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Morphological redescription and DNA barcoding of *Thalassosmittia marina* (Sounders, 1928) (Diptera: Chironomidae: Orthocladiinae) from Kamchatka coast of the Russian Far East

Морфологическое переописание и ДНК-анализ *Thalassosmittia marina* (Sounders, 1928) (Diptera: Chironomidae: Orthocladiinae) с побережья Камчатки российского Дальнего Востока

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***Key words*:** Diptera, Chironomidae, *Thalassosmittia marina*, redescription, DNA barcoding, Kamchatka, Russian Far East.

Ключевые слова: Diptera, Chironomidae, *Thalassosmittia marina,* переописание, ДНК-анализ, Камчатка, российский Дальний Восток.

Abstract. Illustrated redescription of adult male as well as the results of DNA barcoding of *Thalassosmittia marina* (Sounders, 1928) in comparison with the British Columbia population and known species of the genus *Thalassosmittia* Strenzke et Remmert, 1957 from the coast of Kamchatka in the Russian Far East are provided. DNA barcodes obtained for *Orthocladius* sp. (locus COI-5P), belonging to BIN BOLD:ACP8936, as well as the sequence of *Thalassosmittia* sp. 1 (GenBank number HQ441011, BOLD ID number GBDP42937-19, locus COI-3P) are conspecific with *Thalassosmittia marina*.

Резюме. Приведены иллюстрированное переописание имаго самца и результаты ДНК-баркодинга *Thalassosmittia marina* (Sounders, 1928) в сравнении с популяцией из Британской Колумбии и известными видами рода *Thalassosmittia* Strenzke et Remmert, 1957 с побережья Камчатки на Дальнем Востоке России. ДНК-баркоды, полученные для *Orthocladius* sp. (локус COI-5P), относящиеся к BIN BOLD:ACP8936, а также сиквенс *Thalassosmittia* sp. 1 (GenBank номер HQ441011, BOLD номер ID GBDP42937-19, локус COI-3P) являются конспецифичными с *Th. marina.*

Introduction

The genus *Thalassosmittia* Strenzke et Remmert, 1957 was established for chironomid species of the subfamily Orthocladiinae, which inhabit coastal seas and moist soils and were previously included in the genus *Camptocladius* van der Wulp [Strenzke, Remmert, 1957]. The genus *Thalassosmittia* currently includes 12 species [Ashe, O'Connor, 2012; Andersen, Pinho, 2014; Moubayed-Breil1, Dominici, 2019; Li, Tang, 2024], two of which are non-marine and are found in Tibet at an altitude of 2500 m above sea level [Wang, Sæther, 1993] and in the Amazon Rainforest [Andersen, Pinho, 2014]. Finding of representatives of the genus *Thalassosmittia* on the territory of Russia is not indicated but noted that their presence is possible [Pankratova, 1970]. And it turned out to be so. In the summer of 2025, A.A. Semenchenko and A.B. Krasheninnikov during field work on the sea shore of Kamchatka collected adult males of *Th. marina* (Sounders), which was previously known and described only from the type locality in North America (British Columbia) [Saunders, 1928; Sublette, 1967]. All subsequent studies, including those using identification keys, relied only on these descriptions [Morley, Ring, 1972; Cranston et al., 1989; Wang, Sæther 1993]. Having analyzed the original description, we came to the conclusion that it is not informative enough and it is necessary to redescribe *Th. marina* at a modern level, as well as to create a DNA barcoding of this species too.

Below we redescribe adult male of *Th. marina* from the coast of Kamchatka compared to the British Columbia population as well as give the results of DNA barcoding

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of this species in comparison with other species of *Thalassosmittia* and subfamily Orthocladiinae available in BOLD and GenBank.

Materials and methods

The material was preserved in 96 % ethanol for DNA analysis, in 70 % ethanol for further study of morphology. The adult male was slide-mounted in polyvinyl lactophenol. The morphological terminology and abbreviations used below generally follow Sæther [1980].

The photographs were taken using an Axio Lab.A1 (Carl Zeiss) microscope with an AxioCam ERc5s digital camera and an Olympus SZX16 stereomicroscope with an Olympus DP74 digital camera, and then stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software.

Total genomic DNA was extracted from thorax with the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) followed the manufacturer's instructions. A two DNA fragments of the mitochondrial cytochrome oxidase subunit I (COI) gene – COI5P and COI3P was amplified using the polymerase chain reaction (PCR) with the two pair of primers LCO1490 and HCO2198 [Folmer et al., 1994] and s2183 and a3014 [Simon et al., 1994] respectively. Amplification was performed in 10 µl reactions containing 6.0 µl ddH2O, 0.5 µl of each 10 µM primer, 2 µl of 5X ScreenMix-HS DNA polymerase (Evrogen, Moscow, Russia), and 1 µl of DNA template. The PCR product was purified using Cleanup St PCR (Evrogen, Moscow, Russia) and sequenced for both directions. Sequencing reaction was performed using BigDye® Terminator v3.1 Cycle Sequencing Kits and run on an ABI 3500 Genetic Analyzer Sequencer (Applied Biosystems, Foster City, CA, USA). Forward and reverse sequences are manually assembled and edited using Finch TV and MEGA 7 [Kumar et al., 2016]. Based on the Kimura-2-Parameter (K2P) model intraspecific genetic distances are calculated inter- and using MEGA7 [Kumar et al., 2016]. The obtained sequences have been deposited in BOLD systems under process IDs RUCH001-25 and RUCH002-25.

Material from this study is deposited in the Bioresource Collection (reg. number 2797657) of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (FSCEATB FEB RAS).

The present work is registered in ZooBank (www.zoobank.org) under urn:lsid:zoobank.org:pub:BF856DAC-ECA2-4B29-B936-56DDA6B9E45C

Taxonomy

Thalassosmittia marina (Sounders, 1928)

Figs 1–14.

Camtocladius marinus Saunders, 1928: 526;

Thalassosmittia marina (Sounders, 1928): Strenzke, Remmert, 1957: 270; Ashe, O'Connor, 2012: 615;

Spaniotoma (*Smittia*) *marina* (Sounders, 1928): Johannsen, 1937: 59, 62;

Saunderia marina (Sounders, 1928): Sublette, 1967: 321; Morley, Ring, 1972: 1097;

Orthocladius sp., adult, available from: https://portal.boldsystems.org/record/CNGAA393-15, https://www.ncbi.nlm.nih.gov/nuccore/MF730388;

Orthocladius sp., adult female, available from: https://portal.boldsystems.org/record/SMTPJ272-14, https://www.ncbi.nlm.nih.gov/nuccore/KR769049;

Orthocladius sp., adult female, available from: https://portal.boldsystems.org/record/SMTPJ125-14, https://www.ncbi.nlm.nih.gov/nuccore/KR778517;

Thalassosmittia sp. 1, adult, available from: https://portal.boldsystems.org/record/GBDP42937-19, https://www.ncbi.nlm.nih.gov/nuccore/HQ441011.

Material. **Russian Far East, *Kamchatskii Krai:*** 8 adult males, Elizovskii District, Zavoiko Bay, coastal rocks, 16.VI.2025, 52.94104 N, 158.68187 E, leg. A. Krasheninnikov.

Description. *Adult male* (n=4). Total length 2.3–2.6 mm. Total length/wing length 0.99–1.07.

Coloration. Dark brown. Head, pedicel of antenna, thorax and abdomen dark brown; legs and wings grey, antennal flagellomeres greyish brown.

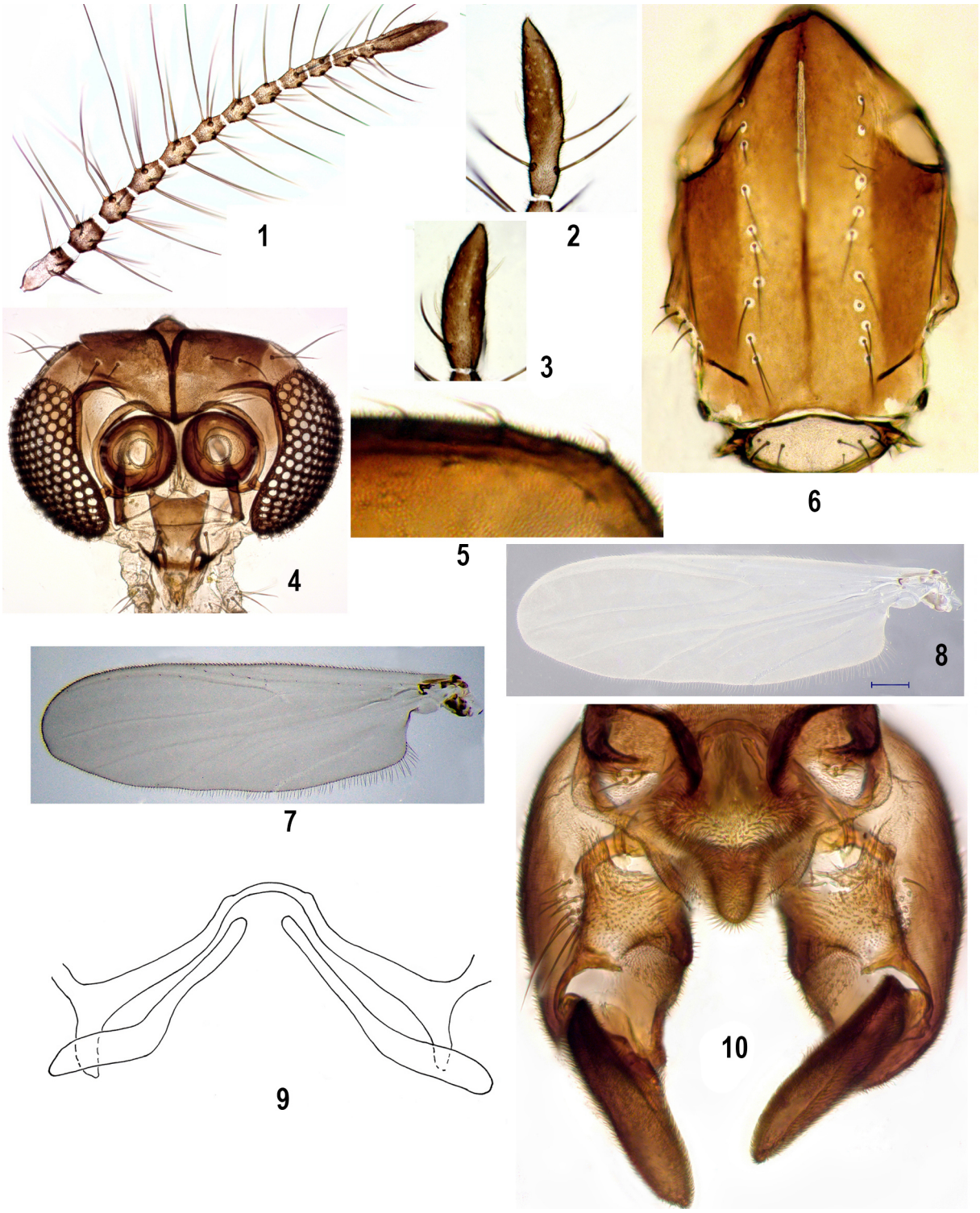
Head (Fig. 4). Eyes hairy, reniform. Temporal setae including 2 frontals and 2–3 verticals. Clypeus with 2 setae. Antenna with 12–13 flagellomeres and reduced plume of setae, maximum length of which 148 µm. It should be noted that antenna with 12 flagellomeres is due to the fusion of 12 and 13 flagellomeres. Length of flagellomeres (µm) and antennal index for antenna with 12 flagellomeres (n=2): 68–80, 48, 48, 48, 44, 40–44, 32–40, 36, 32, 28–32, 28–36, 112–126; AR 0.24–0.26; terminal flagellomere with 3–4 setae and 3–4 sensilla chaetica (Fig. 2). Length of flagellomeres (µm) and antennal index for antenna with 13 flagellomeres (n=1): 72, 40, 44, 40, 44, 44, 40, 40, 36, 32, 40, 32, 100; AR 0.20–0.21. Number of setae on flagellomeres 1–12 as: 1, 2, 2, 2, 2, 4–5, 4, 4, 3–4, 3–4, 3–4, 1; terminal flagellomere with 1 seta in basal part and 3–4 sensilla chaetica (Fig. 3). Palpomere 1–5 length (µm): 24–36, 52–56, 56–80, 56–76, 76–100. Head width/palpal length 1.38–1.76. Antennal length/palpal length 1.36.

Thorax. Antepronotum narrow, weakly developed without setae. Acrostichals 6–8, 16–20 µm long, decumbet, in two rows on anterior scutum (Fig. 5); dorsocentrals 10–11, 76–108 µm long; prealars 4–5, 60–64 µm long, scutellars 6–7, 64–72 µm long (Fig. 6).

Wing (Figs 7–8). Length 2.32–2.52 mm, width 0.60–0.68 mm. Anal lobe slightly reduced, angular or rounded angular. Squama without setae. R and R_1 with 8–11 setae, R_{4+5} with 4–6 setae. The venation is not clearly expressed. R_{2+3} reduced.

Legs. Spur of front tibia 36–40 µm long. Spurs of mid tibia 18–24 µm and 36–40 µm long. Spurs of hind tibia 48–60 µm and 16–18 µm long. Hind tibial comb with 16–18 spines. Length (µm) and proportions of leg segments are as in Table 1.

Hypopygium (Figs 9–14). Tergite IX with 3–4 lateral setae on each side, the anal tergite band (ATB) is wide, but is located only laterally, and is absent in the middle part. Anal point 60–68 µm long, 40–44 µm wide at the apex, broad, tongue-shaped, with a rounded apex, densely covered with microtrichia and short setae. Transverse sternopodeme 68–100 µm long, weakly convex, with weak oral projections. Virga not found. Aedeagal lobe 104–160 µm long, narrow rod-shaped (Fig. 9). Gonocoxite 380–386 µm long, with group of 11–13 dorsal setae (DSG), 28–96 µm long; inferior volsellae poorly developed, in the form of rounded pads covered with microtrichia and short setae. Gonostylus 224–228 µm long, knife-shaped



Figs 1–10. Adult male of *Thalassosmittia marina* (Sounders). 1 — antennal flagellum; 2 — apical flagellomere 13; 3 — apical flagellomere 12; 4 — head; 5 — acrostichal setae of scutum; 6 — scutum and scutellum; 7–8 — wings; 9 — transverse sternapodeme and aedeagal lobe; 10 — hypopygium in dorsal view. Scale bar: 50 μm.

Рис. 1–10. Имаго самец *Thalassosmittia marina* (Sounders). 1 — флагеллюм антенны: 2 — апикальный флагелломер 13; 3 — апикальный флагелломер 12; 4 — голова; 5 — акростихальные щетинки среднеспинки; 6 — среднеспинка и щиток; 7–8 — крылья; 9 — TSA и эдеагальная лопасть; 10 — гипопигий, вид сверху. Масштаб: 50 мкм.

Figs 11–14. Hypopygium in different positions. 11 — dorsal view; 12 — ventral view; 13, 14 — sublateral view. Designations: DSG — dorsal setae of gonocoxite.

Рис. 11–14. Гипопигий в различных положениях. 11 — сверху; 12 — снизу; 13, 14 — сбоку. Обозначения: DSG — дорсальные щетинки гонококсита.

Table 1. Lengths (in μm) and proportions of leg segments of *Thalassosmittia marina* (Saunders), male (n = 4)
Таблица 1. Длина (в мкм) члеников ног самца и их индексы *Thalassosmittia marina* (Saunders) (n = 4)

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	738–752	820–935	420–492	246–262	164–180	98	98	0.52–0.53	3.22–3.39	3.40–3.71
P_2	968–1132	968–1132	426–476	213–246	164–180	98	98	0.42–0.44	4.06–4.41	4,27–4.78
P_3	918–1033	918–1132	476–525	246–262	213–246	98	98	0.46–0.52	3.53–3.82	3.85–4.12

when viewed from above and in the form of a wide lobe when unfolded, densely covered with microtrichia and short setae, maximum width 104–108 μm, megaseta or terminal spine absent. HR 1.67–1.70.

Adult female, pupa and *larva* are described by Sounders [1928].

Remarks. *Th. marina* is distinguished from all known species of the genus by antenna with 12–13 flagellomeres, shape of male gonostylus, which, when straightened, is in the form of a wide lobe without megaseta or terminal spine and poorly developed inferior volsellae, whicn in form of rounded pads covered with microtrichia and short setae. In the key to males of *Thalassosmittia* in the work of Wang, Sæther [1993], the absence of an inferior volsella is erroneously reported for the species, which is apparently related to the drawing of the hypopygium in the work of Sublette [1967], where in Fig. 10 gonocoxite is without inferior volsellae. However, in the original description Sounders [1928] indicates the presence of inferior volsellae — «process on inner surface of sidepieces reduced to soft membranous cushion bearing very short setae». The males from Kamchatka have a similar structure to that of British Columbia, with the exception of some characters given in Table 2. Thus, specimens from Kamchatka are slightly larger (total length 2.3–2.6 mm), wing is longer (2.32–2.52 mm), antennae with 12 and 13 flagellomeres, scutum with 6–8 acrostichals, scutellum with 6–7 setae and squama of the wing naked. Males from British Columbia have a total length of 2.0–2.3 mm, antennae with only 12 flagellomeres, a wing length of 1.5–1.91 mm, scutum without acrostichals, scutellum with 4 setae and a squama with 1 seta. The presence of setae on the squama is described by Sublette [1967], which is not characteristic of the genus *Thalassosmittia*, in whose diagnosis the squama is naked [Cranston et al., 1989]. In our opinion, if additional material from North America is studied, the differences between populations of *Th. marina* will be significantly smaller.

Distribution. Amphipacific species, known from Canada (British Columbia), USA (California) [Ashe, O'Connor, 2012] and the Russian Far East (Kamchatka).

Results and discussion of COI DNA barcoding

In this study we obtained two partial mitochondrion fragments, COI-5P (658 bp in length) and COI-3P (826 bp in length) for two specimens, EAM1663 and EAM1664 of *Th. marina.* Intraspecific sequence divergence between two specimens were 0.77 % and 0.49 % using K2P distances of COI-5P and COI-3P locus respectively. Closely related sequences by COI-5P locus were three specimens *Orthocladius* sp. belonged to BIN BOLD:ACP8936. The average K2P genetic distances between obtained COI-5P sequences and *Orthocladius* sp. were 1.36% that corresponds to intraspecific level for orthocladiinae [Silva, Wiedenbrug, 2014; Makarchenko et al., 2017, 2018, 2019; Kang et al., 2022]. The combined dataset yielded average intraspecific distances equal to 0,92 %. Unfortunately, to date there are no available DNA barcodes identified as *Thalassosmittia*, so we deposited sequences in this study for the first time. In turn, three samples of *Orthocladius* sp. (see taxonomy section for GenBank and BOLD accession numbers) should be transferred to *Th. marina.*

Closely related sequence by locus COI-3P was *Thalassosmittia* sp.1 (GenBank accession number HQ441011, BOLD Process ID GBDP42937-19) [Cranston et al., 2012] which differed from obtained in this study sequences by 3.26 % using K2P model. Unfortu-

Table 2. Comparison of some morphological characters of *Thalassosmittia marina* (Sounders) from type locality in Canada and from Kamchatka (adult males)
Таблица 2. Сравнение некоторых морфологических признаков *Thalassosmittia marina* (Sounders) из типового местообитания в Канаде и с Камчатки (имаго самцы)

Characters	*T. marina* (Sounders) Canada (British Columbia) (n=3) Sounders 1928; Sublette 1967	*T. marina* (Sounders) Russian Far East (Kamchatka) (n=4), our data
Total length, mm	2.0–2.3	2.3–2.6
Number of antennal flagellomeres	12	12–13
AR	0.14–0.23	0.20–0.26
Wing length, mm	1.5–1.91	2.32–2.52
Squamal setae	1	0
Ac	0	6–8
Dc	9	10–11
Pa	–	4–5
Sc	4	6–7
LR_1	0.51–0.56	0.52–0.53
LR_2	0.42–0.46	0.42–0.44
LR_3	0.50–0.57	0.46–0.52
IVo	«Process on inner surface of sidepieces reduced to soft membranous cushion bearing very short setae»	Poorly developed, in the form of rounded pads covered with microtrichia and short setae

nately, this genetic marker is not used for DNA barcoding of chironomids, and the BOLD system did not delimit sequences into molecular taxonomic units. Furthermore, we cannot apply species delimitation methods due to only three available sequences for the genus *Thalassosmittia*. However, we assume similar mutation rates between COI-3P and COI-5P due to these are two ends of the same gene. Interspecific divergence of COI-5P to nearest neighbour for orthocladiinae is above 10 % [Silva, Wiedenbrug, 2014; Makarchenko et al., 2017, 2018, 2019]. On this basis, we conclude that the *Th. marina* from this study and *Thalassosmittia* sp.1 from GenBank and BOLD are conspecific.

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