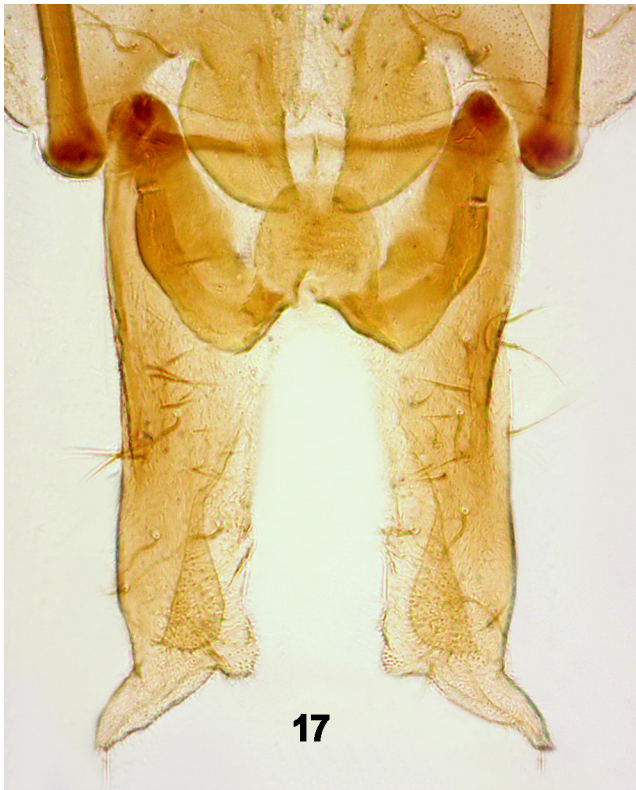
*Nymphomyia rohdendorfi* Makarchenko, 1979: 1070; Courtney 1994: 16; Makarchenko 2006: 739.



**FIGURES 15–16.** Adult male (15) and female (16) terminalia of *Nymphomyia rohdendorfi* Makarchenko in ventral view. fp, fingerlike projection; ivl, inner ventral lobe. Scale bar—50  $\mu$ m.

**Material examined.** RUSSIA: adult male extracted from the exuvium of a mature pupa (holotype), 1 adult female, extracted from the exuvia of a mature pupa, 6 mature pupae, 4 larval skins (paratypes), *Magadan region*, Tenkinsky District, environs of Sibit-Tyellakh Village, Anyuradat Stream (Kolyma River basin) 62.029238 N, 149.971665 E, 3.VII.1977, leg. E. Makarchenko and S. Kocharina; 1 adult male, 1 pupa, the same data except Tenkinsky District, vicinity of the Omchak Village, Intrigan River (Kolyma River basin), 61.591589 N, 147.718341 E, altitude 836 m a.s.l., 19.IX.2009, leg. I. Zasyapkina; 1 mature pupa, the same data except unnamed stream, left tributary of Intrigan River, 61.690687 N, 147.714991 E, 18.VI.2014; 1 mature pupa, the same data except, Intrigan River, 61.657267 N, 147.71865 E, 18.VII.2014; 1 adult male, 1 female, 6 pupae, the same data except 17.VII.2015, leg. E. Khamenkova. 23 adult males, 21 females, 5 pupae, the same data except Khasynsky District, Natural monument of regional significance of the Magadan region “Ola Plateau”, Khurendya River, a tributary of the Maltna River (Kolyma River basin), 60.690241 N, 151.244209 E, alt. 1095 m a.s.l., 14.VII. 2023, leg. A. Krashennnikov; 10 adult male, 10 pupae, *Chukotka*, Anadyr Region, Sredniy Kayemraveem River (tributary of the Mechkerov River, upper reaches of the Anadyr River), 66.841378 N, 169.685671 E, alt. 623 m a.s.l., 18.VII.2004, leg. I. Zasyapkina; 1 pupa, 1 pupal exuvia, the same data except 9.VII.2005, leg. I. Zasyapkina; 2 pupal exuviae, Bilbinsky District, Starichnaya River (tributary of the Maly Anyui River, Kolyma River basin), 66.797778 N, 169.361389 E, altitude 617 m a.s.l., 7.IX.2005, leg. I. Zasyapkina. 1 larva, Amur Region. Zeya District, Allenga River (tributary of the Tynda River), 53.763783 N, 127.826967 E, 14.VII.2012, leg. E. Makarchenko; 5 larvae, the same data except, 28.VI.2013, leg. E. Makarchenko; 6 larvae, *Republic of Altai*, Kosh-Agachsky District, Mukhor-Tarkhatinsky rural settlement, Kuruk

River (Katun River basin), alt. 2273 m a.s.l., 49.58278 N, 88.42767 E, 28.V 2022; 4 mature pupae, 9 pupae, the same data except 5.VII. 2023, leg. L. Yanygina; 1 larva, the same data except Kalanegir River (Katun River basin), alt. 2200 m a.s.l., 49.64237 N, 88.47350 E, 28.V. 2022 leg. L. Yanygina.



**FIGURES 17–20.** Female terminalia of *Nymphomyia rohdendorfi* Makarchenko in ventral view from Anyuradat Stream (Kolyma River basin) (paratype) (17), Intrigan River (Kolyma River basin) (18), Khurendya River (Kolyma River basin) (19) and Altai Mountains (20).

## Description

*Adult male* (n = 5, except when otherwise stated). Pale grey, weak chitinized. Total length 1.96–2.4 mm. Total length/wing length 1.14–1.20.

Head length 200–220 µm long and 140–204 µm wide, gradually narrowed anteriorly and ends rostrum, which is distally expanded, its anterior margin undulating, with a slightly curved ventral side in the beak-shape; rostrum dorsally with 3–4 pairs of setae. Compound eyes on the dorsal side of the head are wide apart, distance between them in 1.8–2.0 times width of rostrum end; on the ventral side of the compound eyes are fused with each other, but at the midline of ommatidia do not touch. Antenna 180–192 µm long, with two rounded basal segments, 1<sup>st</sup> segment 34–40 µm long, 2<sup>nd</sup> segment 24–25 µm long, 3<sup>rd</sup> segment 104–118 µm long, slightly extended to the top, with a terminal sensilla of three rod-like structures, most of which are 16–18 µm long; antenna 1.1–1.6 times shorter than the head. Labium with almost straight or slightly rounded front edge and with tooth-covered spines, laterally with 2 pairs of long setae, medially with a pair of short setae.

Thorax length 574–836 µm; halteres 224 µm long; wing length 1.9–2.0 mm, maximum width 0.24–0.28 mm. Structure of the legs, thorax and wings are typical for genus.

Terminalia (Fig. 15). Abdomen 1.47–1.72 mm long. Tergite VIII with one pair of elongate, posteriorly directed lateral paratergal projections 60–70 µm long. Cerci 64–88 µm long, distally narrowed, digitiform; gonocoxite length 140–168 µm, in 3.2–3.5 times as long as gonostyles; gonostylus length 40–52 µm, lanceolate or rounded (depending on the position in slide), covered with numerous hair-like setae directed inward; aedeagus length of 40–56 µm, width—36–48 µm, with rounded apex.

*Adult female* (n=5) in general similar to male. Total length 2.0–2.3 mm. Wing length 1.88–1.92 mm. Total length/wing length 1.15–1.29.

Head length 180–196 µm, width 132–142 µm. The distance between the compound eyes dorsally 1.5–1.8 times the width of the end of the rostrum. Antenna 176–188 µm long; length of 1<sup>st</sup> segment 36–40 µm, length of 2<sup>nd</sup> segment 24–26 µm; 3<sup>rd</sup> segment 96–100 µm long; terminal sensilla maximum length 20–24 µm; antenna 0.89–0.96 times shorter than the head.

Thorax length 0.57–0.61 mm; halteres 204–248 µm long; wing length 1.88–1.92 mm, maximum width 0.26–0.30 mm.

Terminalia (Figs. 16–20). Segment VIII with small lateral paratergal projection 20–28 µm long. Sternites V–VII simple, without appendages. Sternite VIII consists of a broad middle plate; posterior edge with hollow in the middle, above which is a pair of lobes 48–50 µm long, each with 2 short (about 8 µm long) setae at the apex. The last abdominal segment with cerci with rounded triangular “heel” and their distal part is 24–32 µm long. Internal lobes of gonocoxite widened and rounded distally, and narrow proximally (Figs. 16–20)

**Ecology and biology.** In Altai Mountains larvae and pupae of *N. rohdendorfi* were found at altitude of 2200–2300 m above sea level in the moderately cold-water streams (Fig. 49), with 11.0–11.9°C water temperature during sampling. In sampling sites, width of rivers Kuruk and Kalanagir was 14 and 20 m, respectively, and depth—0.25 m. Bottom sediments were mainly formed by pebbles (50–65% of the bottom area). The studied waters were weakly alkaline (pH 8.1–8.4) and low-mineralized (0.09–0.12 ppt). The nutrient content (below detection limits) and organic substances (BOD<sub>5</sub> 0.54–0.86 mgO/dm<sup>3</sup>) was low. Relatively high COD values (28–33 mgO/dm<sup>3</sup>) were noted, probably, due to a partially waterlogged catchment area. A common feature of the nymphomyids ecology of the Altai is their habitat in streams running through free from tree vegetation landscapes (Yanygina & Makarchenko 2023). In the Allenga River of the Zeya River basin 16 ind./m<sup>2</sup> of this species were collected on June 30, 2015 in a riffle at a water temperature of 8.5°C, at a depth of 20 cm, and 48 ind./m<sup>2</sup> on June 30, 2016 at the same place at a water temperature of 6.9°C. In the Intrigan River of the Kolyma River basin pupae were recorded in June–July at a water temperature of 6.0–11.8 °C, adults—in July at a water temperature of 11.8–14.4 °C, pH 8, in the number of 69 ind./m<sup>2</sup> (pupae) and 23 ind./m<sup>2</sup> (adults) on July 17, 2015 and larvae 11 ind./m<sup>2</sup> on July 1, 2023. In the Kolyma River basin adults of *N. rohdendorfi* were collected also on July 14, 2023 on the banks of the Khurendya River at a maximum altitude of 1095 m above sea level in inclement weather. The first half of the day was characterized by heavy fog and strong winds. In the second half of the day it became cloudy with clearings. At 21:47 local time, the moment of distribution of a swarm of adults over the surface of stones at the water’s edge was recorded (Figs. 50–51). All imagoes were actively vibrating their wings at this moment. The next day, single dead individuals were noted on the stones near the water film. In samples of zoobenthos on July 15, 2023, 5 larvae and 59 pupae per square meter were found.

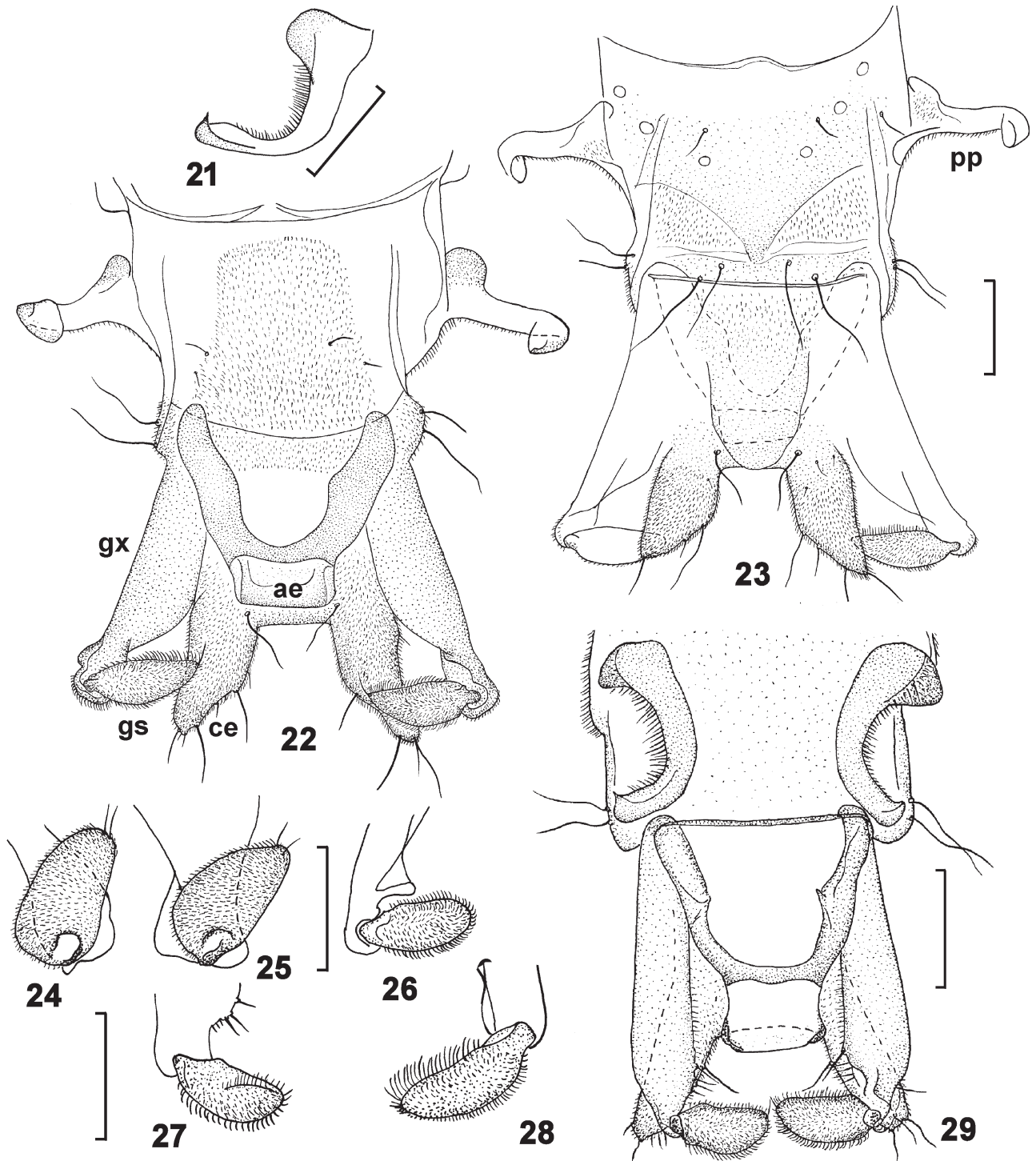
*Nymphomyia kannasatoi* Makarchenko et Gunderina

(Figs. 21–34)

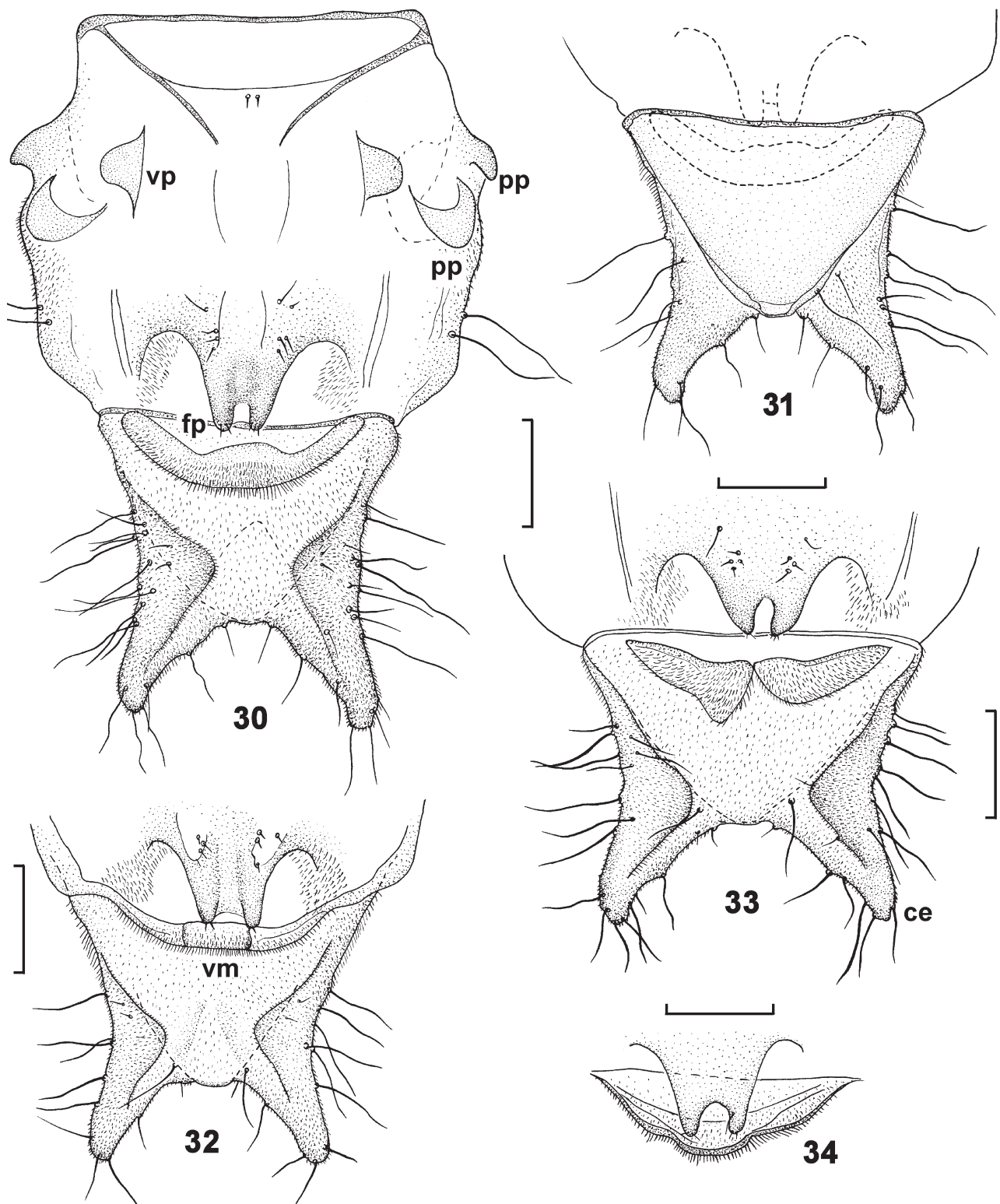
*Nymphomyia kannasatoi* Makarchenko et Gunderina, 2014: 536.

*Nymphomyia rohdendorfi* Makarchenko, 1979; Makarchenko *et al.* 1989: 17 (misidentification).

*Nymphomyia* sp. near *N. rohdendorfi* Saigusa *et al.* 2009: 6.



**FIGURES 21–29.** Adult male terminalia of *Nymphomyia kannasatoi* Makarchenko et Gunderina from Japan (21–28) and Sakhalin Island (29). 21, paratergal projection; 22, 29, in ventral view; 23, in dorsal view; 24–28, gonostylus in varies positions. Scale bars—50  $\mu$ m.



**FIGURES 30–34.** Adult female terminalia of *Nymphomyia kannasatoi* Makarchenko et Gunderina from Japan (30–33) and Sakhalin Island (34). 30, 32–33, in ventral view; 31, in dorsal view; 34, ventral margin and fingerlike projections of sternite VIII. pp—paratergal projections, vp—ventral projection, fp—fingerlike projection, vm—ventral margin, ce—cerci. Scale bars—50  $\mu$ m.

**Material examined.** JAPAN: adult male (holotype), 12 adult males, 14 females (paratypes), Honshu Island, Gunma Prefecture, Katsuyama, Kawawa area, Ueno Village, upper part of Kanna River, alt. *ca* 511 m a.s.l., 36.083619 N, 138.776519 E, 29.III.2014, 9.IV.2014, leg. S. Sato. RUSSIA: 2 adult females, Sakhalin Island, Dolinsk District, Sokol Village, Belaya River, 47.247234 N, 142.737799 E, 10.VII.1986, leg. E. Makarchenko and M. Makarchenko; 1 adult male, 1 female, the same data except 12.VII.1986; 1 adult male, 1 female, the same data except 20.VII.1986, leg. E. Makarchenko and M. Makarchenko.

## Description

*Adult male* (n=8). Pale grey, weak chitinized. Total length 2.0–2.5 mm. Total length/wing length 1.0–1.15.

Head 216–248  $\mu\text{m}$  long and 164  $\mu\text{m}$  wide, gradually narrowed anteriorly and ends rostrum, 52–64  $\mu\text{m}$  long and 40–44  $\mu\text{m}$  wide and which is distally expanded, its anterior margin undulating, with a slightly curved ventral side in the beak-shape; rostrum anteriorly with 3 pairs of setae and with 2 pairs of lateral setae. Compound eyes on the dorsal side of the head are wide apart, the distance between them is 1.3–1.8 times the width of the end of rostrum; on the ventral side of the compound eyes are fused but at the midline of ommatidia do not touch. Antenna 186–190  $\mu\text{m}$  long, 1<sup>st</sup> segment 36–38  $\mu\text{m}$  long, 2<sup>nd</sup> segment 22–24  $\mu\text{m}$  long, 3<sup>rd</sup> segment long (108–112  $\mu\text{m}$ ), slightly extended to the top, with a terminal sensilla of three rod-like structures, most of which are 12–16  $\mu\text{m}$  in length; antenna 1.16–1.31 times shorter than head. Labium with almost straight or slightly rounded front edge and with tooth-covered spines, very apparent when viewed from the side, laterally with 2–3 pairs of long setae, medially with a pair of short setae.

Thorax length 0.58–0.66 mm; wing length 1.92–2.28 mm, on the edge with whip-shape setae 0.34–0.46 mm long; halteres 0.24 mm long.

Terminalia (Figs. 21–29). Abdomen 1.66–1.70 mm long. Segment VIII with one pair of large, elongate, posteriorly directed, lateral paratergal projections 70–72  $\mu\text{m}$  long, that basally with bump (Figs. 21–23, 29). Cerci 60–76  $\mu\text{m}$  long, distally narrowed, digitiform; gonocoxite length 140  $\mu\text{m}$ , in 2.9–3.8 times as long as gonostylus, distally to the top with a little strength; gonostylus length 36–48  $\mu\text{m}$ , shape varies from bean to lanceolate or rounded (depending on the position in preparation), covered with numerous hair-like setae directed inward, sometimes with 1–2 more long setae in apex (Figs. 24–28); aedeagus length of 44–60  $\mu\text{m}$ , width—40  $\mu\text{m}$ , with straight apex (Figs. 22, 29).

*Adult female* (n=10) in general similar to male. Total length 1.6–2.3 mm. Total length/wing length 0.89–1.0.

Head 208–228  $\mu\text{m}$  long and 148  $\mu\text{m}$  wide. Rostrum length 48–52  $\mu\text{m}$ , width 40  $\mu\text{m}$ . The distance between the compound eyes dorsally 1.9–2.0 times the width of rostrum end. Antenna 174–192  $\mu\text{m}$  long; length of 1<sup>st</sup> segment 36–40  $\mu\text{m}$ , length of 2<sup>nd</sup> segment 22–24  $\mu\text{m}$ ; 3<sup>rd</sup> segment 92–104  $\mu\text{m}$  long; terminal sensilla maximum length 14–16  $\mu\text{m}$ ; antenna 0.81–0.84 times shorter than the head.

Thorax length 0.48–0.54 mm. Wing length 2.1–2.3 mm, on the edge with whip-shape setae 0.46–0.54 mm long; halteres 0.27 mm long.

Terminalia (Figs. 30–34). Segment VIII with 2 pairs of lateral paratergal projections of which proximal pair nose-shaped, *ca* 12  $\mu\text{m}$  long, distal pair roundish, 28  $\mu\text{m}$  long. Anteromedial from distal pair of projections also has a pair of rounded projections 12–16  $\mu\text{m}$  long (Fig. 30). Sternite VIII in distal part with a pair of fingerlike projections with 1–2 short setae in apex. Cerci 48–64  $\mu\text{m}$  long, in subapical part with 2–4 pairs of long setae. In proximal part ventrally located transverse margin which covered short hair-like setae; shape of this ventral margin varies and sometimes consists of two parts (Figs. 30, 32–33). Also on the ventral side laterally on each side is weakly expressed rounded-triangular lobe covered with short setae.

**Ecology and biology.** Emergence of adults of *N. kannasatoi* in the Kanna River in Japan (Fig. 56) occurs in the spring—from late March to the first half of April. In South Sakhalin in the Belaya River (Fig. 57) this process begins in mid-June and the mass flight ends at the end of July. During this period, the water temperature in the river is 8–15.4 °C. However, judging by the quantitative census data of the zoobenthos of the Belaya River in 1986–1987, emergence of adults can continue until mid-October. Larvae are extremely in the spring, reaching their maximum number in April (280 ind./m<sup>2</sup>). At this time they are in instars III and IV. By the end of May, the number of larvae gradually decreases due to pupation and consumption by benthic invertebrates. The first pupae were discovered on May 21 at a water temperature of 5°C and were continuously found until mid-October. Swarming of adults on

Belaya River was not observed. All collections of adult insects (wingless) were made from stones in the water. Analysis of the longitudinal distribution of nymphomyiids showed that their numbers in the upper sections of the river are higher than in the lower sections. Thus, in mid-April, the number of larvae in the upper reaches of the river ranged from 346 to 1179 ind./m<sup>2</sup>, in the middle and lower reaches—from 3.2 to 12.5 ind./m<sup>2</sup>. Life cycle of *N. kannasatoi* from the Belaya River is univoltine.

**Distribution.** *N. kannasatoi* is known from Honshu Island of Japan and South part of Sakhalin Island (Fig. 2).

### *Nymphomyia kaluginae* Makarchenko

(Figs. 35–37)

*Nymphomyia kaluginae* Makarchenko, 2013: 292

**Material examined.** RUSSIA: adult male (holotype), 6 adult males, 8 females, including 2 copulating pairs (paratypes), *Amur Region*, Tyndinsky District, Nagima River in 15 km East of the Solovyovsk Village (Zeya River basin), 54.174683 N, 124.639533 E, 18.VII.2009, leg. D. Kotsyuk; 1 mature pupa, the same data except Levaya Burinda River, tributary of the Burinda River, 53.59925 N, 124.9135 E, 20.VII.2009, leg. D. Kotsyuk; 4 larvae, the same data except Selemdzhinsky District, Maristy Stream, tributary of Elgakan River (basin of Selemdzha River), East of Zlatoustovsk Village, 52.933683 N, 133.659019 E, 26.VII.2009, leg. D. Kotsyuk; 8 mature pupae, 12 larvae, the same data except Zeya District, Zeysky State Nature Reserve, Bolshoy Garmakan River (basin of the Zeya Reservoir), 53.886358 N, 127.193588 E, 23.VI.2004, leg. V. Teslenko and T. Tiunova; 7 larvae, the same data except 25.06.2013, leg. E. Makarchenko.

*Adult male* (n=5). Pale gray, slightly chitinized. Total length 1.76–2.28 mm.

Head 212–240 µm long, 152–188 µm wide, gradually narrows anteriorly and ends with a rostrum, which does not expand distally and is approximately the same width along the entire length (36–40 µm), its anterior edge is almost straight, when viewed from the side it is slightly curved ventrally in the form of a beak; rostrum dorsally with 4–5 pairs of setae. The compound eyes on the dorsal side of the head are widely spaced, the distance between them is 2.3–2.6 times greater than width of rostrum end; on the ventral side the compound eyes are close to each other, but along the midline the ommatidia do not touch. Antenna 192–196 µm long, two basal segments rounded, length of 1<sup>st</sup> segment 44 µm, 2<sup>nd</sup>–24 µm; 3<sup>rd</sup> segment 104 µm long, widening towards the apex, with a terminal sensilla consisting of two rod-shaped structures, the largest of which is 20 µm long; antenna 0.80–0.91 times shorter than the head. Labium with rounded anterior edge, its anterior part is covered with spines, laterally with 2 pairs of long setae, medially with 1 pair of shorter setae.

Thorax 0.64 mm long, halteres 0.14 mm long; wings absent.

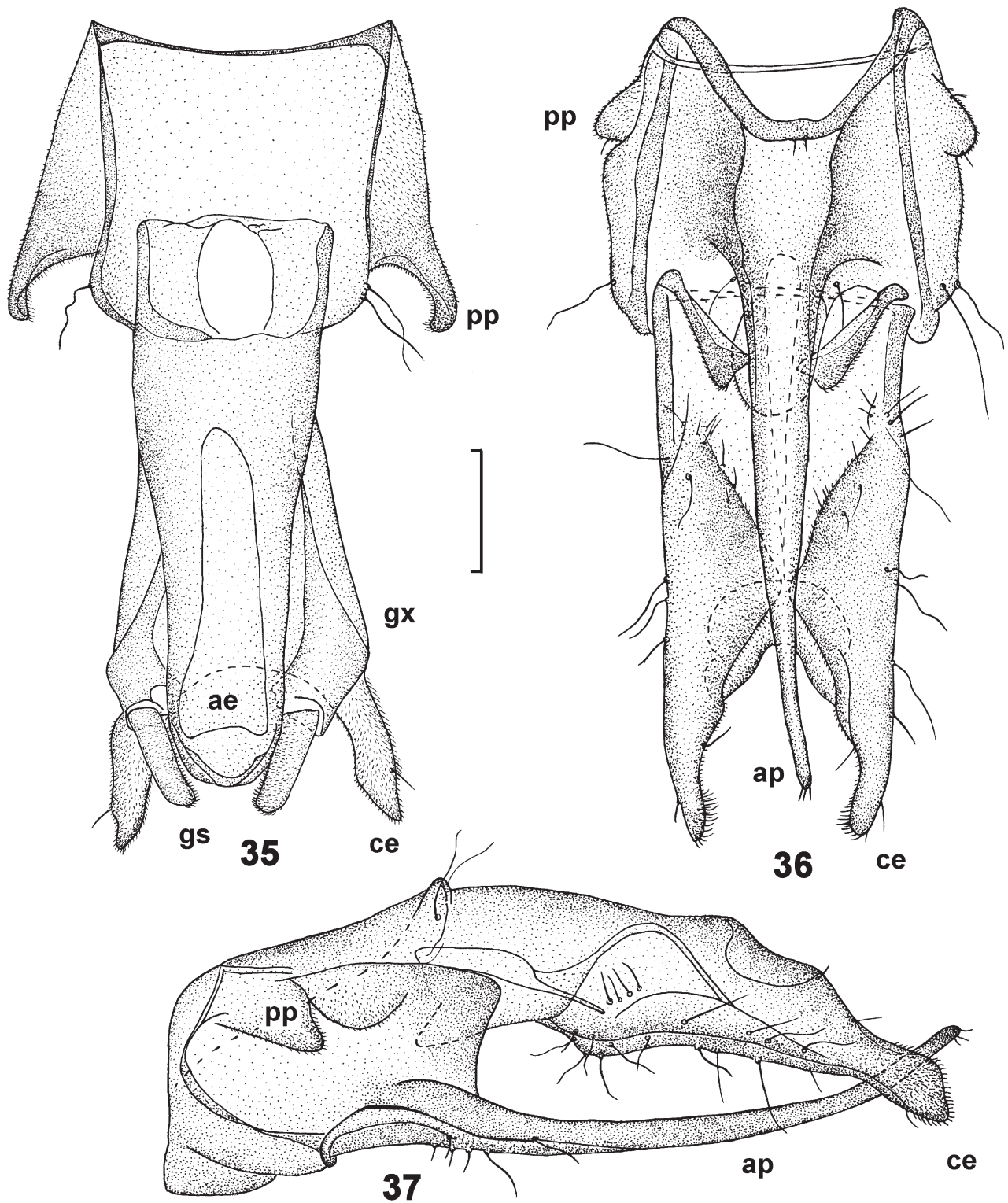
Terminalia (Fig. 35). Abdomen length 0.88–1.50 mm. Segment VIII with long paratergal appendages 44–52 µm long, distally curved inward and covered along the inner edge with short setae, without basal tubercles. Cerci 80–84 µm long, narrow distally; gonostylus 48–56 µm long, approximately same width along entire length, apically rounded, covered with numerous short hair-like setae directed inward; aedeagus 212–228 µm long, 56–60 µm wide in the apical third, with a rounded apex (Fig. 35).

*Adult female* (n=5). Overall similar to the male. Total length 1.76–2.0 mm.

Head 224–228 µm long and 164 µm wide. The distance between the compound eyes dorsally is 2.5–3.0 times width of rostrum end. Antenna 176–180 µm long, length of 1<sup>st</sup> segment 40 µm, 2<sup>nd</sup>–24 µm; 3<sup>rd</sup> segment 96 µm long, maximum length of apical sensilla 16 µm; antenna 0.75–0.83 times shorter than head.

Thorax 640 µm long, typical for genus.

Terminalia (Figs. 36–37). Segment VIII bears small lateral projections shaped like tubercles covered with short setae. Sternites V–VII are simple, without processes or appendages. Sternite VIII with an anal point 232–280 µm long, which gradually narrows towards the apex and apically with 3 short setae 4–5 µm long (Fig. 36); when viewed from the side, the anal point slightly curved in the ventero-dorsal direction (Fig. 37). At the base of the last abdominal segment, anal point adjacent to pair of chitinized sclerites, widening towards apex and covered with short setae (Fig. 36). Last abdominal segment also bears cerci 40–60 µm long, which are pubescent subapically and along inner margin with short setae (Figs. 36–37).



**FIGURES 35–37.** Adult male (35) and female (36–37) terminalia of *Nymphomyia kaluginae* Makarchenko. 35–36, in ventral view; 37, in lateral view. ae, aedeagus; ap, anal point; ce, cerci; gs, gonostylus; gx, gonocoxite; pp, paratergal projection. Scale bar—50  $\mu$ m.

**Ecology and biology.** Larvae and pupae of *N. kaluginae* were collected in the Bolshoi Garmakan River (Fig. 54) on June 23, 2004 at a water temperature of 10.5 °C, at a depth of 15–20 cm on gravel and pebble soil. On the rift, the number of larvae was 1456 ind./m<sup>2</sup>, on the stretch—640 ind./m<sup>2</sup>. Adults, but already wingless, nymphomyids

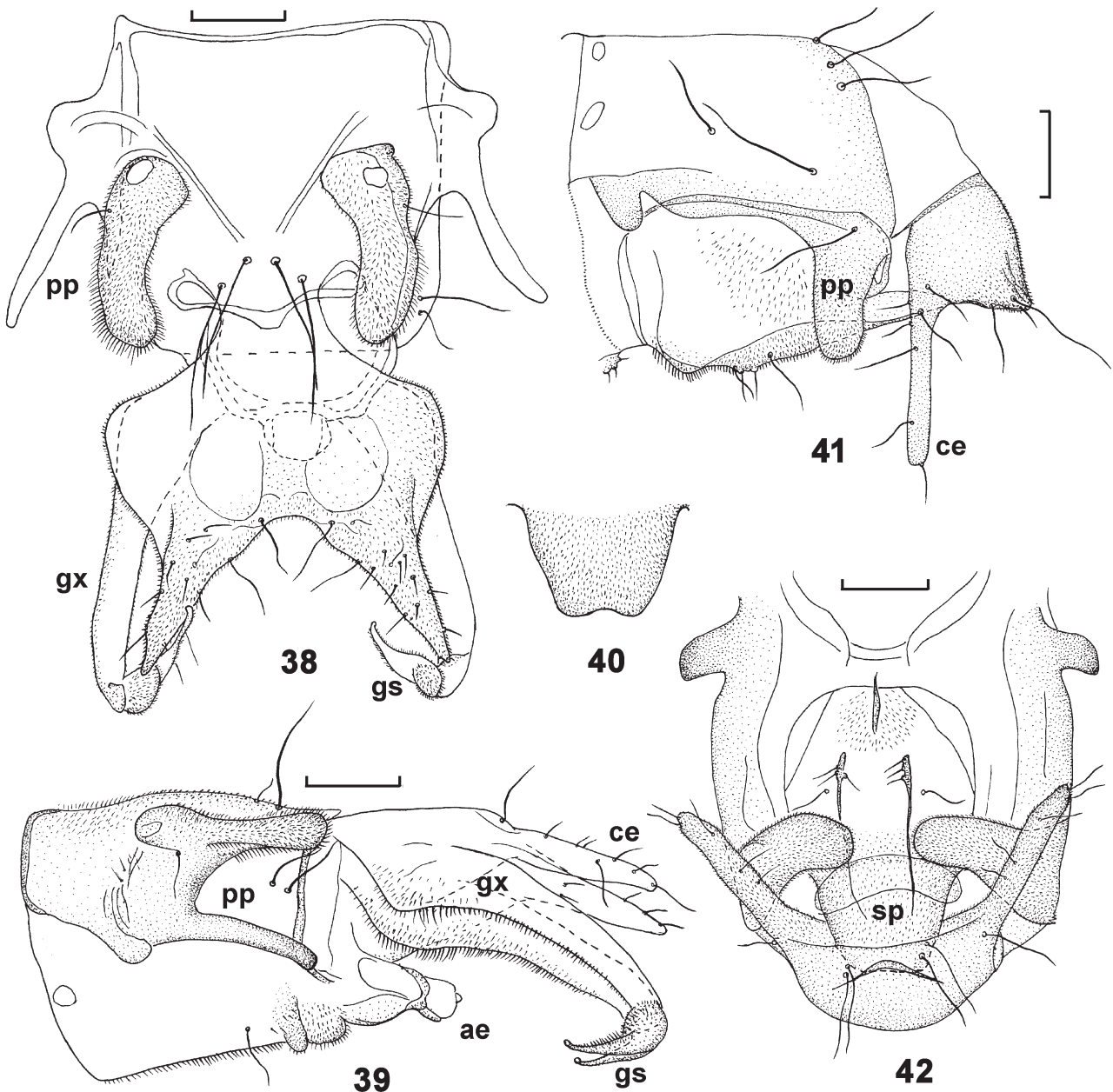
(608 ind./m<sup>2</sup>) were collected only in the Nagima River on July 28, 2008, on a riffle at a water temperature of 14.3 °C, at a depth of 10 cm, and mature pupae were collected in the Levaya Burinda River on July 20, 2008, on a riffle at a water temperature of 8.6 °C, at a depth of 10 cm, on stones and pebble soil.

**Distribution.** *N. kaluginae* is known only from type locality in the Zeya River basin (Amur River basin) (Fig. 2).

***Nymphomyia alba* Tokunaga**

(Figs. 38–42)

*Nymphomyia alba* Tokunaga, 1932: 561; 1935b: 127; Makarchenko *et al.* 1989: 15; Courtney 1994: 14; Takemon & Tanida 1994:120; Makarchenko 1996: 23, 2006: 739; Saigusa 2014: 80; Nakamura *et al.* 2015: 23; Makarchenko & Gunderina 2019: 74.



**FIGURES 38–42.** Adult male (38–39) and female (40–42) terminalia of *Nymphomyia alba* Tokunaga. 38, in dorsal view; 39, 41, in lateral view; 40, sternite IX process; 42, in ventral view. ae, aedeagus; ce, cerci; gs, gonostylus; gx, gonocoxite; pp, paratergal projections; sp, sternite IX median process. Scale bars—50 µm.

**Material examined.** JAPAN: 12 adult males, 10 females, Gunma Prefecture, Nuru River, tributary of the Tone River, 36.499296 N, 138.682426 E, 3–6.V.2020, leg. S. Sato; 5 adult males, 4 females, Kochi Prefecture, Monobe River, 33.762000 N, 134.028806 E, 22.XI.2020, leg. T. Minami. RUSSIA: 23 adult males, 14 females, Kunashir Island, Kuril Islands, Kurilsky State Nature Reserve, Balysheva Creek (Filatovka River basin), about 100 m above the mouth, 44.183026 N, 146.025748 E, 19.VI.1989, leg. T. Tiunova; 46 adult males, 24 females, the same data except unnamed creek of Filatovka River basin (lower reaches), in the area of the Filatovsky cordon, 44.194098 N, 146.020354 E, 26.VI.2013, leg. Yu. Sundukov; 1 adult male, 1 female, 1 pupal exuvia, Kunashir Island, Pacific coast, Prozrachnyi Stream (Kosmodemyanskaya Bay basin), 44.094722 N, 145.890833 E, 24–25.VIII.2016, drift trap, leg. M. Astakhov.

## Description

*Adult male* (n=10). Pale gray, slightly chitinized. Total length 2.1–2.2 mm; total length/ wing length 1.04–1.05.

Head 232–316  $\mu\text{m}$  long and 148–152  $\mu\text{m}$  wide, 1.3–1.4 times longer than wide, gradually narrows anteriorly and ends with a rostrum, which is almost not expanded distally, its anterior edge is straight or slightly rounded and slightly rounded in lateral view; rostrum dorsally with 5 pairs of setae. Compound eyes on the dorsal side of the head are widely spaced, the distance between them is 1.9–2.1 times greater than width of rostrum end; on the ventral side the compound eyes are not fused with each other. Antenna 170–178  $\mu\text{m}$  long, two basal segments rounded, length of 1<sup>st</sup> segment 36  $\mu\text{m}$ , 2<sup>nd</sup>—20–24  $\mu\text{m}$ ; 3<sup>rd</sup> segment 88–199  $\mu\text{m}$  long, widening towards the apex, with a terminal sensilla consisting of three rod-shaped structures, the largest of which is 16  $\mu\text{m}$  long; antenna 0.80–0.89 times shorter than the head. Labium with a rounded anterior edge, laterally with 2–3 pairs of long setae, medially with 1 pair of short setae.

Thorax 0.60–0.64 mm long, bears a pair of wings, halteres and 3 pairs of legs; wing 2.0–2.1 mm long, maximum width 0.26–0.31 mm, boomerang-shaped, with reduced venation, along the margin with long whip-like setae 0.32–0.34 mm long; halteres 0.24–0.28 mm long

Terminalia (Figs. 38–39). Abdomen length 1.39–1.43 mm. Segment VIII with dorsal and ventral pairs of paratergal processes (Figs. 38–39); dorsal pair 84–100  $\mu\text{m}$  long, wider than the ventral one, densely pubescent with microtrichia; ventral pair without microtrichia, 72–76  $\mu\text{m}$  long, with basal tubercle 20  $\mu\text{m}$  high. Cerci in the form of expanded basal triangular outgrowths 84–100  $\mu\text{m}$  long; gonocoxite 132–156  $\mu\text{m}$  long, 3.0–3.8 times longer than gonostylus; gonostylus 36–52  $\mu\text{m}$  long, slightly curved, wider and pubescent basally, glabrous and narrow distally; aedeagus 20–32  $\mu\text{m}$  long, 24–36  $\mu\text{m}$  wide, with a rounded apex.

*Adult female* (n=10) is generally similar to the male. Total length 2.1–2.4 mm; total length/ wing length 1.0–1.1.

The head 197–205  $\mu\text{m}$  long, 146–150  $\mu\text{m}$  wide. The distance between the compound eyes dorsally is 2.0–2.1 times the width of rostrum end. The antenna, like that of a male, is 156–170  $\mu\text{m}$  long, 0.79–0.85 times shorter than the head.

Thorax 0.45–0.48 mm long. Wing length 2.0–2.4 mm, maximum width 0.25–0.30 mm.

Terminalia (Figs. 40–42). Abdomen length 1.48–1.70 mm. Segment VIII distally with pair of tubercles, about 20  $\mu\text{m}$  long, and pair of lateral processes directed downwards and 32–70  $\mu\text{m}$  long. The median process of sternite IX is trapezoidal, without setae, at the posterior edge with small notch in middle (Fig. 40), its length 53  $\mu\text{m}$ , width 64  $\mu\text{m}$ . Cerci 80–116  $\mu\text{m}$  long, slightly expanded basally, in the rest of the part they are narrow rod-shaped and parallel-sided, rarely covered with setae (Figs. 41–42)

**Ecology and biology.** In Japan the larvae and pupae of *N. alba* were collected at Kozugawa along Takami Stream in Nara Prefecture on 16–18 March, 1991 from stony substrata beside the main flow, with 10–100 cm/sec surface current speed, and 6–100 cm deep, with water temperature 6.0 °C. Apparently, the major habitat of this species was the riffle area with compiled debris. It is highly probable that the interstices of pebbles and cobbles (hyporheic zone) in a riffle constitute micro-habitats of larvae and pupae. Reasons for the appearance of wingless adults in the benthos samples are unknown but their restricted micro-distribution which was similar to that of larvae and pupae suggests that the adults cast off the wings after their aerial life and remain alive under water for reproduction (Takemon & Tanida 1994). Our adult material was collected in Manobe River of Kochi Prefecture (Fig. 52) during swarming on November 22, 2020 and in Nuru River of Gunma Prefecture on May 3–6, 2020. Detailed information on the

biology of this species in Japan is given in Saigusa *et al.* (2009). On Kunashir Island nymphomyids were found in the Prozrachnyi Stream (Fig. 53) during drift sampling, at a water temperature of 11.1–13.3 °C at a depth of 20 cm, with a current speed of 0.6–0.7 m/s at rocky and gravel-pebble soil August 24–25, 2016 (Astakhov 2021). Swarming of adults was observed in the Filatovka River basin on June 19, 1989 and June 26, 3013.

**Distribution.** *N. alba* is known from Japan (Honshu and Shikoku Islands, Prefectures Kyoto, Nara, Kochi, Tokushima, Tochigi, Gunma, Mie and Kunashir Island (Kurile Islands) of Russia (Takemon & Tanida 1994; Makarchenko 1996; Saigusa 2014; Nakomura *et al.* 2015; Makarchenko & Gunderina 2019).

### ***Nymphomyia levanidovae* Rohdendorf et Kalugina**

(Figs. 43–45)

*Nymphomyia levanidovae* Rohdendorf et Kalugina, 1974: 687; Makarchenko & Makarchenko 1983: 92; Makarchenko *et al.* 1989: 15; Courtney 1994:15; Makarchenko 1996: 23, 2006: 739; Makarchenko & Gunderina 2019: 75.

**Material examined.** RUSSIA: 58 adult males, 46 females, Primorye Territory, Khasansk District, Kedrovaya Pad Nature Reserve, Kedrovaya River, 43.097900 N, 131.556867 E, 27.VIII.1986, leg. T. Arefina; 14 adult males, 12 females, the same data except 3.VI.2012, leg. Makarchenko; 23 adult males, 16 females, the same data except 26.VIII.2013, leg. E. Makarchenko; 1 adult male, Primorye Territory, Pozharskyi District, Antonovskiy Spring, tributary Zeva River, (Bikin River basin), 46.740729 N, 137.387804 E, 24.VII.1995, leg. T. Tiunova.

*Adult male* (n=10). Pale gray, slightly chitinized. Total length 2.0–2.6 mm; total length/ wing length 1.09–1.45.

Head 180–213 µm long and 136–148 µm wide, 1.3–1.4 times longer than wide, gradually narrower anteriorly and ends with a rostrum, which is not expanded distally, its anterior edge is straight or slightly rounded, as in the previous species when viewed from the side, it is slightly curved ventrally in the form of a beak; rostrum dorsally with 7 pairs of setae. The compound eyes on the dorsal side of the head are widely spaced, the distance between them is 2.5–2.6 times the width of the rostrum end; on the ventral side the compound eyes are not fused with each other. Antenna 154–172 µm long, length of 1<sup>st</sup> segment 28–40 µm, 2<sup>nd</sup>—20–22 µm; 3<sup>rd</sup> segment 86–92 µm long, widening towards the apex, with a terminal sensilla consisting of three rod-shaped structures, the largest of which is 14 µm long; antenna 0.78–0.81 times shorter than the head. Labium with a rounded anterior edge, as in the previous species.

Thorax 0.61–0.62 mm long; wing 1.7–1.8 mm long, maximum width 0.26–0.31 mm, boomerang-shaped, with reduced venation, along the margin with long whip-like setae 0.28–0.39 mm long; halteres 0.19–0.22 mm long.

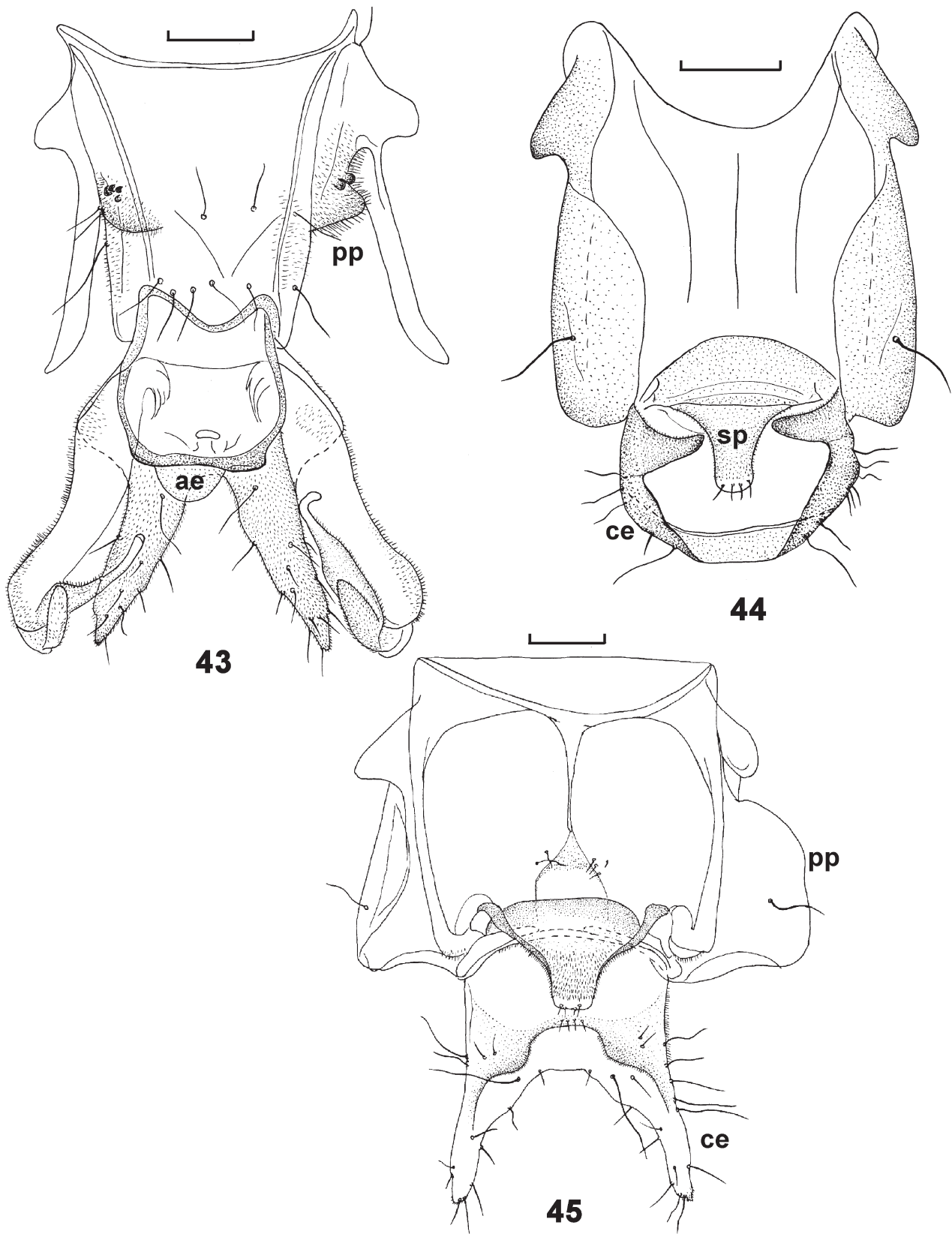
Terminalia (Fig. 43). Abdomen length 1.32–1.82 mm. Segment VIII with a pair of unsclerotized, wide and short internal paratergal projections, equipped at the base with 2–3 close rounded sclerotized tubercles, as well as a pair of sclerotized bare and long (112–116 µm), weakly curved and rounded lateral paratergal projections at the apex, with large basal tubercles 24–32 µm long and protruding to the sides (Fig. 43). Cerci triangular, pubescent, 92–96 µm long; gonocoxite is arcuate, 140–160 µm long, 1.7–2.0 times longer than gonostylus; gonostylus 72–84 µm long, slightly curved, wider and pubescent basally, glabrous and narrow distally; aedeagus 20–24 µm long, 32–36 µm wide, with a rounded apex (Fig. 43).

*Adult female* (n=8) is generally similar to the male. Total length 2.2–2.8 mm; total length/ wing length 1.25–1.77.

Head 197–205 µm long and 140 µm wide. The distance between the compound eyes dorsally 3.5–4.0 times the width of end of rostrum. Antenna, like that of a male, 110–116 µm long, 0.75–0.80 times shorter than head.

Thorax 0.57–0.64 mm long. Wing length 1.56–1.76 mm, maximum width 0.20–0.24 mm.

Terminalia (Figs. 44–45). Abdomen length 1.52–1.61 mm. Segment VIII distally with a pair of paratergal nasal projections, 26–28 µm long, and pair of wide and long lateral lobe-shaped projections, 68–70 µm long, directed posteriorly (Figs. 44–45). The median process of sternite IX is elongated, with rounded apex, along the edge of which there are 4 short setae (µm) (Figs. 44–45). The last segment of the abdomen bears elongated triangular cerci with rounded apices, 84–88 µm long, with sparse setae.



**FIGURES 43–45.** Adult male (43) and female (44–45) terminalia of *Nymphomyia levanidovae* Rohdendorf et Kalugina, in ventral view. For abbreviations see Figs. 11–16, 30–42. Scale bars—50  $\mu$ m.

**Ecology and biology.** As a result of hydrobiological research in Southern Primorye *N. levanidovae* was found in the Kedrovaya River of Kedrovaya Pad Nature Reserve in Khasansk District (Fig. 55), in connection with which it was possible to study its biology. Quantitative collections and observations of *N. levanidovae* were carried out in the Kedrovaya River from May to October 1978, from June 1979 to August 1980 and in June 1981. It turned out that *N. levanidovae* has a bivoltine life cycle. Mass emergence of adults of the first generation occurs from the end of May to the beginning of July at a water temperature of 10.2–19 °C, of the second generation—from early September to early November at a water temperature of 8.5–13.8 °C. Adults are also found during the period between mass flights. After emergence, nymphomyiids swarm above the water at a height of about 2 m, here they copulate in the air, then the copulating pairs descend onto damp stones in a watercourse or the surface of the water, lose their wings, plunge into the water and for some time (at least a few hours) live at the bottom of the river. At this time the female lays eggs. Swarming of nymphomyiids occurs during sunset. The larvae lead an active lifestyle, moving along the stones with the help of abdominal pseudopods. The larvae feed on microscopic algae, which they scrape from the surface of stones. Pupae and adult insects do not feed (Makarchenko & Makarchenko 1983).

**Distribution.** *N. levanidovae* is known only from South Primorye of the Russian Far East and upper stream of Bikin River (Amur River basin) (Fig. 2).

## Updated key on adults by Courtney (1994) for known species of *Nymphomyia* of the world

### Males

1. Ommatidia of compound eyes contiguous ventrally; abdominal segment VIII without elongate paratergal projections; gonocoxites and cerci fused indistinctly. . . . . 2
- Ommatidia of compound eyes separated ventrally; abdominal segment VIII with elongate paratergal projections; gonocoxites and cerci readily separable, at least distally . . . . . 6
2. Abdominal segment VIII dorsolaterally and/or laterally set with numerous setiform sensilla or patches of short setae; gonocoxites enlarged, extended anteroventrally to beyond posterior margin of abdominal segment VIII, tapered apically; gonostylus bifurcate . . . . . 3
- Abdominal segment VIII not invested with setiform sensilla or patches of short setae; gonocoxites reduced, extended posteriorly or medially for only a short distance; gonostylus variable. . . . . 5
3. Ventral eye bridge with contact between three pairs of ommatidia . . . . . 4
- Ventral eye bridge with contact between two pairs of ommatidia; gonostylus of two lobes, posterior lobe curved basally, bent abruptly at middle, narrowly tapered apically, anterior lobe thick, invested with microtrichia on outer margin, with short, blunt, medially directed apical lobe bearing short sensilla at tip . . . . . *N. brundini* (Kevan)
4. Gonostylus of two similarly shaped, curved lobes, anterior lobe relatively more tapered apically than posterior lobe . . . . . *N. holoptica* Courtney
- Gonostylus with two branches—bare sickle-shaped and serrate elongated, slightly curved, with numerous setae along the inner edge. . . . . *N. aijuanae* Makarchenko et Tang
5. Compound eyes with microtrichia between all facets; cranium almost entirely covered with microtrichia; terminal sensilla of antenna subapical; empodium of foretarsus not extended beyond tarsal claws; abdominal stemites V and VI each with a pair of posteriorly or medially projected processes; gonostylus simple; aedeagus markedly elongate, aedeagal rod, when not exerted, extended anteriorly to abdominal segment V. . . . . *N. walkeri* (Ide)
- Compound eyes without microtrichia between ventral facets; cranium glabrous anterodorsally and posteroventrally (behind eyes); terminal sensilla of antenna apical; empodium of foretarsus elongate, extended beyond tarsal claws; abdominal stemites V and VI without elongate processes; gonostylus bifurcate; aedeagus short, aedeagal rod, when not exerted, extended anteriorly only to abdominal segment VIII. . . . . *N. dolichopeza* Courtney
6. Gonocoxites not extended posteriorly beyond apex of cercus; gonostylus short, blunt, extended posteriorly . . . . . 7
- Gonocoxites extended posteriorly to near or beyond apex of cercus; gonostylus elongate, tapered, curved anteroventrally. . . 9
7. Segment VIII with 1 pair of large elongate, posteriorly directed lateral paratergal projections with blunt apex and without basal bump. Aedeagus with rounded apex. . . . . 8
- Paratergal projections with basal bump. Aedeagus with almost straight apex (Figs. 21–22) . . . . . *N. kannasatoi* Makarchenko et Gunderina
8. Aedeagus 212–228 µm long, gonostylus along the entire length is approximately same width (Fig. 35) . . . . . *N. kaluginae* Makarchenko
- Aedeagus 40–60 µm long, gonostylus lanceolate or rounded (Fig. 8). . . . . *N. orientalis* sp. nov.  
*N. rohdendorfi* Makarchenko
9. Dorsal and ventral paratergal projections distinct, of approximately equal length (Fig. 38). . . . . *N. alba* Tokunaga
- Dorsal paratergal projections poorly developed (Fig. 43) . . . . . *N. levanidovae* Rohdendorf et Kalugina



**FIGURES 46–51.** Localities of *Nymphomyia orientalis orientalis* subsp. nov. (46), *N. orientalis makcha* subsp. nov. (47), *N. rohdendorfi* Makarchenko (48–51). 46, Polovinka River, Amur River basin (photo by N. Yavorskaya); 47, Makcha River, Zeya River basin (photo by E. Makarchenko); 48, Intrigan River, Kolyma River basin (photo by E. Khamenkova); 49, Kalanegir River, Katun River basin, of Altai Mountains (photo by A. Kotovshchikov); 50–51, Khurendya River, Kolyma River basin, adults wait out bad weather on coastal rocks (photo by A. Krasheninnikov).



**FIGURES 52–57.** Localities of *Nymphomyia alba* Tokunaga (52–53), *N. kaluginae* Makarchenko (54), *N. levanidovae* Rohdendorf et Kalugina (55), and *N. kannasatoi* Makarchenko et Gunderina (56–57). 52, Monobe River, Honshu, Japan (photo by S. Sato); 53, Prozrachnyi Stream, Kunashir Island, Russia (photo by M. Astakhov); 54, Bolshoi Garmakan River, Zeya River basin (photo by E. Makarchenko); 55, Kedrovaya River, South Primorye (photo by E. Makarchenko); 56, Kanna River, Honshu, Japan (photo by S. Sato); 57, Belaya River, South Sakhalin (photo by E. Makarchenko).

## Females

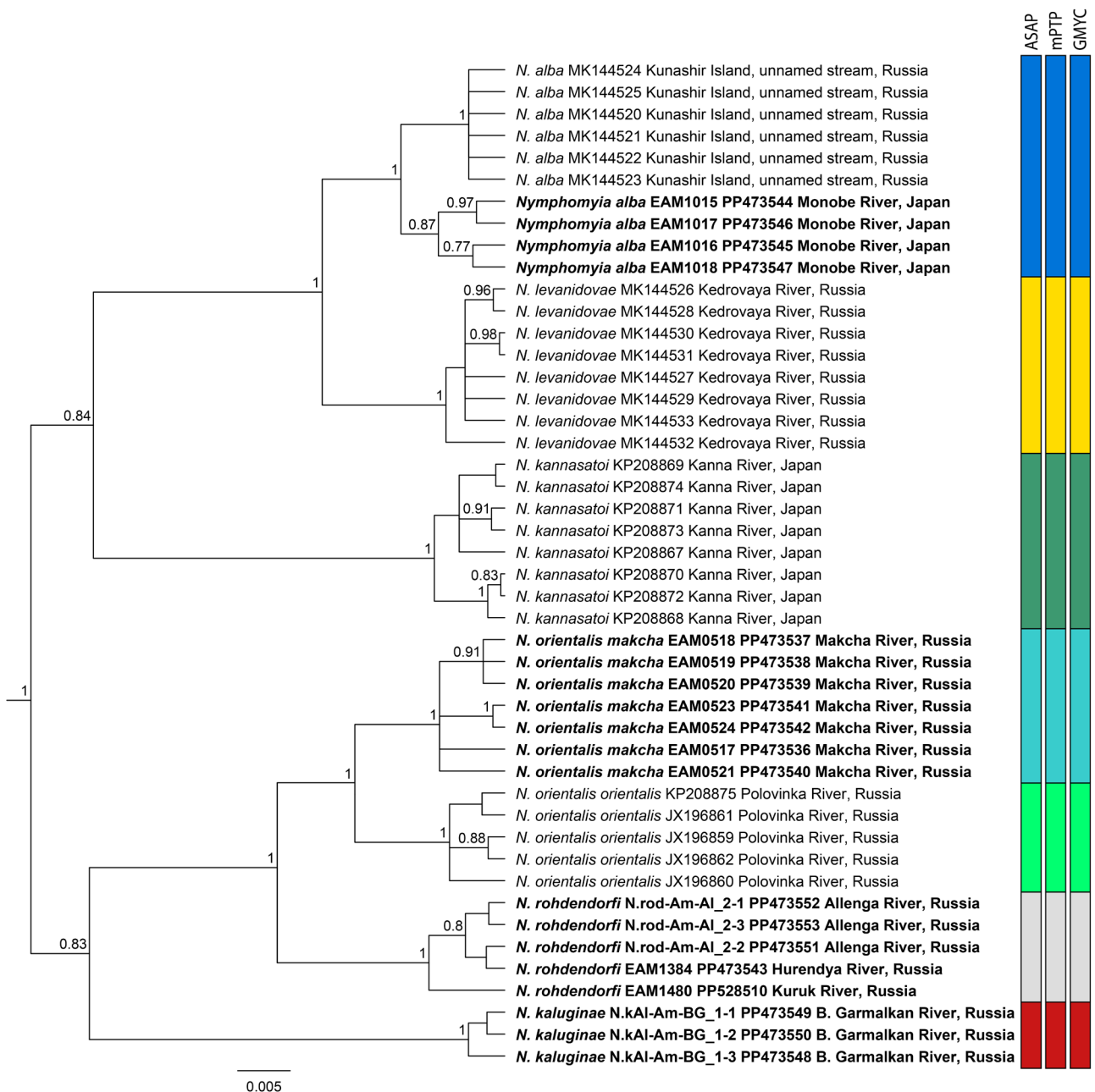
1. Ommatidia of compound eyes contiguous ventrally ..... 2
- Ommatidia of compound eyes separated ventrally ..... 5
2. Ventral eye bridge with contact between three pairs of ommatidia ..... 3
- ventral eye bridge with contact between two pairs of ommatidia ..... 4
3. Abdominal segment VIII dorsolaterally and/or laterally set with numerous setiform sensilla, glabrous anterolateral tubercle, and elongate, ventrally projected lobe. Cerci broad, directed posteroventrally. Segment VII without any tubercles in anterolateral part ..... *N. holoptica* Courtney
- Abdominal segment VIII not invested with setiform sensilla, with pair of small lateral paratergal projections. Cerci distally expanded, ending in narrow projection. Segment VII with pair of small tubercles in anterolateral part. .... *N. aijuanae* Makarchenko et Tang
4. Compound eyes with microtrichia between all facets; cranium almost entirely covered with microtrichia; terminal sensilla of antenna subapical; abdominal segment VIII with lateral flap bearing several thick, elongate, ventrally projecting, hair-like processes. .... *N. walkeri* (Ide)
- Compound eyes without microtrichia between ventral facets; cranium glabrous anterodorsally and posteroventrally (behind eye); terminal sensilla of antenna apical; abdominal segment VIII laterally with complex, glabrous receptacle for male gonostylus. .... *N. dolichozeza* Courtney
5. Sternite VIII with long anal point, which gradually narrows towards apex and apically with 3 short setae (Figs. 36–37). .... *N. kaluginae* Makarchenko
- Sternite VIII without anal point ..... 6
6. Segment VIII with 1 pair of short lateral paratergal projects ..... 7
- Segment VIII with 2 pairs of lateral paratergal projects different shape and size ..... 8
7. Cerci with rounded “heel” and their distal part is 12–16  $\mu\text{m}$  long. Internal lobes of gonocoxite approximately same width or slightly widen proximally (Figs. 11–14) ..... *N. orientalis* **sp. nov.**
- Cerci with rounded triangular “heel” and their distal part is 24–32  $\mu\text{m}$  long. Internal lobes of gonocoxite widened and rounded distally, and narrow proximally (Figs. 16–20) ..... *N. rohdendorfi* Makarchenko
8. Segment VIII with 2 pairs of lateral paratergal projections of which proximal pair nose-shaped, ca 12  $\mu\text{m}$  long, distal pair roundish, 28  $\mu\text{m}$  long. Anteromedial from distal pair of projections also has a pair of rounded projections 12–16  $\mu\text{m}$  long (Figs. 30–34). .... *N. kannasatoi* Makarchenko et Gunderina
- Shape of projections different. .... 9
9. Segment VIII distally with a pair of paratergal nasal projections, 26–28  $\mu\text{m}$  long, and a pair of wide and long lateral lobe-shaped projections, 68–70  $\mu\text{m}$  long, directed posteriorly. Tergite VIII with broad, ventrally projected lobe that does not overlap with lobe from opposite side; cerci broad (Figs. 44–45) ..... *N. levanidovae* Rohdendorf et Kalugina
- Segment VIII distally has a pair of tubercles, about 20  $\mu\text{m}$  long, and a pair of lateral processes directed downwards and 32–70  $\mu\text{m}$  long. Tergite VIII with narrow, ventrally projected lobe that overlaps with lobe from opposite side; cerci thin (Figs. 41–42). .... *N. alba* Tokunaga

## Results of DNA barcoding

In this study we obtained 19 DNA barcodes of cytochrome c oxidase subunit I (658 bp) that belong to 4 species, *N. alba*, *N. orientalis makcha* **subsp. nov.**, *N. rohdendorfi* and *N. kaluginae*. Intraspecific p-distances within these taxa were 0.75%, 0.49%, 0.64% and 0.51% respectively (Table 2). Such values are average for the genus. The largest intraspecific distances were observed in *N. alba* as a result of divergence between samples from the Monobe River, Japan and Kunashir Island.

**TABLE 2.** Inter- intraspecific sequence divergence (%) based on p-distances for the cytochrome c oxidase subunit I (COI) mitochondrial DNA of *Nymphomyia* spp.

Species	BIN BOLD	N	Intra-specific	1	2	3	4	5	6
1. <i>N. alba</i>	AED2646	10	0.75						
2. <i>N. kannasatoi</i>	ACV8873	8	0.49	5.95					
3. <i>N. kaluginae</i>		3	0.51	7.48	6.45				
4. <i>N. levanidovae</i>	AED7119	8	0.32	2.20	5.45	7.69			
5. <i>N. rohdendorfi</i>		5	0.64	6.52	5.63	6.38	6.53		
6. <i>N. orientalis makcha</i>		7	0.49	6.83	5.44	6.18	7.00	2.34	
7. <i>N. orientalis orientalis</i>	ACD5003	5	0.56	6.71	5.58	6.55	6.62	3.07	1.99



**FIGURE 58.** Ultrametric Bayesian inference (BI) tree inferred from the cytochrome *c* oxidase I (COI) gene fragment and the results of species delineation of the genus *Nymphomyia* Tokunaga, 1932. Bayesian posterior probabilities (higher than 0.7) are given above the tree nodes. Vertical bars correspond to molecular operational taxonomic units by ASAP, mPTP, GMYC. The sequences obtained in this study are in bold.

Species of the genus *Nymphomyia* differed from each other by an average of 5.65% (ranging from 1.99 to 7.69) (Table 2). The mean intraspecific p-distance distribution of the whole data did not overlap with Interspecific distances. Previously, the closest species were considered *N. alba* and *N. levanidovae* with a genetic divergence of 2.2% (Makarchenko & Gunderina 2019, Table 2). In the present study we obtained slightly lower distances of 1.99% between *N. orientalis makcha* **subsp. nov.** and *N. orientalis orientalis* **subsp. nov.** These subspecies appear to be cryptic (see above), but ASAP, mPTP and GMYC approaches place them in different molecular operational taxonomic units (mOTUs), assuming their heterospecificity (Fig. 58).

The Bayesian phylogeny revealed two well-supported (Bayesian PP, BPP = 1) primary clades, one including *N. kaluginae*, *N. rohdendorfi*, *N. orientalis orientalis* **subsp. nov.**, *N. orientalis makcha* **subsp. nov.** the other includes

*N. kannasatoi*, *N. levanidovae* and *N. alba* (Fig. 58). *N. kaluginae* was placed (BPP = 0.83) as sister to the clade uniting *N. rohdendorfi* and both subspecies of *N. orientalis* **sp. nov.** The latter are sister taxa with high posterior probability (BPP = 1). Obtained sequences of *N. alba* from Monobe River (Japan) placed it as sister taxon to *N. alba* from Kunashir Island (Russia), and the results of species delimitation confirm their conspecificity (Fig. 58). The remaining species belong to separate mOTUs according to the ASAP, mPTP and GMYC analysis.

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