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Towards the taxonomy of *Corynoneura* Winnertz (Diptera: Chironomidae: Orthocladiinae) from the Russian Far East and Eastern Siberia

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Abstract

The genus *Corynoneura* Winnertz is partly revised on the basis of materials from the Russian Far East and Eastern Siberia. Adult male of a new species, *C. sikhotealinensis* **sp. nov.**, as well as pupa of *C. schleei* Makarchenko *et* Makarchenko and pupa with associated pharate male of *Corynoneura* sp. are described. Adult male of *C. fujiundecima* Sasa, earlier erroneously determined as *C. lacustris* Edwards, is briefly redescribed and annotated. DNA barcoding results of *C. kadalinka* Makarchenko *et* Makarchenko from Eastern Siberia (Baikal Lake basin) are also provided in comparison with closely related *C. carriana* Edwards from Sweden. As a result of the analysis, supported with sufficiently significant morphological differences between males of these two distant populations, the two species were combined into one, *C. carriana*, with two subspecies: *C. carriana carriana* Edwards (nominotypical) and *C. carriana kadalinka* Makarchenko *et* Makarchenko **stat. nov.** A morphological redescriptions of the adult male and pupa, as well as the 4th instar larva (here described for the first time) of *C. carriana kadalinka* in comparison with specimens of populations of the *C. carriana carriana* from Sweden and the Urals are presented. A key to adult males and pupae of the *Corynoneura* species from the Russian Far East and Eastern Siberia is also provided.

Key words: Diptera, Chironomidae, Orthocladiinae, *Corynoneura,* new species, key, East Siberia, Russian Far East

Introduction

The genus *Corynoneura* was established by Winnerz (1846) with the type species *Corynoneura scutellata* Winnertz, 1846. Chironomids of this genus are among the most peculiar in the subfamily Orthocladiinae. The length of adult males rarely exceeds 2 mm. Veins R_1 and R_{2+3} are short, wide and fused with the costal vein into a broad so-called clavus, ending in the middle of the wing, while R_{4+5} is weak. On the basis of such the distinct wing venation Goetghebuer (1939) separated this genus, as well as the closely related *Thienemanniella* Kieffer into an independent subfamily Corynoneurinae. Later, Brundin (1956) revising the system of the family Chironomidae paid more attention in the diagnosis of chironomids chaetotaxy of thorax, rather than the wing venation and abolished the subfamily Corynoneurinae, by inclusion of these two genera in the Orhocladiinae subfamily, where they are to this day.

In the world fauna, 98 *Corynoneura* species are known, of which 46 were recorded for the Palaearctic, 19—for the Nearctic, 25—for the Neotropical, 17—for the Oriental, 4—for the Afrotropical, and 5—for the Australasian Region (Ashe & O'Connor 2012; Fu & Sæther 2012; Fu *et al*. 2009, 2017; Wiedenbrug & Trivinho-Strixino 2011; Wiedenbrug *et al.* 2012).

By now in the Russian Far East and Eastern Siberia 21 species of *Corynoneura* were found (Makarchenko & Makarchenko 2010, 2017).

In the present paper, the genus *Corynoneura* is revised in part on the basis of materials from the Russian Far East and Eastern Siberia. As a result of our study, adult male of the a species *C. sikhotealinensis* **sp. nov.** as well as pupa of *C. schleei* Makarchenko *et* Makarchenko and pupa with associated pharate male of *Corynoneura* sp. are described. Adult male of *C. fujiundecima* Sasa, which we erroneously determined earlier as *C. lacustris* Edwards,

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is briefly redescribed and annotated. A key to adult males and pupae of the *Corynoneura* species from the Russian Far East and Eastern Siberia is given. DNA barcoding results of *C. kadalinka* Makarchenko *et* Makarchenko from Eastern Siberia (Baikal Lake basin) are also provided in comparison with closely related *C. carriana* Edwards from Sweden. DNA barcoding using a standard fragment of the mitochondrial cytochrome c subunit 1 gene (COI) has proven a successful method for species-level identification in many animal groups including chironomids in the subfamily Orthocladiinae (Hebert *et al.* 2003, Makarchenko *et al.* 2015, 2017a-b, 2018) as well as in the genus *Corynoneura* (Silva & Wiedenbrug 2014). In our case now, based on low genetic distances, common clade in Maximum Likelihood tree, as well as single operational taxonomic unit (OTU) in ABGD analysis we consider it reasonable to combine two species into one species *C. carriana* but with two subspecies, *C. carriana kadalinka* Makarchenko *et* Makarchenko and *C. carriana carriana* Edwards, because adult males of the two studied populations have sufficiently significant morphological differences. In confirmation of this, we also presented a morphological description of the adult male, pupa and larvae of *C. carriana kadalinka* in comparison with populations of the *C. carriana carriana* from Sweden and the Urals. The description of the 4th instar larva of *C. carriana kadalinka* is given for the first time.

Materials and methods

The material was preserved in 96% ethanol for DNA-analysis and in 70% ethanol for further study of morphology and slide-mounting, following the methods by Makarchenko (1985). The larva, pupa and adult male were associated using the DNA barcoding. The morphological terminology follows Sæther (1980).

Holotypes 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).

For comparative morphological characterization of *Corynoneura carriana* and *C. kadalinka* was used information on the specimens and some photos of males *C. carriana* from Sweden, which were used for DNA barcoding (Brodin *et al.* 2013, GenBank accession numbers: KC250767–KC250770), kindly provided by Dr. Yngve Brodin. Some photos of males from Finland which were determined by Dr. Lauri Paasivirta and kindly sent to us by Dr. Marko Mutanen, and also material of Dr. Andrei Krasheninnikov from the Ural Region of Russia.

DNA was extracted from specimens using the Invitrogen PureLink Genomic DNA Mini Kit (Invitrogen corp, Carlsbad, CA 2007) according with the protocol, and the resultant DNA was eluted in 70 µl. according with the protocol. Following DNA extraction, 1 µl of each DNA extract was added to a PCR mixture consisting of 5 µl Go Taq Green Master Mix (Promega corp, Madison, WI, USA), 0.5 µM of each primer, 3 µl of nuclease-free water (Ambion) and 1 µl of genomic DNA. The primers for amplification of the 658 bp fragment were LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG -3') and HCO2198 (5' - TAAACTTCAGGGTGACCAAAAAATCA - 3'), obtained from Folmer *et al.* (1994). The reactions were cycled at 95°C for 1 min, followed by 35 cycles of (95°C for 30 s, 50°C for 30 s, 72°C for 1 min), and a final extension of 72°C for 5 min. PCR products were visualized on a 1.5% TBE agarose gel GelDoc XR+ imaging systems (BioRad). Each PCR fragment was purified using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA). PCR products were cycle sequenced using BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.), and sequenced bidirectionally on an ABI 3130XL automated 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 Maximum likelihood tree (T92) using MEGA7. The reliability of the internal branches in the ML-tree was assessed by 500 bootstrap replicates. ABGD analysis (wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html, Puillandre *et al.* 2012) was used for species delamination and establish taxonomic status of *C. kadalinka*, using relative gap width (X = 1.0) and intraspecific divergence (P) values between 0.005 and 0.100 with the K2P model. These settings were successfully used for chironomids by Song *et al*. 2018. All sequences of *C. carriana kadalinka* have been deposited in GenBank (accession numbers MK482345-MK482354) and Barcode of Life Data System (BOLD: RCHIR001-19 - RCHIR010-19).

Descriptions

Corynoneura carriana Edwards, 1924

Corynoneura carriana carriana Edwards, 1924 [nominotypical, by present denotation (ICZN art. 47)]
Corynoneura carriana kadalinka Makarchenko *et* Makarchenko, **stat. nov.**

Corynoneura carriana kadalinka Makarchenko *et* Makarchenko, stat. nov.

(Figs. 1–7, 12–22)
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Corynoneura kadalinka Makarchenko *et* Makarchenko, 2010: 360, Figs. 26–27; Ashe & O'Connor 2012: 195; Fu *et al.* 2017: 158.

Material. *Eastern Siberia of Russia*: 1 adult male, Zabaikalsk Territory, Chita City neighborhood, Kadalinka River (Amur River basin), 29.VII.2009, leg. N. Saltanova; 4 adult males, 2 pupal exuviae, Baikal Lake, Zmeevaia Cover of Chivyrkuy Bay, 08.VI.2012, leg. N. Bazova; 2 adult males, 4 pupal exuviae, 4 larvae and 2 skin of larvae, the same region, Gusinoe Lake of Baikal Lake basin, 20.V.2017, leg. N. Bazova.

Adult male (n = 4). Total length 1.30–1.95 mm. Total length/wing length 1.23–1.86. Total coloration brownish, thorax dark brown.

Head. Clypeus with 7–9 setae. Antenna with 10 flagellomeres and well-developed plume (Fig. 3); length of 2–9 flagellomeres setae 140–208 µm, length of ultimate flagellomere setae in basal part 60–120 µm; ultimate flagellomere 168–248 µm long, shape and sensilla chaetica as in *Corynoneura edwardsi* group (Hirvenoja & Hirvenoja1988, Fig. 4); sensilla chaetica of ultimate flagellomere sometimes almost reaching tip of flagellomere (Figs. 4–6); length of ultimate flagellomere equal to combined length of seven previous flagellomeres; AR 0.60–0.90. Length (in µm) of palpomeres: 12–14, 16–28, 24–36, 28–40, 44–52.

Thorax. Antepronotum with 0–2 lateral setae; dorsocentrals 4–7; prealars 2; scutellars 2.

Wing. Typical of the genus. Length 0.75–1.23 mm. Costa length/wing length 0.30–0.35.

Legs. Fore trochanter with dorsal keel. Spur of fore leg tibia 26–28 µm long, spurs of mid leg tibia 10–12 µm long, spur of hind leg tibia 40 –44 µm long. Hind leg tibia with comb of 19–20 setae and with slightly curved apical seta; a/d=1.37–2.0; b/d=1.0–1.8. Lengths and proportions of leg segments as in Table 1.

TABLE 1. Lengths (in µm) and proportions of leg segments of *Corynoneura carriana kadalinka* **stat. nov.**, male (n=4)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	248–352	288–400	164–220	92–112	52–68	20–28	36–48	0.52–0.57	3.50–3.84	3.27–3.68	1.2–1.4
P_2	340–480	320–432	176–236	84–108	44–60	20–28	36–48	0.52–0.55	4.36–5.12	3.75–4.0	1.7–2.3
P_3	280–400	296–416	172–220	100–136	40–52	20–28	36–48	0.53–0.58	3.82–4.05	3.35–3.71	1.3–1.5

Hypopygium (Figs. 1–2, 7). Tergite IX with 3–4 setae on each side; laterosternite IX with 2–4 setae. Sternapodeme inverted V-shaped, with wide middle part. Phallapodeme high, narrowing towards apex, connected to sternapodeme by "lock". Gonocoxite 128–152 µm long; inferior volsella absent; superior volsella in form of naked and small rounded triangular plate; at least 10 long setae at convexity over superior volsella dorsally. Gonostylus 72–76 µm long, tapering towards tip, megaseta 4–8 µm long.

Pupa (n=3). Coloration of exuviae. Cephalothorax light-brown, abdomen lighter in middle part and darker on sides.

Cephalothorax. Frontal apotome with 2 taeniate setae 80–164 µm long. Thorax smooth, in middle part dorsally near suture with rugulose area. Antepronotum with 2 narrowly taeniate median and 1 hair-like lateral antepronotals 40–60 µm long. Precorneals 52–64 µm long, arranged in one row; Pc_1 and Pc_2 narrow taeniate, Pc_3 widely taeniate. Dc_1, Dc_3 and Dc_4 arranged in one row, Dc_2 displaced ventrally and far from other precorneals. Dc_1 hair-like, 20–40 µm long; Dc_3 widely taeniate, twisted on top (Fig. 12), 128–170 µm long; Dc_4 narrowly taeniate or hair-like, 40–60 µm long. Distance between Pc_1 and Pc_3 60–80 µm; distance between Pc_3 and Pc_4 16–28 µm. Wing sheaths apically with 3 rows of good visible pearls and sometimes 4–5 rows of poorly visible pearls in middle part.

Abdomen. Tergite I without shagreen. Tergites II with shagreen of small spinules in middle part. Tergites III–IX with more distinct shagreen of spinules than tergite II, located in central part and without shagreen in lateral part (Fig. 15). Sternite I with sparse colorless needle-like spinules, located mainly in antero-lateral corners. Colorless needle-like spinules covering entire surface of sternite II, in middle part spinules thicker and longer (Fig. 13). Sternites III–VIII with same kind of shagreen as tergites III–VIII in central part, but less intense. Sternite IX with shagreen of small spinules in central part anteriorly. Pupae of males with rows of 10–20 relatively strong hooklets situated on tergites II–VII (Fig. 15), with 11–26 hooklets on sternites III–VII and 4 hooklets on sternite VIII. Pupae of females with 7–19 hooklets on tergites III–VII and with 11–17 hooklets on tergites III–VII. Segment I with 0–2 pairs of short hair-like lateral setae. Segment II usually with 3 pairs of short hair-like lateral setae, but pupae of 2 males in one side have 3 hair-like setae, in other side 3 hair-like setae and 1 taeniate seta; pupa of 1 male with 2 pairs of hair-like setae and 2 pairs of taeniate setae. Segments III–VIII with 4 pairs of taeniate setae. Anal lobe 188–220 μm long and 102–120 μm wide, with fringe of 45–52 setae 380–500 μm long, 1 taeniate inner seta 44–176 μm long and 3 taeniate macrosetae 280–300 μm long. Anal lobe 204–220 μm long and 102–120 μm wide (index of anal lobe 1.5–1.96). Male genital sac extending 16–24 μm beyond anal lobe (Fig. 14).

Fourth instar larva (n=4). Total length 2.7–3.2 mm.

Head. Head capsule yellowish, dorsally with mesh pattern; 275–300 μm long and 150–175 μm wide (Fig. 16). S_I–S_{II} and S_{IV} of labrum short and hair-like, S_{III} stick-shaped and longer than other labral setae. Pecten epipharyngis consists of 3 scales. Premandible with one apical tooth, on which some very small teeth (poorly visible), brush with long and thin setae (Fig. 22). Antenna 574–602 μm long, longer than head in 1.9–2.1 times; with 4 segments, from which segments 2–3 brown, while the rest are yellowish. Length antennal segments (in μm): 292–320, 128–136, 144–160, 5–6. Basal segment with one ring organ in middle part or sometimes little more distally, with 2 setae in distal half and with short antennal blade in apex. Lauterborn organs absent (Figs. 17–18); AR 1.04–1.08. Mandible with 4 inner teeth, apical tooth shorter than first inner tooth; seta subdentalis absent; seta interna with some branches (Fig. 20). Mentum with 3 median teeth and 5 pairs of lateral teeth; middle tooth smaller than adjacent teeth (Fig. 19).

Anal tubules shorter than posterior parapods bearing simple hooks. Procercus in shape of small tubercle, bearing 4 apical anal setae which 368–448 μm long; 2 lateral setae short and thin. Ventral basal part of posterior parapods with plumose dark brown seta (Fig. 21), longest branches of which 68–84 μm.

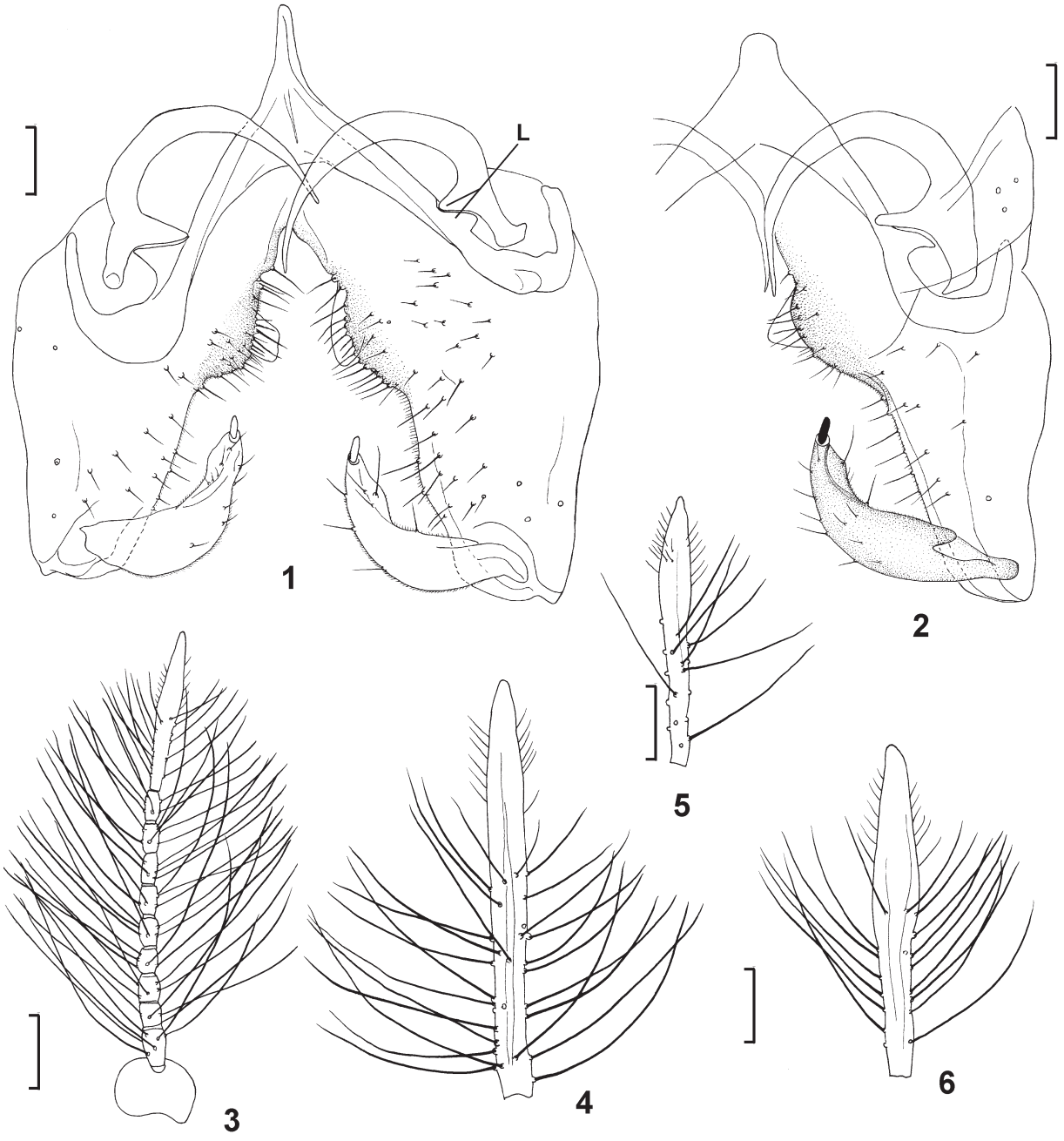
Results of DNA barcoding

Overall, we have sequenced fragments of the cytochrome oxidase I (658 bp) of 10 specimens of *C. kadalinka*. Totally, we have determined 7 synonymous substitutions, 6 of which were transitions and one transversion. The sequences were heavily AT-biased (67.9%), especially in the third position where 88.7 were A or T. The obtained DNA barcodes formed 6 haplotypes with 5 mutational steps between the most distant samples on the haplotype network. The average intraspecific pairwise distance was 0.28%, and ranged from 0% to 0.76%. Studied sequences have been compared with sequences of other *Corynoneura* species. The closest species was *C. carriana,* interspecies distances (K2P) between two species was 0.36% or 1–5 mutations. The average nucleotide divergence between *C. kadalinka* and other described species of the genus *Corynoneura* in Genbank was 14.3%, and ranged from 8.8% to 17.2%.

We used Maximum likelihood approach to present *C. kadalinka* and *C. carriana* relationships with *C. scutellata* as an outgroup. We used four *C. carriana* specimens from GenBank (accession numbers KC250767- KC25070) collected in Sweden. Samples of *C. kadalinka* formed polyphyletic on a tree with low bootstrap support. Thus, based on the ML tree 13 DNA barcodes of two morphospecies clustered into one clade despite the high geographical distance of collection localization.

The high similarity of the two species is also confirmed by ABGD analysis. We create a dataset of all described species of genus *Corynoneura* including *C. carriana* and obtained sequences. The ABGD analysis using a 1.9–2.6% maximum intraspecific divergence, yielded only 1 operational taxonomic unit (OTU) contained *C. kadalinka* and *C. carriana.*

Thus, based on low genetic distances, common clade in ML analysis, as well as one OTU in ABGD analysis we consider it reasonable to combine two species into one *C. carriana* with two subspecies—*C. carriana kadalinka* and *C. carriana* s. str. (Fig. 23).



FIGURES 1–6. Adult male of *Corynoneura carriana kadalinka.* **1–2,** hypopygium in dorsal view; **3,** antenna; **4–6,** ultimate flagellomere. L—"lock". Scale bars: Figs. 1–2—20 μm; Fig. 3—100 μm; Figs. 4–6 –50 μm.

Remarks and comments. Some years ago we described *Corynoneura kadalinka* Makarchenko *et* Makarchenko, 2010 from East Siberia (Amur River basin). This species was closely related to *C. carriana* but separable from the *later* by some features of antenna and hypopygium. This year we have got for this species additional adult, pupae and larvae material from the same region and decided to prepare redescription of this species with immature stages and with results of DNA-barcoding. Results of barcoding of *C. kadalinka* were compared with data of *C. carriana* from GenBank and found that our species have more than 99% similarities with data on *C. carriana* and with those published by Brodin *et al.* (2013). That is, we have one species but with two geographical forms which we have considered as subspecies *C. carriana carriana* Edwards and *C. carriana kadalinka* Makarchenko *et* Makarchenko. Most features of adult males and pupae of both subspecies are similar but males of *C. carriana carriana* has the reduced antennal plume, setae of which are not longer than 112 μm, the ultimate flagellomere pin-shaped (Figs. 9–10), AR 0.37–0.46, the hypopygium without a strong rounded protrusion along the inner edge in the basal part of gonocoxite (Figs. 8, 11), while males of *C. carriana kadalinka* has the antennal plume well-developed,

setae of which are 140–208 μm long, the ultimate flagellomere spindle-shaped (Figs. 3–6), AR 0.60–0.90, and the hypopygium with more strong rounded protrusion along the inner edge in the basal part of gonocoxite (Figs. 1–2) (Table 2 and see key for species). Pupae of both subspecies are closely related but differs by some features listed in the key for the species given below.

Distribution. *C. carriana kadalinka* is known only from East Siberia—Baikal Lake basin and upper part of Amur River basin.

Corynoneura carriana carriana Edwards, 1924

(Figs. 8–11)

Corynoneura carriana Edwards, 1924: 188; Hirvenoja & Hirvenoja 1988: 231; Langton & Wisser 2003: 319; Langton & Pinder 2007: 88; Ashe & O'Connor 2012: 189; Fu *et al.* 2017: 63.

New material. *Ural Region of Russia*: 4 adult males, Permskyi Territory, Suksunskyi District, Bukina Yama Lake, N 57.194306, E 57.297306, 08.VII.2009, leg. A. Krasheninnikov.

Remarks. Males collected in the Urals fully correspond to the description of Hirvenoja & Hirvenoja (1988) and other data published for *Corynoneura carriana* from Finland and Sweden, as given above, and in Table 2.

Distribution. *C. carriana carriana* apparently inhabits the territory from Scandinavia to the Urals.

TABLE 2. Comparison of adult males of *Corynoneura carriana carriana* Edwards and *C. carriana kadalinka* Makarchenko et Makarchenko

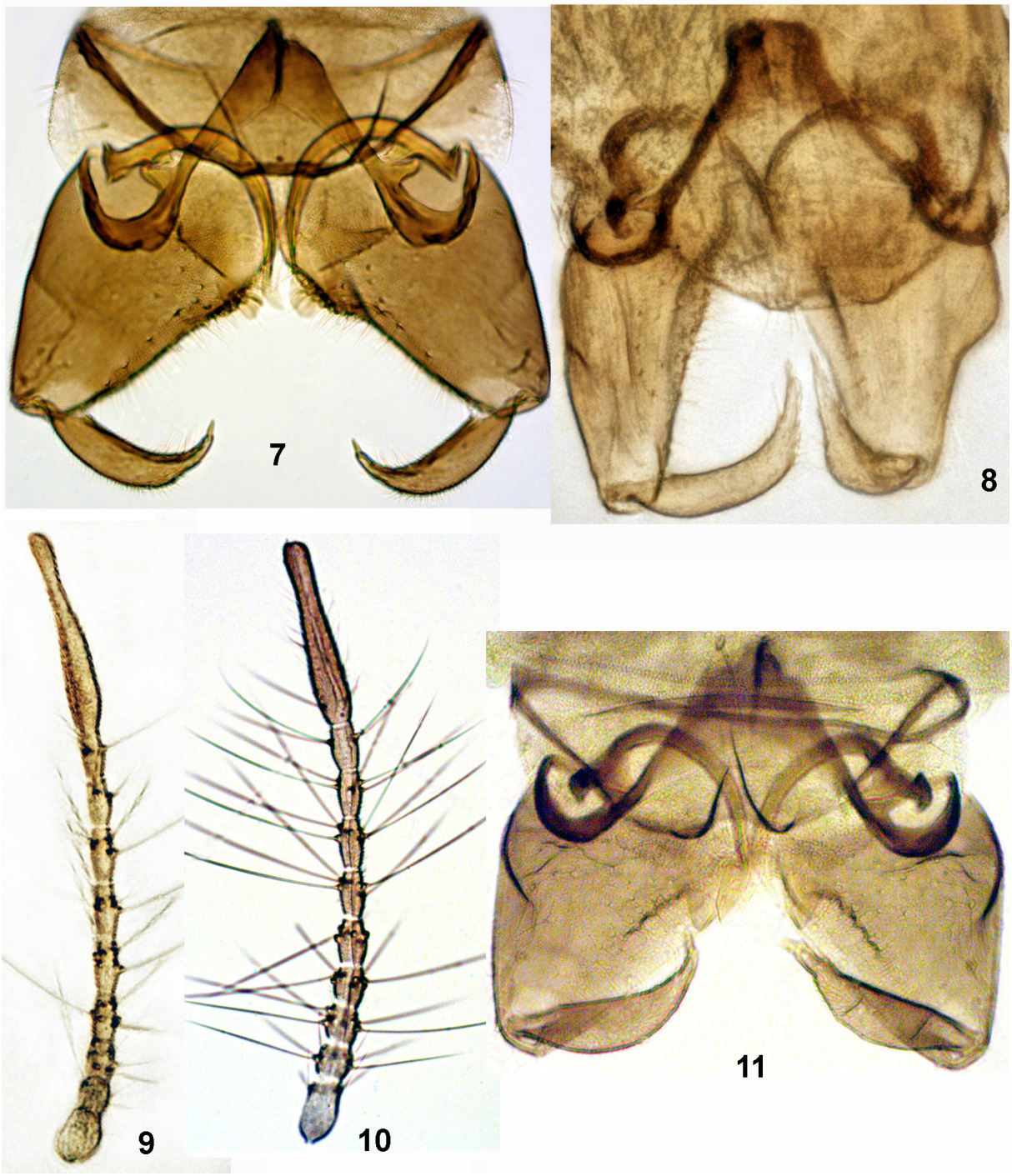
Characters	*C. carriana* s. str. from Finland and Sweden (n = 6–8). After: Hirvenoja & Hirvenoja 1988, and original data of Y. Brodin.	*C. carriana* s. str. from Ural Region of Russia (n = 4). Original data.	*C. carriana kadalinka* from Baikal Lake basin (n = 4). Original data.
Antennal plume	Reduced; length of 2–9 flagellomeres setae *ca* 100 μm	Reduced; length of 2–9 flagellomeres setae 60–112 μm	Well-developed; length of 2–9 flagellomeres setae 140–208 μm
Shape of ultimate antennal flagellomere	Pin-shaped	Pin-shaped	Spindle-shaped
AR	0.37–0.45	0.41–0.46	0.60–0.90
PR (length of palpomere 5/length of palpomere 4)	1.3–1.4	1.3–1.7	1.1–1.3
Wing length, mm	0.7–1.1	0.6–0.7	0.75–1.23
Costa length/wing length	0.5	0.5	0.30–0.35
LR_1	0.47–0.52	0.45–0.55	0.52–0.57
LR_2	0.48–0.55	0.51–0.55	0.55–0.52
LR_3	0.50–0.57	0.50–0.53	0.53–0.58

Corynoneura "carriana" Edwards *sensu* Makarchenko & Makarchenko

Corynoneura carriana Makarchenko & Makarchenko 2010: 358, Figs. 6–10.

Remarks. We agree with Dr. Lauri Paasivirta (pers. comm.), believing that the *C. "carriana"* specimens from Baikal Lake with 6 flagellomeres antenna belong to the local aberrant population of this species which is closely related to male of Finnish endemic species *C. gynocera* Tuiskunen; however, their hypopygia clearly differs, namely by size and shape of the superior volsella (Tuiskunen 1983). An additional material on *C. "carriana"* in Baikal Lake is expected to be collected and performed using the DNA-barcoding, after which it will be possible to evaluate the individuals from this population.

Distribution. Known only from Baikal Lake.



FIGURES 7–11. Adult males of *Corynoneura carriana kadalinka* from Baikal Lake basin (7) and *C. carriana* s. str. From Sweden (8–9) and Ural Region (10–11). **7–8, 11,** hypopygium in dorsal view; **9–10—**antenna.

Corynoneura sikhotealinensis **Makarchenko *et* Makarchenko, sp. nov.**
(Figs. 24–26)
urn:lsid:zoobank.org:act:21CCBA5A-45D4-4803-8308-7613D380C05E

Type material. Holotype: adult male, *Russian Far East*, Primorye Territory, Terneiskyi District, Sikhote-Alin Biosphere Nature Reserve, Verkhnee Lake, 25.VII.2004, leg. O. Zorina. Paratypes: 1 adult male, same data as holotype except, 17.VIII.2007, leg. O. Zorina; 1 adult male, same data as holotype except, Dlinnoe Lake, 25.VII.2007, leg. O. Zorina.

Adult male (n = 3). Total length 1.05–1.40 mm. Total length/wing length 1.49–1.88. Total coloration brown, tergites VI–VIII with light areas near top.

Head. Eye bare but front part pubescent. Clypeus with 7–9 setae. Antenna with 10 flagellomeres and well-developed plume; ultimate flagellomere 192–208 μm long, with short sensilla chaetica reaching to apex; AR 0.68–0.76. Length of 2–5 palpomeres (in μm): 16–18, 22–24, 32–36, 52–56.

Thorax. Antepronotum with 0–1 lateral setae; dorsocentrals 4–6; prealars 2; scutellars 2.

Wing. Typical of the genus. Length 0.72–0.94 mm.

Legs. Fore leg trochanter with dorsal keel. Spur of fore leg tibia 24 μm long, spurs of mid leg tibia 8–12 μm long, spur of hind leg tibia 32 μm long. Hind leg tibia with comb of 14–18 setae plus single strongly hooked apical seta; a/d=1.8–2.6; b/d=1.8–2.0. Lengths and proportions of leg segments as in Table 3.

Hypopygium (Figs. 24–26). Tergite IX with 1–4 setae on each side; laterosternite IX with 2–3 setae. Sternapodeme inverted V-shaped. Phallapodeme high, connected to sternapodeme by "lock". Gonocoxite 80–88μm long; inferior volsella absent; superior volsella triangular or rounded triangular, without setae. Gonostylus 20–32 μm long, basally with inner lobe, megaseta 4–8 μm long.

Diagnosis. The adult male of *C. sikhotealinensis* is most closely related to *C. scutellata* and *C. arctica*, but can separated from the first species by the lack of inferior volsella and the presence of the rounded-triangular superior volsella, and from the second species by the S-shaped apical seta of hind leg tibia and the shape of superior volsella (*C. arctica* with the straight or slightly curved apical seta of hind leg tibia and with the angular superior volsella).

TABLE 3. Lengths (in μm) and proportions of leg segments of *Corynoneura sikhotealinensis* **sp. nov.**, male (n=3)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	248–280	308–324	176–196	92–100	52–60	20	36–38	0.57–0.60	3.54–3.72	3.08–3.23	1.7–2.0
P_2	308–352	320–432	188–204	80–96	44–52	20	36–40	0.58–0.61	4.50–4.60	3.37–3.59	2.7
P_3	272–308	308–332	184–196	100–104	40–44	20	36–38	0.58–0.60	4.0–4.08	3.06–3.33	2.7–2.8

Corynoneura fujiundecima Sasa

(Figs. 27–28)

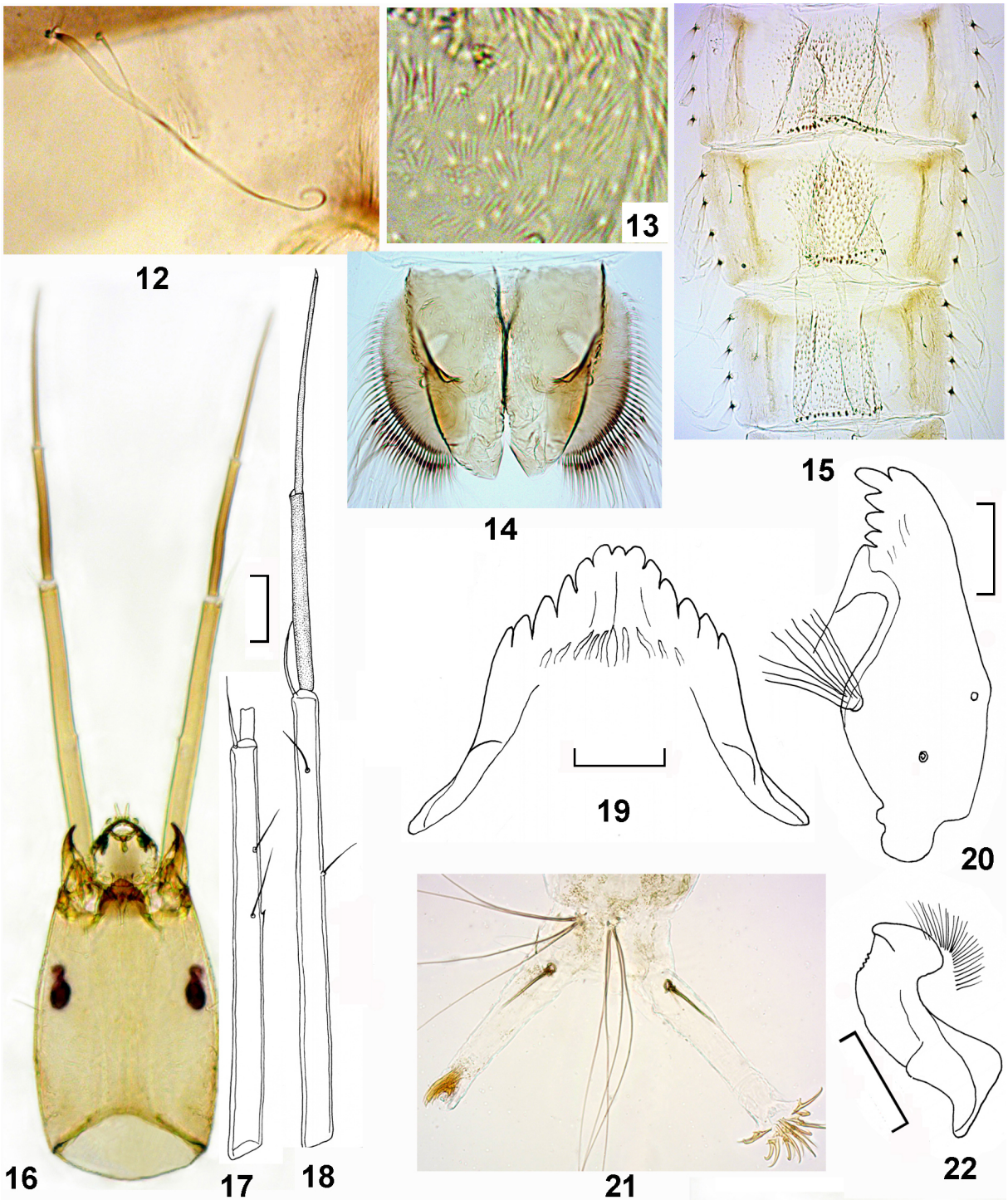
Corynoneura fujiundecima Sasa, 1985: 130; Yamamoto 2004: 17; Fu *et al.* 2009: 10, 2017: 132; Ashe & O'Connor 2012: 193.
Corynoneura lacustris Edwards 1924; Makarchenko & Makarchenko 2006: 154 (misidentification).

Material. *Russian Far East, Primorye Territory*: 5 adult males, *Putyatin Island (*Strelok Bay, near 50 km East of Vladivostok and 35 km West of Nakhodka), Gusinoe Lake, 9–11.VII.1999, leg. E. Makarchenko; 4 adult males, Khasanskyi District, Lotos Lake near Khasan Village, 3.VI.2007, leg. E. Makarchenko; 3 adult males, Terneiskyi District, Sikhote-Alin Biosphere Nature Reserve, Golubichnoe Lake 31.VIII.2002, leg. O. Zorina; 1 adult male, same data except 9.VII.2006, leg. O. Zorina.

The total length (1.15–1.65 mm) and wing length (0.82–0.88 mm) values for *C. fujiundecima* from the Russian Far East are lower than that of specimens from Japan (total length 1.86–2.13 mm, wing length 1.14–1.24) (Sasa 1985).

Remarks and comments. The adult male of *C. fujiundecima* is very closely related to *C. lacustris* Edwards, and we mistakenly identified this species from the Russian Far East as *C. lacustris* (Makarchenko & Makarchenko 2006: 154). The main differences between these species are as follows: *C. fujiundecima*: apical seta of hind leg tibia slightly curved (Fig. 27), AR 0.47–0.66, sternapodeme width-length ratio 1.2–2, except for one male from Lotos Lake, whose height of sternapodeme is almost equal to its length, gonostylus short and broadened in middle part (Makarchenko & Makarchenko 2006, Fig. 8); *C. lacustris*: apical seta of hind leg tibia S-shaped, AR 0.32–0.42, sternapodeme height shorter than the length, gonostylus longer and slender, slightly widening in middle (Schlee 1968).

Distribution. East Palaearctic species. Known from the Primorye Territory of the Russian Far East and Japan.



FIGURES 12–22. Pupa (12–15) and larva of fourth instar (16–22) of *Corynoneura carriana kadalinka.* **12,** Dc_3 and Dc_4 setae; **13,** colorless needle-like spinules of sternite II; **14,** anal segment of male; 15, tergites III–V; 16, head; 17, basal segment of antenna; 18, antenna; 19, mentum; 20, mandible; **21,** posterior parapods and procercus; **22,** premandible. Scale bars 20 μm.

***Corynoneura* sp.**
(Fig. 29–32)

Material. *Russian Far East*: 1 mature pupa (male), Khabarovsk Territory, Kiva River, between villages Elban and Volochaevka (tributary of Nuskhi River, Amur River basin), 28.VIII.2010, leg. N. Yavorskaya.

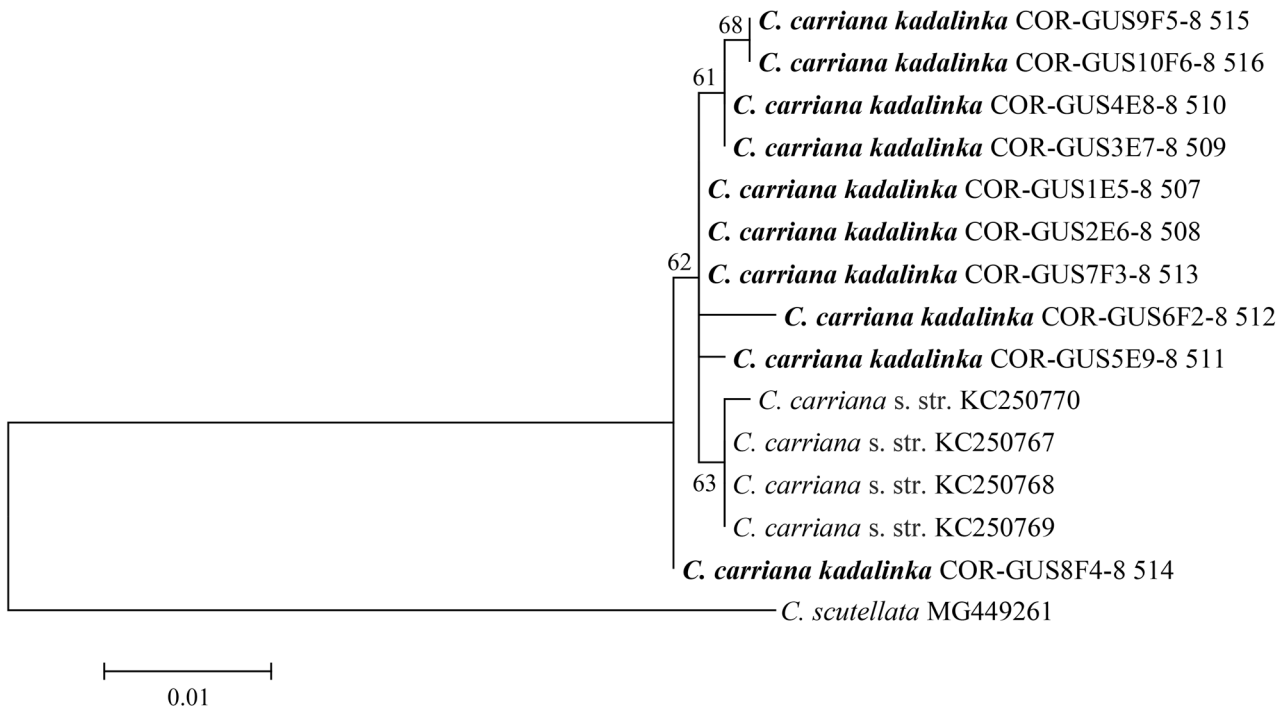


FIGURE 23. Maximum likelihood (ML) tree (-Ln likelihood = 1170.95) of the *C. carriana kadalinka, C. carriana* s. str. and *C. scutellata* (outgroup) inferred from the cytochrome c oxidase I (COI) nucleotide sequence data (658 bp). Numbers are bootstrap support of 500 replicates. Specimens obtained in this study are in bold.

Adult male (extracted from pupal exuviae, n = 1). Clypeus with 7–9 setae. Antenna with 11 flagellomeres and well-developed plume; AR 0.73–0.77. Length of palpomeres 2–5 (in μm): 24, 30, 32, 48. Dorsocentrals 3; prealars 2; scutellars 2. Legs. Fore leg trochanter with dorsal keel. Hind leg tibia with comb of 10–11 setae and with long and straight apical seta.

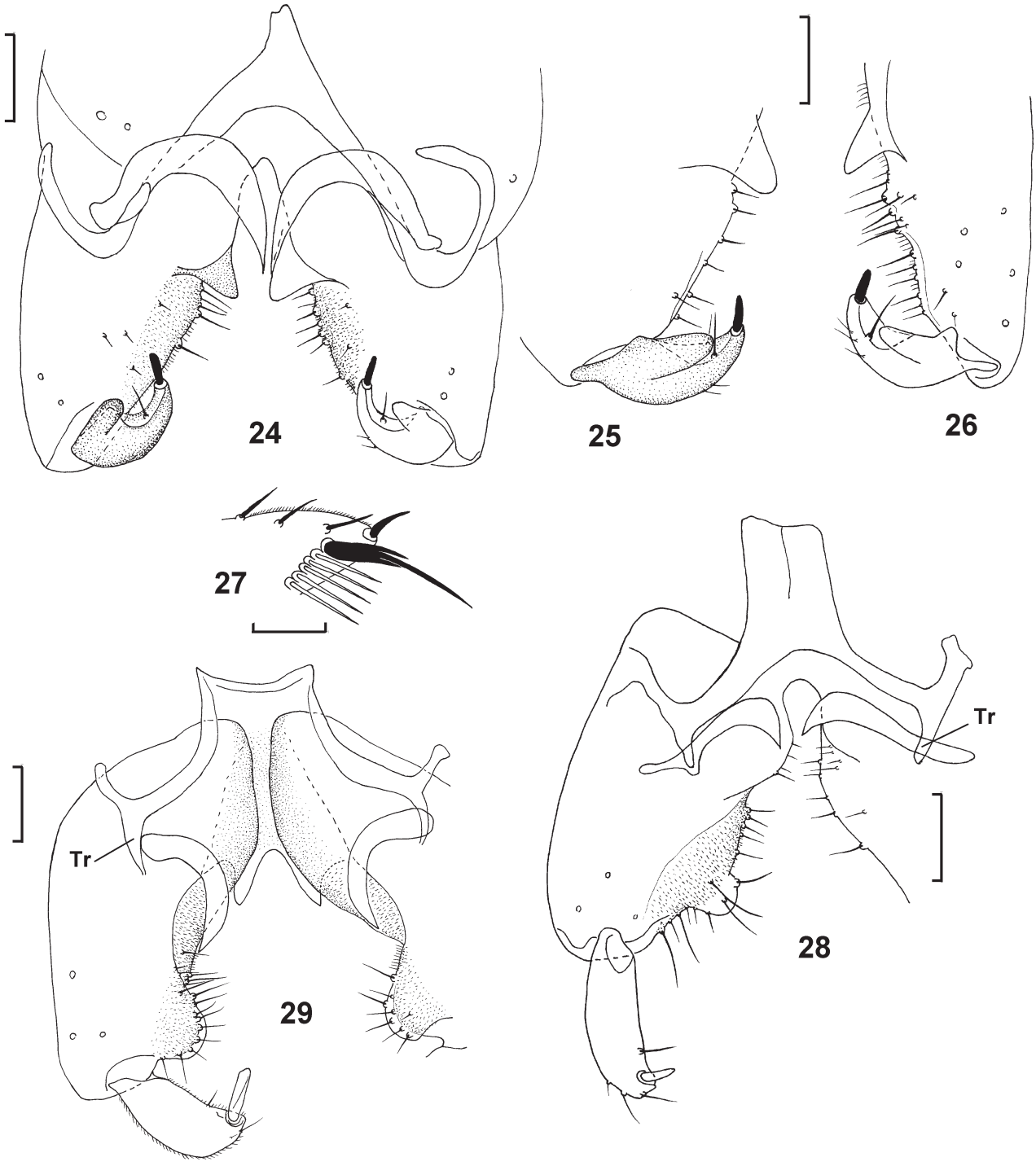
Hypopygium (Fig. 29). Tergite IX with 3–4 setae on each side; laterosternite IX with 2–3 setae. Sternapodeme wide, 32 μm long. Phallapodeme low, sickle-shaped, located on trunnion. Gonocoxite 104 μm long; inferior volsella with hyaline apex, located near the top of gonocoxite; superior volsella bare and triangular. Gonostylus 32 μm long and 16 μm wide; megaseta 12 μm long.

Pupa (n = 1). Exuviae light-brown.

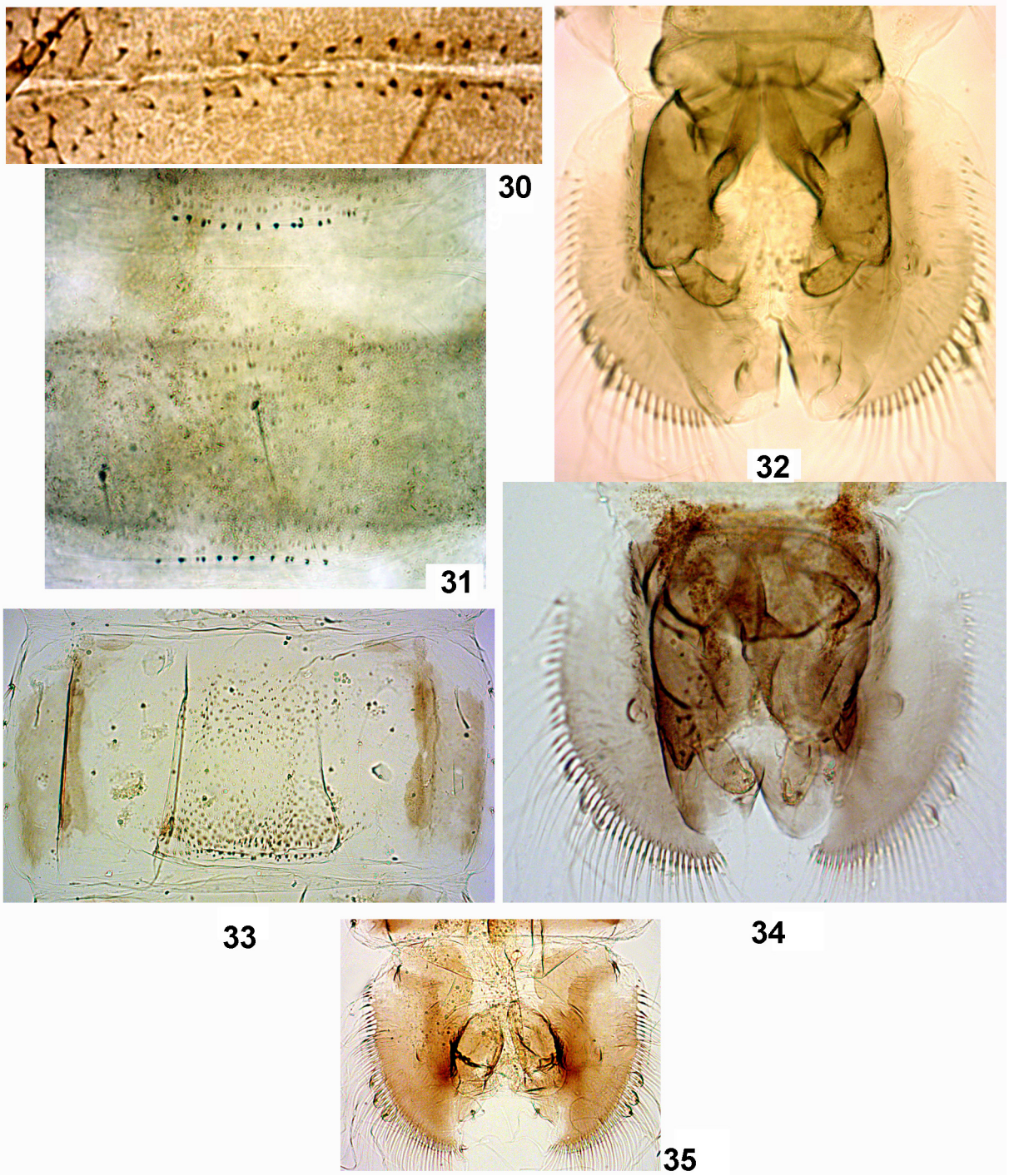
Cephalothorax. Frontal apotome with 2 simple setae. Thorax with small teeth in 1–5 rows in front part dorsally, near suture (Fig. 30). Antepronotum with 2 simple median and without lateral antepronotals. Precorneals simple, uniserial. Three dorsocentrals simple and short. Distance between Pc_1 and Pc_2 46–48 μm; distance between Pc_2 and Pc_3 16–68 μm. Wing sheaths with 4–5 short rows of pearls.

Abdomen. Tergite I without shagreen. Tergites II–VIII with shagreen of small spinules. Sternite I naked. Sternite II with very tender shagreen in central part and without colorless needle-like spinules. Sternites III–VIII with shagreen of small spinules. Tergites II–VI with posterior rows of relatively strong hooklets, number of which is 4, 11, 12, 6 respectively (Fig. 31). Sternites III–VII also with posterior rows of relatively strong hooklets, number of which is 6, 10, 15, 13, 6 respectively. Segment I with 1 pair of short hair-like lateral setae. Segment II with 2–3 pairs of short hair-like lateral setae. Segments III–VIII with 4 pairs of taeniate setae. Anal lobe 160 μm long and 100 μm wide (index of anal lobe 1.6), with fringe of 32–36 setae, 1 taeniate inner seta and 3 taeniate macrosetae. Male genital sac reaching posterior margin of anal lobe (Fig. 32).

Remarks. *Corynoneura* sp. is closely related to *C. lacustris* but differs by some features of adult male and pupa listed in the key for this species given below.



FIGURES 24–29. Adult males of *Corynoneura sikhotealinensis* **sp. nov.** (24–26), *C. fujiundecima* Sasa (27–28) and *Corynoneura* sp. (29). **24, 28–29,** hypopygium in dorsal view; **25–26,** gonocoxite and gonostylus; **27,** apex of hind leg tibia. Tr—"trunnion". Scale bars 20 μm.



FIGURES 30–35. Pupae of *Corynoneura* sp. (30–32) and *C. schleei* Makarchenko *et* Makarchenko (33–35). **30,** teeth in front part of thorax, dorsal view; **31,** tergites III–IV; **32, 34,** anal segment of male; **33,** tergite III; **35,** anal segment of female.

Corynoneura schleei Makarchenko *et* Makarchenko

(Figs. 33–35)

Corynoneura schleei Makarchenko *et* Makarchenko, 2010: 363; Ashe & O'Connor 2012: 198; Fu *et al.* 2017: 221.

New material. *Russian Far East, Khabarovsk Territory*: 2 mature pupae of male and 1 pupa of female, Khabarovsk district, Bolshekhekhtsirsky Nature Reserve, Ussuri River basin, Golovina Stream, N 48°11'131", E 134°41'039", 6.V. 2016, leg. N. Yavorskaya.

Remarks. This species was described based on adult male from the Khabarovsk Territory (Makarchenko & Makarchenko 2010). Herein, we present a first description of the pupa.

Description.

Pupa (n = 2). Coloration of exuviae. Cephalothorax light-brown, abdomen lighter in middle part and darker on sides.

Cephalothorax. Frontal apotome with 2 hair-like short setae. Thorax with small teeth in front part dorsally, near suture. Antepronotum with 2 median and 1 lateral hair-like antepronotals. Three precorneals hair-like and short. Three dorsocentrals hair-like and short. Wing sheaths with some short rows of pearls.

Abdomen. Tergite I with tender shagreen near posterior margin. Tergites II–VIII with shagreen of small spinules in central part. Tergites III–V with rows of relatively strong hooklets near posterior margin (Fig. 33). Sternites I–II without colorless needle-like spinules and shagreen of spinules. Sternite 3 with shagreen of small sparse spinules. Sternites IV–VIII with same kind of shagreen as tergites II–VIII in central part. Sternites III–VII with rows of relatively strong hooklets near posterior margin. Segment II with 2 pairs of short hair-like lateral setae. Segments III–VIII with 4 pairs of taeniate setae. Anal lobe 196–212 μm long and 106–126 μm wide (index of anal lobe 1.68–1.85), with fringe of 31–45 seate, 1 taeniate inner seta and 3 taeniate macrosetae (Figs 34–35). Male genital sac not extending beyond anal lobe (Fig. 34).

Remarks. Pupa is closely related to *Corynoneura* sp. but differs by some features listed in the key for the species given below.

Distribution. Known from Amur River basin.

Key to species of *Corynoneura* Winnertz from the Russian Far East and East Siberia

Adult males

1. Inferior volsella present . . . 2
- Inferior volsella absent . . . 16
2. Phallapodeme “low”, sternapodeme inverted U-shaped . . . 3
- Phallapodeme “high”, sternapodeme inverted V-shaped or U-shaped . . . 13
3. Inferior volsella relatively narrow, expands slightly to apex, located almost in the middle of gonocoxite or slightly distal, subapically bare and weakly chitinized. Superior volsella rounded triangular. Antenna with 10–12 flagellomeres. . . . *C. aurora* Makarchenko *et* Makarchenko (Makarchenko & Makarchenko 2010, Figs. 1–5)
- Inferior volsella usually wide and located in distal half of gonocoxite, often not far from the apex of the gonocoxite. Superior volsella triangular, other shape or absent. Antenna with 7–12 flagellomeres . . . 4
4. Antenna with 12 flagellomeres. . . . 5
- Antenna with 7–11 flagellomeres. . . . 8
5. Sternapodeme height greater than its length, very rarely equal . . . *C. fujiundecima* Sasa (Fig. 28)
- Sternapodeme height less than its length . . . 6
6. Superior volsella in form of pointed peg; inferior volsella subapical weakly chitinized; inner basal angle of gonocoxite with long setae arranged fan-like . . . *C. fittkaui* Schlee (Makarchenko & Makarchenko 2010, Figs. 20–23, 25)
- Superior volsella in other form; inferior volsella subapically strongly chitinized; inner basal angle of gonocoxite without long setae, only sometimes with short setae. . . . 7
7. Superior volsella triangular, above it in basal part of gonocoxite are located moderately long setae. Phallapodemes wide and slightly curved . . . *C. schleei* Makarchenko *et* Makarchenko (Makarchenko & Makarchenko 2010, Figs. 37–38, 40–42)
- Superior volsella round triangular, setae above it in basal part of gonocoxite absent. Phallapodemes narrow, distally strongly curved and directed posteriorly . . . *C. sundukovi* Makarchenko *et* Makarchenko (Makarchenko & Makarchenko 2010, Figs. 43, 45–48)
8. Ultimate flagellomere of antenna apically with setae assembled into socket . . . 9
- Ultimate flagellomere without socket of setae . . . 10
9. Antenna with 7–9 flagellomeres. Phallapodemes length 24–26 μm. Superior volsella wide and rounded; inferior volsella low and rounded . . . *C. prima* Makarchenko *et* Makarchenko (Makarchenko & Makarchenko 2006, Figs. 10–12)
- Antenna with 9–10 flagellomeres. Phallapodemes length 32–36 μm. Superior volsella in shape of narrow collar; inferior volsella convex and well marked. . . . *C. collaris* Makarchenko *et* Makarchenko (Makarchenko & Makarchenko 2010, Figs. 11–14)
10. “Trunnion” absent, phallapodemes situated above bend of lateral part of sternapodeme . . . *C. kibunelata* Sasa (Makarchenko & Makarchenko 2010, Figs. 28–30, 32)
- Phallapodemes situated on “trunnion” of sternapodeme . . . 11
11. Inferior volsella long and narrow; phallapodemes large and sickle-shaped; antenna with 8–10 flagellomeres . . . *C. tertia* Makarchenko *et* Makarchenko (Makarchenko & Makarchenko 2010, Figs. 54–55)
- Inferior volsella never long and narrow, phallapodemes small; antenna with 11 flagellomeres . . . 12
12. Inferior volsella apically weakly chitinized; superior volsella in form of small bare plates . . . *Corynoneura* sp. (Fig. 29)

- Inferior volsella well chitinized; superior volsella large angular, covered with microtrichia . . . . . *C. tenuistyla* Tokunaga (Makarchenko & Makarchenko 2006, Fig. 25)
13. Sternapodeme inverted U-shaped. 14
- Sternapodeme inverted V-shaped. 15
14. Overall length of phallapodemes of equal width, with rounded apex. "Lock" in connection of phallapodeme with sternapodeme absent. Antenna with 8–9 flagellomeres; ultimate flagellomere apically with numerous long setae assembled into socket . . . . . *C. kedrovaya* Makarchenko *et* Makarchenko (Makarchenko & Makarchenko 2006, Figs. 5–6)
- Phallapodemes gradually tapering to pointed apex. Connection of phallapodeme with sternapodeme by the "lock". Antenna with 12 flagellomeres; ultimate flagellomere apically and subapical with setae which not assembled into socket . . . . . *C. doriceni* Makarchenko *et* Makarchenko (Makarchenko & Makarchenko 2006, Figs. 2–3)
15. Antenna with 9–10 flagellomeres; ultimate flagellomere apically rounded with group of sensilla chaetica on apex; males with 10 flagellomeres have AR 0.42–0.59 and length of ultimate flagellomere is equal to combined length of 4–5 previous flagellomeres; males with 9 flagellomeres, AR 0.40–0.44, length of ultimate flagellomere equal to combined length of three previous flagellomeres. Inferior volsella well-visible, often in the form of small rectangular projection; gonostylus simple . . . . . *C. lobata* Edwards (Makarchenko & Makarchenko 2006, Fig. 4)
- Antenna with 10 flagellomeres; ultimate flagellomere narrowing to tip, with numerous sensilla shaetica in apex and laterally; length of ultimate flagellomere equal to combined length of six previous flagellomeres; AR 0.63–0.65. Inferior volsella small and hyaline, poorly visible. Gonostylus basally with inner lobe . . . . . *C. scutellata* Winnertz (Makarchenko & Makarchenko 2006, Fig. 16)
16. Antenna with reduced setae of plume; ultimate flagellomere skittle-shaped, without sensilla chaetica and any setae. 17
- Antenna with well-developed setae of plume; ultimate flagellomere of other shape, apically with sensilla chaetica or setae . 18
17. Antenna with 6 flagellomeres. . . . . *C. "carriana"* Edwards sensu Makarchenko & Makarchenko 2010, Figs. 6–10.
- Antenna with 10 flagellomeres. . . . . *C. carriana carriana* Edwards (Figs. 8–11)
18. Ultimate flagellomere with 11–15 long, thin and pale setae assembled into socket. Antenna with 8–9 flagellomeres . . . . . *C. secunda* Makarchenko *et* Makarchenko (Makarchenko & Makarchenko 2006, Figs. 13–15)
- Ultimate flagellomere without setae assembled into socket. Antenna with 10 flagellomeres 19
19. Connection of phallapodeme with sternapodeme by the "lock" 20
- Connection of phallapodeme with sternapodeme without "lock" . . . . . *C. gratias* Schlee (Schlee 1968, Figs. 54–59)
20. Gonostylus with inner lobe basally 21
- Gonostylus simple 22
21. Superior volsella angular. Apical seta of hind leg tibia straight or slightly curved . . . . . *C. arctica* Kieffer (Hirvenoja & Hirvenoja 1988, Fig. 2)
- Superior volsella triangular or rounded triangular. Apical seta of hind leg tibia S-shaped . . . . . *C. sikhotealinensis* **sp. nov.** (Figs 24–26)
22. Superior volsella in the form of rounded triangular plates. . . *C. carriana kadalinka* Makarchenko *et* Makarchenko (Figs. 1–7)
- Superior volsella finger-shaped . . . . . *C. edwardsi* Brundin (Schlee 1968, Figs. 66–69; Hirvenoja & Hirvenoja 1988, Fig. 2)

Pupae

1. Tergites III–VI with large spines in posterior half 2
- Tergites III–VI with small, almost one-dimensional spinules 5
2. Sternite II usually without shagreen, sometimes with sparse small spinules. 3
- Sternite II with shagreen of colorless needle-like spinules 4
3. Tergite II with 18–26 middle and small spines, not collected near posterior margin in row. Tergites III–V with spines of diverse size, collected near posterior margin in 2 rows. Segment II with 4 pairs of lateral setae of which one pair is taeniate. Fringe occupy 75% of the anal lobe margin . . . . . *C. prima*
- Tergite II with 12 large, middle and small spines, collected near posterior margin in 1 row. Tergites III–V with spines of diverse size, collected near posterior margin in 1 row. Segment II with 3 pairs of taeniate lateral setae. Fringe occupy 60% of the anal lobe margin. . . . . *C. secunda*
4. Shagreen of tergites III–VI fine and not occupying entire surface of tergites. Sternite VIII with shagreen of spinules of diverse size, near posterior margin large spines collected in row. . . . . *C. collaris*
- Shagreen of tergites III–VI more distinct and occupyng almost entire surface of tergites. Sternite VIII with shagreen of one-dimensional spinules evenly covering the surface of sternite . . . . . *C. tertia*
5. Sternite II without shagreen or with few very small spinules 6
- Sternite II with colorless needle-like spinules 11
6. Tergites near of posterior margin without hooklets 7
- Tergites and sternites near of posterior margin with hooklets 8
7. Anal lobe 160 µm long, with 32–40 setae of fringe ca 160 µm long long in posterior part and with 7 setae of fringe 8–16 µm long in anterior part . . . . . *C. fittkaui*
- Anal lobe 130 µm long, with 29–31 setae of fringe 240–260 µm long . . . . . *C. doriceni*
8. Frontal and dorsocentral setae of cephalothorax hair-like and short. Segment II with 3 pairs of hair-like lateral setae 9
- Frontal and Dc_3 taeniate. Segment II with hair-like or taeniate setae. 10
9. Male genital sac reaching posterior margin of anal lobe . . . . . *Corynoneura* sp.
- Male genital sac never reaching posterior margin of anal lobe . . . . . *C. schleei*
10. Segment II with 2 pairs of taeniate and 2 pairs of hair-like lateral setae. Frontal setae 80 µm long; Dc_3 narrow-taeniate, 60 µm long. . . . . *C. lobata*

- All lateral setae of segment II hair-like. Frontal setae 150 µm long; Dc_3 taeniate, 135 µm long . . . . . *C. gratias*
11. Sternite II with colorless long hair-like setae in middle part and with colorless long spinules in lateral parts. Anal lobe with 20–24 setae in fringe occupying half of side margin . . . . . *C. kibunelata*
- Sternite II completely covered with colorless spinules, whose length is more than 10 µm. Setae of fringe occupying entire lateral margin of anal lobe 12
12. Hooklets near posterior margin present only on sternites. Frontal and dorsocentral setae short and hair-like . . . . . *C. lacustris*
- Hooklets near posterior margin present on tergites and sternites. Dc_3 seta long and taeniate 13
13. Sternite I naked or with single small spinules in anterolateral corners 14
- Sternite I covered with long colorless spinules 16
14. Male genital sac reaching posterior margin of anal lobe or slightly extending beyond anal lobe. Anal lobe fringe with 32–55 setae 15
- Male genital sac terminate between bases of apical setae and margin of anal lobe. Anal lobe fringe with 31–40 setae. . . . . *C. scutellata*
15. Tergite I with few spinules or very fine shagreen. Segment II with 4 pairs of long taeniate setae. Male genital sac reaching posterior margin of anal lobe (after Hirvenoja & Hirvenoja 1988, Langton & Visser 2003) . . . . . *C. carriana carriana*
- Tergite I naked. Segment II with 3 pairs of short hair-like lateral setae. Male genital sac slightly extending beyond anal lobe . . . . . *C. carriana kadalinka*
16. Entire surface of sternite I covered with long colorless spinules. First row of pearls on wing sheaths long. . . . . *C. edwardsi*
- Spinules small in middle of sternite and large and long in antero-lateral corners. First row of pearls on wing sheaths short . . . . . *C. arctica*

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