Zootaxa 4686 (1): 119–126
https://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4686.1.6
http://zoobank.org/urn:lsid:zoobank.org:pub:1B726DDF-8EE8-4339-85C7-998F68C49266

Morphological description and DNA barcodes of adult males of *Tanytarsus heliomesonyctios* Langton, 1999 (Diptera, Chironomidae) in northeast of Russia

OKSANA V. OREL[1] & ALEXANDER A. SEMENCHENKO[2]
[1]*Federal research center of biodiversity of terrestrial biota East Asia, Russian Academy of Science, Far East Branch, 100-letya Vladivostoka av. 159, Vladivostok, 690022, Russia. E-mail: zorina@biosoil.ru*
[2] *School of Natural Sciences, Far Eastern Federal University, Suhanova St. 8, 690950 Vladivostok, Russia. E-mail: semenchenko_alexander@mail.ru*

Abstract

An illustrated morphological description of the adult males of *Tanytarsus heliomesonyctios* Langton, 1999, is provided for the first time. The males were found in mountain lakes Bolshoi Darpir (Momsky District of the Republic of Sakha (Yakutia)) and Momontai (Susumansky District of the Magadan Region), located in the Kolyma River basin. Females, pupae and larvae of *T. heliomesonyctios* was previously described from Spitsbergen, Jan Mayen (Norway) and Ellesmere Island (Arctic Canada), and considered parthenogenetic. *Tanytarsus heliomesonyctios* is here for the first time noted for the fauna of Russia. Comparison of DNA barcodes shows high K2P nucleotide distances (1.7%) between the sexual populations (Norway and Russia) and the parthenogenetic populations (Svalbard and Canada). In the Bayesian tree, the COI- sequences from adult males group as sister to a strongly supported clade of sequences from parthenogenetic populations. This apparently indicates a single origin of parthenogeneticity, perhaps due to extreme environmental conditions.

Key words: Diptera, Chironomidae, *Tanytarsus*, DNA barcoding, Russia

Introduction

Tanytarsus heliomesonyctios was described by Langton in 1999 based on the morphology of the pupae and females collected by Dr. Kate Silvester (medical officer to the British Joint Services Expedition to Borup Fiord), together with other colleagues who investigated the fauna of four lakes in the Ellesmere Islands, Arctic Canada (Langton 1999). Later, in 2008, pupae, females and larvae were collected as a result of the study of amphibiotic insects in water bodies and watercourses of the polar archipelago Spitsbergen, Jan Mayen Islands (Norway). Drs E. Stur and T. Ekrem first obtained COI sequences for *T. heliomesonyctios,* linked all stages of development and described the larva of *T. heliomesonyctios* (Stur & Ekrem 2011). Recent studies indicate a close relationship between *T. heliomesonyctios*, *T. bathophilus* and *T. lugens* which relates to the *T. lugens* species group (Lin *et al*. 2015). The parthenogenetic mechanism of reproduction of *T. heliomesonyctios* remains unidentified. In most cases parthenogenesis in non-biting midges is a strategy adopted to survive extreme environmental conditions (e.g. high altitudes and latitudes) (Lencioni 2004; Nondula *et al.* 2004, Donato & Paggi 2008). Asexual populations have many advantages over sexual lineages such as high fecundity, effective population size and colonization rate. Cytogenetic studies on chironomids reveals that parthenogenesis is often associated with hybridization that leads to meiotic disruption and asexual production of diploid female offspring from unfertilized eggs (Porter 1971; Porter & Martin 2011; Carew *et al.* 2013). This type of parthenogenetic mechanism is called apomictic thelytoky, with a restitutional division during oogenesis (Porter 1971, Porter & Martin 2011).

Polyploid parthenogenesis occurs in non-biting midges, mostly in the subfamilies Chironominae and Orthocladiinae. However, for most species of parthenogenetic chironomids, sexual populations are unknown (Porter & Martin 2011, Carew *et al*. 2013, Donato & Paggi 2008, Edward 1963) so that only the larvae, pupae and imago females are described. At least three species of chironomids include sexual and asexual populations—*Corynoneura scutel-*

Accepted by B. Rossaro: 11 Sept. 2019; published: 14 Oct. 2019

lata Winnertz (Edwards 1919, as *C. innupta* n.sp.; Edward & Colless 1968), *Abiskomyia virgo* Edwards (Lindeberg 1974) and *Paratanytarsus laccophilus* (Edwards) (Lindeberg 1958, as *Monotanytarsus boreoalpinus* Thienemann). *Tanytarsus heliomesonyctios* is the second nonbiting midge from subfamily Chironominae for which both parthenogenetic populations (Lindeberg 1958, Langton 1999, Stur & Ekrem 2011) and sexual ones (based on the presence of males in the population) are described (our data). As a result of processing unique material on aquatic insects collected by the staff of the Institute of Biological Problems of the North of the Far East Branch of the Russian Academy of Sciences in 2018 in mountain lakes Bolshoi and Malyi Darpir, Momontai, Ui, and using morphological analysis and DNA barcoding, we describe the males of *T. heliomesonyctios* Langton for the first time. The phylogenetic relationship of sexual and parthenogenetic populations has been reconstructed using partial COI sequences, and the prospects for studying the evolution of parthenogeneticity in this species is discussed.

Materials and methods

Imaginal material was collected by E.V. Khamenkova in 2018 by cutting with a net of coastal vegetation on the lakes of Big Darpir (north point—64°11′18.75″ N, 148°02′39.33″ E; south point—64°05′35.44″ N, 148°01′48.42″ E) Momsky district of the Republic of Sakha (Yakutia) and Momontai (north point—63°44′18.06″ N, 148°07′27.26″ E; south point—63°38′29.96″ N, 148°11′04.24″ E) Susumansky district of the Magadan region, related to the Kolyma River basin.

Material was fixed with 80% ethanol for morphological studies and 96% ethanol for DNA analysis.

The descriptions of the species use terminology and abbreviations according to Sæther (1980). Types of distribution are given by Gorodkov (1984). Also, in refining the distribution, the most updated catalogs were used (Ashe & O'Connor 2009, 2012; Sæther & Spies 2013).

Total DNA was extracted from each sample using the Invitrogen PureLink Genomic DNA Mini Kit in accordance with the protocol in a final elution volume of 70 µL. For PCR mixture 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 were added. Fragments of the mitochondrial cytochrome oxidase c subunit 1 (COI) genes were amplified with the primers LCO1490 and HCO2198 (Folmer *et al.* 1994) which amplifies a fragment of ca 658 bp. 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 bidirectionally sequenced on an ABI 3130XL automated sequencer using BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.). MEGA7 (Kumar *et al.* 2016) and FinchTV were used to edit and assemble double stranded sequences. Also MEGA7 was used for calculated inter- and intraspecific K2P distances.

PartitionFinder 2.1.1 (Lanfear *et al*. 2012) was used to select the best-fit partitioning scheme and models separately for each codon position of COI using the greedy algorithm with linked branch lengths for the corrected Bayesian Information Criterion. The best models of nucleotide substitution for 1 COI codon positions was K80 (Kimura 1980), for 2 codon - F81 (Felsenstein 1981) and GTR for 3 codon position (Tavare 1986). Bayesian phylogenetic analyses were conducted with MrBayes v. 3.2.7 (Ronquist & Huelsenbeck 2003). Bayesian Inference was performed with two independent runs of Metropolis-coupled Markov chain Monte Carlo analyses. The chains were run for 3 million generations and sampled every 500 generations. A burn-in of 300000 generations (or 10% of the sampled trees) was used. Moreover, trace files were visually inspected in Tracer 1.7 (Rambaut *et al.* 2018). FigTree v. 1.4.4 was used to visualize phylogenetic trees after analysis. Sequences of *T. heliomesonyctios* obtained in this study have been deposited in GenBank (accession numbers MN017717-MN017719)

All the material is stored in the Laboratory of Freshwater Hydrobiology of the Federal Scientific Center for Biodiversity of the Terrestrial Biota of East Asia of the Far East Branch of the Russian Academy of Sciences (Vladivostok).

Results

Family Chironomidae Newman, 1834

Subfamily Chironominae Newman, 1834

Tribe Chironomini Zavřel, 1917

Genus Tanytarsus van der Wulp, 1874

***Tanytarsus heliomesonyctios* Langton, 1999**
Figures 1–4.

Tanytarsus sp. Langton, 1992 (ecology, parthenogenesis, phenology).
Tanytarsus heliomesonyctios Langton, 1999: 212, Fig. 1 b, d, f (female); Fig. 2 b, e, f (pupa).
Tanytarsus heliomesonyctios Langton, 1999; GU073186– GU073192|Female, pupa, larva, Stur & Ekrem 2011: 32, Figs 27–32.
Tanytarsus heliomesonyctios Langton, 1999; AAC2863|Male, available from: http://www.boldsystems.org/index.php/Public_RecordView?processid=CHRFI417-11
Tanytarsus heliomesonyctios Langton, 1999; Makarchenko *et al*., 2019, Figs 10–13.

Material examined. 4 males, Russia, Magadan region, Monomtai Lake, 27–28.vii.2018, leg. E. Hamenkova; 4 males, same data, 2.viii.2018, leg. E. Hamenkova; 4 males, Republic of Sakha (Yakutia), Big Darpir Lake, 4.viii.2018, leg. E. Hamenkova.

Description. Adult male (n=3). Total length 3.6–4.7 mm; wing length 2.8–3.7 mm. Total length / wing length 1.17–1.61.

Colouration. Ground colour of thorax, scutellum, maxillary palpomeres, haltere, legs and abdomen brown; antenna, scutal stripes and postnotum dark brown.

Head. Frontal tubercles cone-shaped, 10–24 μm long and 7–17 μm wide. Temporal setae 16. Clypeus with 16–20 setae. Antenna with 13 flagellomeres, 1638–1701 μm long; ultimate flagellomere 1050–1071 μm. AR 1.67–1.79. Maxillary palpomeres 2–4 combined 752–928 μm long, their individual lengths (in μm): 80–96 : 216–248 : 200–240 : 256–344. Antenna length/palp length 1.81–2.18.

Thorax. Acrostichals 4–10, dorsocentrals 8–14, prealars 2. Scutellum with 18 setae.

Wing width 1.0–1.05 mm. VR 0.94–1.18. R with 23–29, R_1 with 3–23, R_{2+3} with 10–30, R_{4+5} with 45–65, M_{3+4} with 26–50, Cu_1 with 9 setae. VR 0.94–1.18. Brachiolum with 1 seta. Membrane covered with sparse macrotrichia in distal half.

Legs (see Table 1). Spur of fore tibia straight, slightly curved apically 24–31 μm long. Combs of mid and hind tibiae separated; each comb bears straight or slightly curved spur, 27–31 μm (mid tibia) to 31–41 μm long (hind tibia). Basitarsus of mid leg with 2–5 sensilla chaetica.

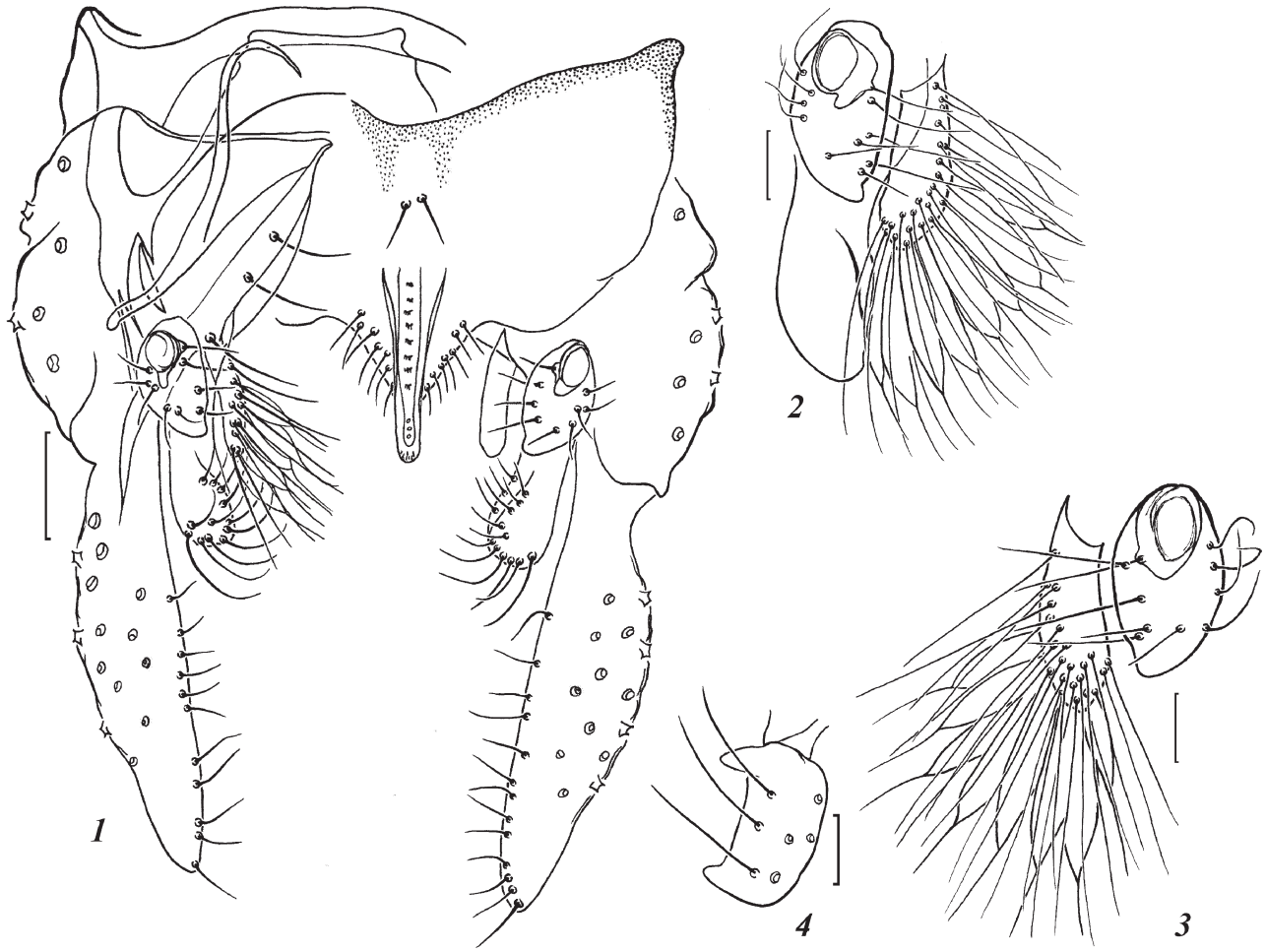
TABLE 1. Lengths (in μm) and proportion of legs *Tanytarsus heliomesonyctios* Langton

P	f	t	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1218–1596	819–1009	1365–1722	798–882	588–735	378–504	179–221
P_2	1176–1407	1113–1281	546–609	399–462	315–336	189–231	126–168
P_3	1470–1827	1428–1785	924–1176	651–777	525–630	315–399	168–210

P	LR	SV	BV	BR
P_1	1.65–1.71	1.49–1.51	1.81–1.93	6.36–6.75
P_2	0.48–0.49	4.19–4.41	2.75–2.76	6.25–6.67
P3	0.63–0.66	3.07–3.20	2.30–2.38	8.00–9.00

Hypopygium (Figs 1–4). Anal tergite with 2–3 median setae (rarely without setae) and great microtrichia-free area surrounding of base. Dark tergite bands separated medially, not reaching anal point crests. Lateral teeth and lateral setae absent. Anterior margin of tergite IX with small protrusions. The anal point is triangular shape with a wide base (85–102 μm long) and a narrower apical part (17 μm), armed with 10–12 spinulae placed in row between crests (102–133 μm long and height 7–10 μm in lateral view); microtrichia absent between crests; 16 lateral setae on each side of anal point. Gonocoxite 231–238 μm long, along the inner margin with 3–4 setae. The width of the transverse

sternapodeme 119–126 μm. Phallapodeme 187–221 μm long. Superior volsella oval-shaped (65–85 μm long, 41–44 μm wide in dorsal view and 31 μm wide in lateral view), bearing 3–4 strong anteromedian and 6–8 fine dorsal setae, microtrichia absent. Digitus short (14 μm long and 7 μm wide). Stem of median volsella 78–92 μm long, bearing long simple and foliate setae that extend beyond the top of the inferior volsella. Inferior volsella 102–153 μm long, with 21–24 setae. Gonostylus straight 255–262 μm long and expanded in the proximal third (71–85 μm), tapering to widely rounded apex. HR 0.91.



FIGURES 1–4. Details of the structure of hypopygium the male *T. heliomesonyctios* Langton. 1—total view of hypopygium, dorsal view; 2, 3—volsellae of gonocoxite; 4—superior volsella, lateral view. Scale bar 50 μm.

Remarks. Adult males of *T. heliomesonyctios* keys to the *T. lugens* species group due to the missing or poorly developed digitus, usually the lack of median tergite setae, a large microtrichia free area around the anal point base, and foliate lamellae with long tips on the median volsella (Ekrem 2003). Currently, the *T. lugens* species group includes six species, namely, *T. bathophilus* Kieffer, 1911, *T. lugens* (Kieffer, 1916), *T. konishii* Sasa & Kawai, 1985, *T. latiforceps* Edwards & Thienemann, 1941, *T. trux* Gilka & Paasivirta, 2007 and *T. heliomesonyctios* Langton, 1999 (Ekrem 2003, Gilka & Paasivirta 2007). Adult males of *T. heliomesonyctios* most similar with two species of *T. lugens* species group—*T. bathophilus* and *T. lugens*. Table 2 shows the main morphological features of males. Males of *T. heliomesonyctios* are distinguished by a large body size, a longer last antenna segment and maxillar palp, thoracic chetotaxy, the presence of medial setae on tergite IX (rarely medial setae are absent), the presence of poorly developed digitus and elongated median volsella (see Table 2).

Distribution. Holarctic species. Previously known from Ellesmere Island in the Canadian high Arctic and the archipelagos Spitsbergen and Jan Mayen (Norway) as well as in Finnmark, northern Norway (males). For the first time noted the fauna of Russia. This is the second record of adult males.

TABLE 2. Comparison of adult male characteristics for species in the *T. lugens* species group

Characters	*T. bathophilus* Kieffer (from Ekrem *et al*. 2003)	*T. lugens* (Kieffer) (from Reiss & Fittkau 1971)	*T. heliomesonyctios* Langton (own data)
Total length, mm	3.3	–	3.6–4.7
Wing length, mm	1.89–2.80	3.0	2.8–3.7
Frontal tubercles length, µm	36	–	10–24
Palpomere lengths, µm	36/126/137/205	–	88/232/220/300
AR	1.27–1.79	1.7–1.8	1.67–1.79
Ac	20	–	4–10
Dc	13	–	8–14
Scts	8	–	18
LR_1	1.53–1.90	–	1.65–1.71
BR_1	4.1	5–7	6.36–6.75
Anal point length, µm	61–76	–	85–102
Number of spinulae on anal point	9–32	–	10–12
Number of median tergite setae	0	–	2–3 (rarely 0)
Median volsella length, µm	65–72	48–54	78–92
Digitus	poorly developed	missing	poorly developed
Inferior volsella length, µm	79–90	–	102–153
Gonocoxite length, µm	166–180	–	231–238
Gonostylus length, µm	151–162	–	255–262
HR	1.04–1.14	–	0.91

Results from DNA barcoding

A total of 3 specimens of *T. heliomesonyctios* were sequenced. The final alignment of the COI barcode region yielded 658 base pairs. The nucleotide composition of the studied sequences of *T. heliomesonyctios* deviated from an equilibrium one, comprising 29.2% of A, 39.2% of T, 16.3% of C, and 15.3% of G. All three sequences were identical and formed one haplotype.

The average interspecies K2P distances between the obtained sequences and specimens of *T. heliomesonyctios* in GenBank and BOLD were 1.6% (1.2%—2.0%). Such values are less than the suggested 4–5% threshold for Tanytarsus species delimitation (Lin *et al.* 2015), which supports that the three sequences relate to *T. heliomesonyctios.* The closest specimen to the Russian samples of *T. heliomesonyctios* was a male collected from the northeast Norway by Ekrem & Stur (BOLD id CHRFI417–11, GenBank accession number JN265037). Distances between two sexual populations were 0.7%. In turn, the average intraspecific distance between parthenogenetic and sexual forms was 1.7%.

The interspecific K2P distance (COI) between *T. heliomesonyctios* and two close species of *T. lugens* group—*T. lugens* and *T. bathophilus* were 3.2 and 5.3% on average respectively. Such interspecific values are low within the genus *Tanytarsus*, as noted in Lin *et al.* (2015). However, each species forms well supported monophyletic clades except the paraphyletic *T. bathophilus* (Lin *et al.* 2015).

The Bayesian inferred phylogeny of *T. heliomesonyctios* revealed two sister clades reflecting populations with different modes of reproduction—parthenogenetic and sexual (Fig. 5). The parthenogenetic clade was strongly-supported (Bayesian posterior probability (PP) = 1) while the clade with sequences from sexual populations was poorly-supported (PP = 0.54). The tree constructed by the Maximum likelihood analysis using a GTR+G model (not shown) had a different topology and revealed specimen CHRFI417-11 (JN265037) as the earliest branching lineage. The sexual clade was well-supported (bootstrap value 87%) in a Neighbor-joining tree (not shown).

The obtained picture of phylogenetic relationships is a good basis for further research. Increased sampling and using nuclear DNA sequences (such as ITS1, ITS2, EF-1a, CAD etc.) in future molecular studies may be able to firmly resolve the relationships of parthenogenetic and sexual groups. The high support of the

parthenogenetic node suggests that parthenogenetic reproduction in *T. heliomesonyctios* appeared once from sexual chironomids. However, increased sampling throughout the full distribution range, and analyses of more molecular markers is necessary to confirm this. In addition, a calibrated tree can be constructed and established the divergence time between sexual and asexual forms. Using paleoclimate data, it becomes possible to clarify the abiotic and climatic conditions for the formation of chironomids parthenogenesis. Unfortunately, the limited sampling (especially sexual populations) and using only single COI locus do not currently allow to perform such analysis.

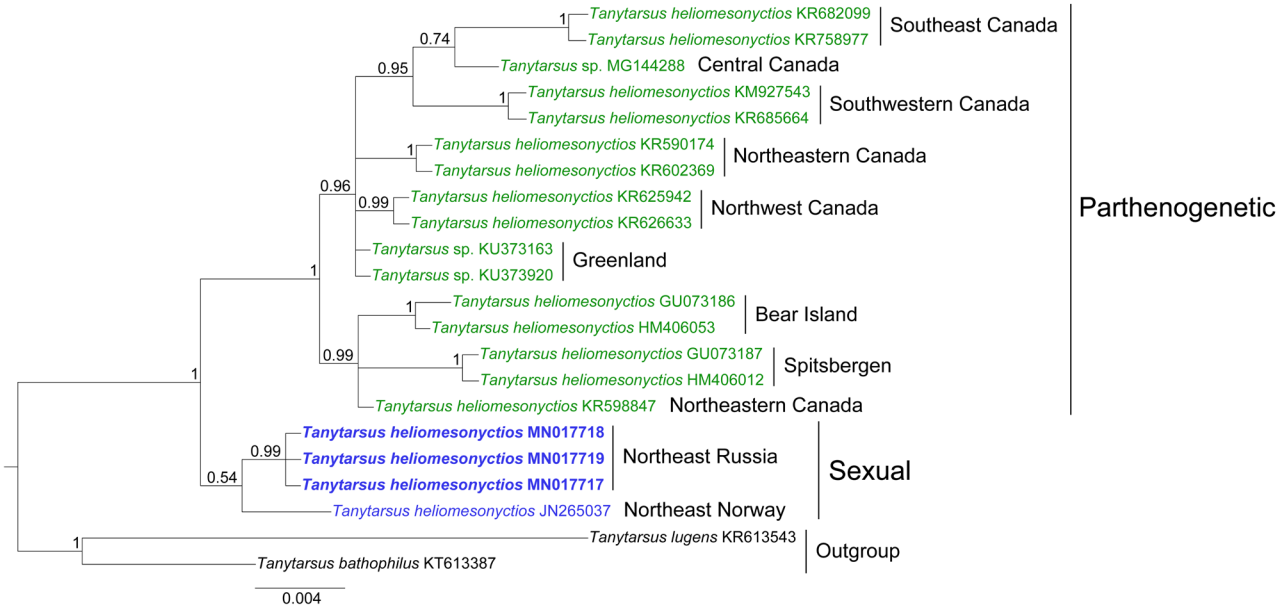


FIGURE 5. Bayesian phylogeny of the *T. heliomesonyctios* from 20 taxa based on the mitochondrial cytochrome c oxidase I (COI) barcode gene sequences (658 bp). T. lugens and T. bathophilus were used as outgroup to root tree. Bayesian posterior probabilities (PP) are given above tree nodes. The specimens obtained in this study are in bold.

Acknowledgements

The authors are deeply grateful to E.V. Hamenkova (Institute of Biological Problems of the North of the Far East Branch of the Russian Academy of Sciences) for the material collected and submitting it to us for further study. We thank Drs. T. Ekrem and P.H. Langton for editing our English and valuable comments in this manuscript.
The expedition was carried out within the framework of the grant project of the Russian Geographical Society (contract No. 07/2018-P).

References

Ashe, P. & O'Connor, J.P. (2009) *A world Catalogue of Chironomidae (Diptera). Part 1. Buchonomyiinae, Chilenomyiinae, Podonominae, Aphroteniinae, Tanypodinae, Usambaromyiinae, Diamesinae, Prodiamesinae and Telmatogetoninae.* Irish Biogeographical Society & National Museum of Ireland, Dublin, 445 pp.

Ashe, P. & O'Connor, J.P. (2012) *A world catalogue of Chironomidae (Diptera). Part 2. Orthocladiinae. Two Vols. Sections A & B.* Irish Biogeographical Society, Dublin, [14] + xvi + 468 pp. + [6] + 500 pp.

Carew, M., Gagliardi, B. & Hoffmann, A. (2013) Mitochondrial DNA suggests a single maternal origin for the widespread triploid parthenogenetic pest species, *Paratanytarsus grimmii*, but microsatellite variation shows local endemism. *Insect science*, 20, 345–357.
https://doi.org/10.1111/j.1744-7917.2012.01564.x

Donato, M. & Paggi, A.C. (2008) *Polypedilum parthenogeneticum* (Diptera: Chironomidae): a new parthenogenetic species from Eryngium L. (Apiaceae) phytotelmata. *Aquatic Insects*, 30, 51–60.
https://doi.org/10.1080/01650420701829633

Edward, D.H.D. (1963) The biology of a parthenogenetic species of Lundstroemia (Diptera: Chironomidae), with descriptions

of the immature stages. *Proceedings of the Royal Society of London*, Series A, 38, 165–170.
https://doi.org/10.1111/j.1365-3032.1963.tb00745.x
Edwards, F.W.E. (1919) Some parthenogenetic Chironomidae. *Annals and Magazine of Natural History*, 9 (3), 222–228.
https://doi.org/10.1080/00222931908673815
Edward, D.H.D. & Coless, D.H. (1968) Some Australian Parthenogenetic Chironomidae (Diptera). *Australian Journal of Entomology*, 7 (2), 158–162.
https://doi.org/10.1111/j.1440-6055.1968.tb00724.x
Ekrem, T. (2003) Towards a phylogeny of *Tanytarsus* van der Wulp (Diptera: Chironomidae). Is morphology alone sufficient to reconstruct the genealogical relationship? *Insect* Systematics & *Evolution,* 34, 199–219.
https://doi.org/10.1163/187631203788964845
Ekrem, T., Sublette, M.F. & Sublette, J.E. (2003) North American *Tanytarsus* I. Descriptions and keys to species in the *eminulus*, *gregarius*, *lugens* and *mendax* species groups (Diptera: Chironomidae). *Annals of the Entomological Society of America*, 96 (3), 265–328.
https://doi.org/10.1603/0013-8746(2003)096[0265:NATIDA]2.0.CO;2
Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, 17 (6), 368–376.
https://doi.org/10.1007/BF01734359
Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299
Gilka, W. & Paasivirta, L. (2007) Two new species of the genus *Tanytarsus* van der Wulp (Diptera: Chironomidae) from Fennoscandia. *In*: Andersen, T. (Ed.), *Contribution to the Systematics and Ecology of Aquatic Diptera—A Tribute to Ole A. Saether*, 2007, pp. 107–113 pp.
Gorodkov, K.B. (1984) *Types of habitats of tundra insects and forest zones of the European part of the USSR*. L.: Science, 3–20 pp. [in Russian]
Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 2, 111–120.
https://doi.org/10.1007/BF01731581
Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33 (7), 1870–1874.
https://doi.org/10.1093/molbev/msw054
Lanfear, R., Calcott, B., Ho, S.Y. & Guindon, S. (2012) Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29 (6), 1695–1701.
https://doi.org/10.1093/molbev/mss020
Langton, P.H. (1992) The Chironomidae collected on Ellesmere Island by Dr. Kate Silvester. *In:* Hankinson, K.W. (Ed.), *Ellesmere*, 1991, pp.1– 87. [privately published]
Langton, P.H. (1999) *Micropsectra silvesterae* n.sp. and *Tanytarsus heliomesonyctios* n.sp., (Diptera: Chironomidae), two parthenogenetic species from Ellesmere Island, Arctic Canada. *Journal of the Kansas Entomological Society*, 71 (3), 208–215.
Lencioni, V. (2004) Survival strategies of freshwater insects in cold environments, *Journal of Limnology*, 63, 45–55.
https://doi.org/10.4081/jlimnol.2004.s1.45
Lin, X., Stur, E. & Ekrem, T. (2015) Exploring Genetic Divergence in a Species-Rich Insect Genus Using 2790 DNA Barcodes. *PLoS ONE*, 10 (9), e0138993.
https://doi.org/10.1371/journal.pone.0138993
Lindeberg, B. (1974) Parthenogenetic and normal populations of *Abiskomyia virgo* Edw. (Diptera, Chironomidae). *Entomologisk Tidskrift*, 95 (Supplement), 157–161.
Lindeberg, B. (1958) A parthenogenetic race of *Monotanytarsus boreoalpinus* Th. (Dipt., Chironomidae) from Finland. *Suomen Hyönteistieteellinen Aikakauskirja*, 24, 35–38.
Makarchenko, E.A., Makarchenko, M.A., Orel, O.V. & Khamenkova, E.A. (2019) Preliminary Data On The Chironomid Fauna (Diptera, Chironomidae) Of The Mountain Lakes Of Cherskiy Ridge (Magadan Region And Sakha Republic (Yakutia)). *Vladimir Ya. Levanidov's Biennial Memorial Meetings*, 8, 73–90.
https://doi.org/10.25221/levanidov.08.09
Nondula, N., Marshall, D.J., Baxter, R., Sinclair, B.J. & Chown, S.L. (2004) Life history and osmoregulatory ability of Telmatogeton amphibius (Diptera, Chironomidae) at Marion Island, *Polar Biology*, 27, 629–635.
https://doi.org/10.1007/s00300-004-0619-z
Porter, D.L. (1971) Oogenesis and chromosomal heterozygosity in the thelytokous midge, Lundstroemia pathenogenetica (Diptera: Chronomidae). *Chromosoma*, 32, 332–342.
https://doi.org/10.1007/BF00284841
Porter, D.L. & Martin, J. (2011) Cytogenetics of a parthenogenetic Arctic species of *Micropsectra* (Diptera, Chironomidae). *Comparitive Cytogenetics*, 5, 315–328.
https://doi.org/10.3897/compcytogen.v5i4.1356
Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarisation in Bayesian phylogenetics

using Tracer 1.7. *Systematic Biology*, 67 (5), 901–904.
https://doi.org/10.1093/sysbio/syy032
Reiss, F. & Fittkau, E.J. (1971) Taxonomy and ecology European distributed *Tanytarsus*-species (Chironomidae, Diptera). *Archive Hydrobiology*, Supplement, 40 (1–2), 75–200.
Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
https://doi.org/10.1093/bioinformatics/btg180
Sæther, O.A. (1980) Glossary of chironomid morphology terminology (Chironomidae, Diptera). *Entomologica scandinavica*, 14 (Supplement), 1–51.
Stur, E. & Ekrem, T. (2011) Exploring unknown life stages of Arctic Tanytarsini (Diptera: Chironomidae) with DNA barcoding. *Zootaxa*, 2743 (1), 27–39.
https://doi.org/10.11646/zootaxa.2743.1.2
Tavaré, S. (1986) Some Probabilistic and Statistical Problems in the Analysis of DNA Sequences (PDF). Lectures on Mathematics in the Life Sciences. *American Mathematical Society*, 17, 57–86.