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## First record of *Ephemera separigata* Bae, 1995 (Insecta, Ephemeroptera) from Russia and Russian Far East

# Первая находка *Ephemera separigata* Bae, 1995 (Insecta, Ephemeroptera) в России и на Дальнем Востоке

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*Key words:* Ephemeroptera, mayfly, new record, Russia, Far East. *Ключевые слова:* Ephemeroptera, поденки, новая находка, Россия, Дальний Восток.

*Abstract.* The first record of *Ephemera separigata* Bae, 1995 from Russia and Far East is given. The mitochondrial COI gene of *E. separigata* and *E. strigata* Eaton, 1892 occurring in Russian Far East were analyzed to confirm the species identification.

**Резюµе.** Для фауны России и Дальнего Востока (Приморский край) приводится первая находка подёнки *Ephemera separigata* Вае, 1995. Митохондриальный ген COI *Ephemera separigata* и *E. strigata* Eaton, 1892 встречающихся на Дальнемм Востоке России, был проанализирован для подтверждения вида.

## Introduction

At the present time, in the Far East of Russia, there are five species of mayflies belonging to the genus *Ephemera* Linnaeus 1758: East Asian Island *E. japonica* McLachlan 1875; East Palaearctic *E. sachalinensis* Matsumura 1911, *E. strigata* Eaton 1892, *E. transbajkalica* Tshernova 1973, and Palaearctic *E. orienta*lis McLachlan 1875 [Tiunova, 2009].

The first record of *Ephemera separigata* Bae, 1995 is reported from Russian Far East on the basis of specimens collected in the Primorskiy Kraii. We made a comparison between Far Eastern *E. separigata* and *E. strigata* and sequences from GeneBank and BOLD systems. To confirm monophyly of each species phylogenetic tree was also provided.

The material is deposited in the collection of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, Russia.

## Material and methods

Nine Ephemera specimens were used for molecular study (Table 1). Total DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) from thorax or legs of larvae and imago and the resultant DNA was eluted in 100 µl. The COI fragment was amplified using LCO1490 (5'-GGTCAACAAATCAT-AAAGATATTGG-3') and HCO2198 (5'-TAAACT-TCAGGGTGACCAAAAAATCA-3') [Folmer et al., 1994]. The 10 µl PCR reaction mix contained 3 µl of ultrapure water, 1 µl of DNA additionally diluted 10x,  $0.5 \,\mu$ l of each primer (10  $\mu$ M), and 5 Go Taq Green Master Mix (Promega corp, Madison, WI, USA). The reaction was conducted with an initial step of 94 °C for 5 min, followed by a total of 30 cycles at 94 °C for 30 sec, 48 °C for 30 sec, 72 °C for 60 sec, and 72 °C for 5 min. Successful amplifications (checked by TBE Gel Electrophoresis on a 1.5 % agarose gel) were purified using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA). Purification and bi-directional Sanger sequencing was performed by ABI 3130xl (Applied Biosystems, Carlsbad, CA) using Big Dye Terminator ver. 3.1 and BigDye 5x Sequencing Buffer.

PartitionFinder 2.1.1 [Lanfear et al., 2012] is used to select the best-fit partitioning scheme and models separately for each codon position of COI gene using the greedy algorithm with linked branch lengths for the corrected Bayesian Information Criterion as the optimality criterion for model selection. A Bayesian Inferences (BI) analysis was performed with MrBayes v.3.2.7 [Ronquist et al., 2012] under the following conditions:

Geographic GeneBank accession Stage/sex Country, collection locality Species Isolate coordinate number 42.823900, Ephemera strigata TMT170 Larva Primorsky Krai, Ryazanovka River ON505834 131.234048 43.693182, ON505835 Ephemera strigata TMT342 Primorsky Krai, Krivov Stream Imago, 3 132.163682 Jewish Autonomous Oblast, Bira 49.007156, ON505836 Ephemera strigata TMT46 I arva 131.884017 River 43.460720, ON505837 Ephemera strigata **TMT540** Primorsky Krai, Nezhinka River Larva 131.731141 43.302552, ON505838 Ephemera strigata TMT700 Primorsky Krai, Tigrovava River I arva 133.057668 Primorsky Krai, Avvakumovka River 43.825636. ON505839 Ephemera strigata **TMT843** Larva Basin 134.905104 43.176817, Ephemera strigata TMT851 Larva Primorsky Krai, Barabashevka River ON505840 131.532742 43.460720, ON505841 Ephemera separigata **TMT539** Larva Primorsky Krai, Nezhinka River 131.731141 43.460720. Ephemera separigata **TMT593** Larva Primorsky Krai, Nezhinka River ON505842 131.731141

Table 1. List of sequenced specimens *Ephemera separigata* Bae, 1995 and *E. strigata* Eaton, 1892 Таблица 1. Список секвенированных образцов *Ephemera separigata* Bae, 1995 и *E. strigata* Eaton, 1892

5 million generations with sampling every 500 generations, four chains and a burn-in of 25 % trees. Sequences were deposited in GeneBank under accession numbers ON505834 - ON505842 (Table 1).

## Descrciption

#### Ephemera separigata Bae

Figs 1-3.

*Ephemera separigata* Bae, 1995: 160; Quan Y.T. et al., 2002: 250; Bae Y.J., Liu G.C. 1999:5.

*Material.* Russia: Primorskiy krai, Khasanskiy district: 4 larvae, Nezhinka River, about 3 km above the Vladivostok– Khasan highway, 14.VII.2017, leg. T. Tiunova; 6 larvae, same place, 28.VII.2017, leg. T. Tiunova; 3 larvae, same place, 24.VII.2018, leg. T. Tiunova; 3 larvae, Khasanskiy district, Gryaznaya River, road bridge, Vladivostok–Khasan highway, 20.VI.2017, leg. T. Tiunova.

*Ephemera separigata* is morphologically very similar to *E. strigata* and *E. japonica*, but distinguished by a pair of relatively narrow, separated, and laterally oriented longitudinal stripes on the abdominal terga 7–9 in larval (Figs 1–2), subimaginal, and adult stages of both sexes. Forefermora with rows of hair like setae along anterior and posterior margins, and with longitudinal hair like setal field on dorsal surface (Fig. 3).

Overall, we have sequenced fragments of the cytochrome oxidase I (658 bp in length) of 9 specimens belonging to 2 species (Table 1). *E. strigata* includes 7 specimens, which made it possible to evaluate the intraspecific distances which were 0.13% in average.

We compared obtained sequences with GeneBank and BOLD systems data. For each species, we found conspecific samples in databases and calculate interspecific distances. The closest sequence to Far Eastern *E. strigata* were *E. strigata* collected from Japan (MN961290-MN961294, LC644376, BIN BOLD:AED3237). The average p-distances between them were 5.71 % which shows their conspecificity [Morinière et al., 2017]. Obtained sequences of *E. separigata* were close to *E. separigata* (HQ257232-HQ257234, MH823275-MH823279) from South Korea, the average p-distance was 0.6 %. Interspecific distances between *E. strigata* — *E. japonica*, *E. japonica* — *E. separigata* and





Figs 1–3. *Ephemera separigata* Bae, 1995. 1 — larva, dorsal view; 2 — larva, ventral view; 3 — foreleg, dorsal view.

Рис. 1–3. *Ephemera separigata* Вае, 1995. 1 — личинка, вид сверху; 2– личинка, вид снизу; 3 — передняя нога.



0.05

Fig. 4. Bayesian tree based on mitochondrion COI gene for obtained sequences and each available BIN number of three *Ephemera* species. *Hexagenia limbata* Servill was used as outgroup to root the tree. Bayesian posterior probabilities (PP) are given above tree nodes. Specimens obtained in this study are in bold.

Рис. 4. Байесовское дерево на основе митохондриального гена СОІ для полученных последовательностей и каждого доступного номера ВІN трех видов *Ephemera*. *Нехаgenia limbata* Servill использовалась как внешняя группа для укоренения дерева. Байесовские апостериорные вероятности (PP) даны над узлами дерева. Образцы, полученные в этом исследовании, выделены жирным шрифтом.

*E. strigata* — *E. separigata* were 10.51 %, 11.66 % and 11.14 % respectively which support the distinctiveness of each species [Morinière et al., 2017].

We used Bayesian Inference to reconstruct relationships of the three related *Ephemera* species (Fig. 4). To do this, we selected one sample from each BIN number from the BOLD systems and *Hexagenia limbata* Servill as outgroup. *E. strigata* was placed as sister to *E. japonica* (BIN BOLD: AED4371) with high support (Bayesian posterior probability, PP = 0.97). *E. separigata* was the earliest branching lineage and sister to two other species (PP = 1.00).

*Distribution.* Russian Far East (Primorye Territory), Korea, Northeast China.

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## An interesting finding of limnephilid caddisfly (Trichoptera, Limnephilidae, Limnephilinae, Limnephilini) on Wrangel Island

# Интересная находка лимнефилидного ручейника (Trichoptera, Limnephilidae, Limnephilinae, Limnephilini) на острове Врангеля

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Key words: new records, Trichoptera, Limnephilidae, High Arctic tundra, freshwater ecosystems.

*Ключевые слова:* новые находки, фауна Trichoptera, Limnephilidae, арктическая тундра, пресноводные экосистемы.

*Abstract.* The data on the limnephilid caddisfly species from the arctic tundra lake of Wrangel Island are presented. A description of IV instar larva is given, attempts are made to associate the found larvae with one of the related genera of the *Asynarchus-Limnephilus-Philarctus* complex, and it is concluded that the larvae belong to the *Philarctus bergrothi* McLachlan, 1880.

**Резюме.** Приводятся данные о находке лимнефилидного ручейника из арктического тундрового озера острова Врангеля. Даётся описание личинки IV возраста, предприняты попытки ассоциации найденных личинок с родом комплекса *Asynarchus–Limnephilus–Philarctus* и делается заключение о принадлежности личинок к виду *Philarctus bergrothi* McLachlan, 1880.

## Introduction

Our knowledge of the aquatic invertebrates of the Russian arctic islands is still insufficient due to the difficulties in reaching these hard-to-reach places, in organizing expeditions and a short season convenient for collecting. Therefore, to date, information about the fauna of freshwater invertebrates, especially amphibiotic insects, is scarce for many groups. However, in regions where protected areas are being created, faunal «white spots» disappear very quickly, thanks to wellorganized regular research by reserve staff and attracted taxonomists.

The first information about the freshwater invertebrates of Wrangel Island appeared after the organization of the «Wrangel Island» State Nature Reserve on March 23, 1976 and subsequent hydrobiological expeditions, which were attended by E.A. Makarchenko in 1978, and then E.A. and M.A. Makarchenko in 1979, researches of the Laboratory of Freshwater Hydrobiology of the Institute of Biology and Soil Sciences FEB RAS, Vladivostok (now Federal Scientific Center of Biodiversity FEB RAS). As a result, a first list of amphibiotic insects was compiled from 35 species [Makarchenko et al., 1980], among which the only one species of caddisfly was listed: *Micrasema gelidum* McLachlan, 1876 based on the materials earlier collected by K. Gorodkov (1 male, imago), without specifying the date and locality.

Then, from 1984 to 1986, collections of terrestrial and aquatic fauna were made by O. A. Khruleva (Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Moscow), who added two limnephilids *Lenarchus expansus* Martynov, 1914 and Limnephilidae gen. sp. collected in the raw willow-sedgemoss community in the lower reaches of the Gusinaya River [Khruleva, 1987].

Since that time, regular studies of the biota on the Wrangel Island have continued, which has significantly enriched knowledge about the aquatic invertebrates of the island. However, Trichoptera findings were extremely rare. Only one more species has added later in the Annals of Nature of the «Wrangel Island» Nature Reserve in 2013: *Limnephilus* sp. In the final species list, which summarized data for all the years of research,

only two species are included: *Micrasema gelidum* and *Limnephilus* sp., while *Lenarchus expansus* is removed from the list.

Recently, A.A. Novichkova (Department of Hydrobiology, Faculty of Biology, Moscow State University) transferred to us three larvae of limnephilid caddisflies collected in the island tundra lake of Tundra of the Academy. Below the description of the larvae is given and the results of attempts to associate them with the genera of the tribe Limnephilini are presented.

## **Materials and Methods**

Three larvae were collected in 2013 during the fieldwork of A.A. Novichkova in the period from June 18 to August 5, 2013 on the territory of the Federal State Budgetary Institution «Wrangel Island State Nature Reserve». She examined 72 water bodies: 23 streams, 2 marshes, 2 brackish lakes and 45 lakes of various sizes and (it is interesting to note) there were only 3 larvae caddisflies revealed among 100 collected samples. This confirms that Trichoptera are extremely rare on the island and represent a very small proportion in the freshwater communities.

For sampling of zoobenthos, scrapers were used, with the help of which the top layer of bottom sediments was sampled, which then was washed through a washing sieve, and obtained invertebrates were fixed with 96 % alcohol.

The material is deposited in the Laboratory of Freshwater Hydrobiology Federal Scientific Center of East Asia Terrestrial Biodiversity (Vladivostok) with collection number ICEM-301 and locality code RS001353 (according to East Russia Aquatic Invertebrate Database).

The photographs were taken using 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.

#### Description

The studied larvae are associated with the Limnephilini tribe (subfamily Limnephilinae) based on following characters: (1) location of the antenna approximately midway between the eye and the anterior edge of the head capsule, (2) the prosternal horn is present; (3) chloride epithelia is present, (4) mandibles toothed; (5) mesanotal plates not subdivided; (5) mesanotal setae are reduced in number, (6) primary setal areas are separated, (7) middle and hind femora have only two major setae on ventral edge, (8) setae on dorsal sclerite of segment IX are reduced in number, (9) most abdominal gills of dorsal and ventral series arise in clusters of three filaments according to the definition of the tribe by G.B. Wiggins [1996].

*Philarctus bergrothi* McLachlan, 1880 Figs 1–12.

*Material.* Chukotka, Wrangel Island, nameless lake, Tundra of the Academy, N71°21'57.7", W179°51'32.8", 30–32 m a.s.l., 12.VII.2013, stony bottom with large boulders,

water temperature 14 °C, pH 9, Field Sample No. 57.2, leg. A.A. Novichkova — 3 larvae of IV instar.

**Description.** Larva of IV instar. Body length 6.6 mm; case length 6.7 mm (Figs 1–3).

Head. Width 944 mkm (the widest), length 1037.5 mkm (from the frontoclypeal anterior edge to the posterior head capsule edge). Head brown dorsally, with three light blotches in narrow part of frontoclypeus (laterally and posteriorly), that is often occur in Asynarchus-Limnephilus (part)-Philarctus related genera group of the Limnephilini [Wiggins, 1996], and with darker dots (muscle scars) forming a central anchor-like (T-shaped) figure; the central portion of the «trend anchor» is not light (as in some Anabolia and Asynarchus) but the same color as lateral dark bands (as in some Asynarchus and *Philarctus*); laterally of frontoclypeus there are darker bands protruding from eye area to the mediooccipital portion with scattered dots distributed in parietal and genal areas (Fig. 4) head ventrally brown with darker brown areas along the head occipital margin and with some unclear expressed spots in genal area (Fig. 5). Surface of the head dorsally and ventrolaterally rough, covered with microbeads. Antenna positioned almost 1/2 the distance between anterior edge of parietal and eye. Ventral apotome is single, triangular and extends not reaching 1/7 to the occipital edge (Fig. 5), anterior portion of the ventral apotome 1/3 its total length, and posterior portion 3/4. Cardo plates are oval with length 1/4 shorter than width; length of cardo relatively of ventral apotome almost 1/2 of its total length; cardo with blackish medial edge, dark brown lateral portion, and lighter brown intermedial portion (Fig. 5). Mandible black with blackishbrown distal margin, apical edge with 4 teeth, a brush of light



Figs 1–3. Larva of *Philarctus bergrothi* McLachlan, 1880 (with the aquatic mites attached to the abdomen): 1 - in lateral view (1), 2 - in its case, in lateral view, 3 - in dorsal view.

Рис. 1–3. Личинка *Philarctus bergrothi* McLachlan, 1880 (с водными клещами, прикрепленными к брюшку): 1 — вид сбоку, 2 — в домике, вид сбоку, 3 — вид сверху.



Figs 4–12. Morphology of *Philarctus bergrothi* larva. 4 — head, dorsal view; 5 — the same, ventral view; 6 — thorax, dorsal view; 7 — anal prologs, dorsal view; 8 — the same, ventral view; 9 — outer view of foreleg; 10 — the same of midleg; 11 — the same of hind leg; 12 — left mandible, ventral view.

Рис. 4–12. Морфология личинки *Philarctus bergrothi*. 4 — головная капсула, вид сверху; 5 — то же, вид снизу; 6 — грудь, вид сверху; 7 — анальные ножки, вид сверху; 8 — вид снизу; 9 —наружная поверхность передней ноги; 10 — то же, средней ноги; 11 — то же, задней ноги; 12 — левая мандибула, вид снизу.

brown-yellow setae on ventral edge (Fig. 12), and 2 black setae on lateral edge. Labrum with a deep oval incision on anteromedial margin, the width of the incision at least 1/6 of total labrum width; with a pair of short pale brown setae at anterolateral margin (setae #2), another pale brown pair setae on anterior edge laterally the incision (setae #1), a pair pale brown setae posteriorly the incision (#4), and a transverse row of 3 pairs of brown to black setae at half the distance between anterior and posterior edges (setae #3, 5 and 6) (numbering of setae according S.G. Lepneva [1966].

Thorax. Pronotum brown, a transverse furrow separates the anterior 1/3 from a posterior 2/3 (Fig. 6); anterior part almost unicolourous light yellowish-brown, dark brown dots in blotches or bands occur on posterior 2/3; setation on pronotum in 2 transverse rows; about 15 setae from each side in anterior part of pronotum: a pair of black and very long (longest on pronotum) conspicuous setae positioned at anterior edge of each half of pronotum, about 3 pair of medium sized black setae at the pronotum anterior edge, other setae are smaller black or yellow; on posterior part there is 1 very long seta on each side close to the ecdysial suture, and on lateral edge 1 more long seta and about 6-7 smaller ones; prosternal horn long and slender, extended ventrally to distal edge of head capsule; mesonotum (Fig. 6) brown darker blotches or bands of dots; following the G.B. Wiggins terminology [Wiggins, 1996] sa 1 has 2 setae (posterior setae longer than anterior in 3.5 times), sa 25, and sa 38–12 setae; metanotum (Fig. 6) with 3 pairs of sclerotized plates with setae, the number of setae on sa 1 is 2 (plus 1 inconspicuous seta laterally of sa 1 sclerite), on sa 2 5-6, and on sa 3 10 setae.

Legs. First pair of legs shortest, second and third pairs about equal in length (Figs 9-11). First coxa outer surface anteriorly with 1 long black, and 6 short ones, posteriorly with 2 long and 1 shorter setae; ventral and inner surface with 10-12 setae scattered on the coxa surface, and with 2 setae at distal edge. Trochanter divided in 2 parts: basal and distal portions, with no setae on inner part; 3 black setae at, or close to, lateral edge on outer part of trochanter; 1 short seta close to distal edge trochanter, and a brush of yellow microsetae distoventrally. Femur, on its ventral portion with 2 yellow setae and a row (comb) of small microsetae, 4 black setae dorsally, 1 seta posteriorly on outer face; and 4 setae on dorsal edge. Tibia almost twice shorter than femur, with a row of small microsetae (comb) on part of ventral edge; distally 2 yellow tibial spurs, and 1 pale, 2 black setae dorsally. Tarsus with 2 rows of yellow setae on the entire length of ventral side; distally 2 small pale setae ventrally and 1 black dorsally; basal seta on tarsal claw shorter than the claws on all 3 pairs of legs. Second pair of legs with 2 long and 5 shorter black setae on distal edge of coxa (towards trochanter), on outer half of dorsal and anterolateral area are 14-15 black setae, and a variable number on dorsal edge towards trochanter; inner trochanter without setae; basal portion of trochanter with 1 black seta on the edge; distal portion of trochanter with 4-5 black setae scattered ventrally and with 2 yellow setae distally on ventral edge, a little brush of small yellow setae distally on ventral edge; femur with a row of small yellow setae (comb) along the entire length of its ventral face, the comb curving to dorsal side distally; 4 small accessory setae on the inner (anterior) surface, 3 on the dorsal edge, and 1 long seta distally; 3 on the ventral edge. Tibia with a row of small yellow setae on outer 2/3 of ventral edge; distally 2 yellow tibial spurs on ventral edge, 1 anterolateral and 4 black setae dorsally. Tarsus with a row of small setae; distally 1 dorsal long black seta. Third pair of legs have 16Table 1. Gill position of IV larval instar of Philarctusbergrothi

Таблица 1. Расположение жабр у личинки IV возраста *Philarctus bergrothi* 

Abdominal segments	Dorsal	Lateral		Ventral
2	2 3	2	2	2 3
3	3 3	2	2	2 3
4	3 1	1		2 2
5	1 1			2 2
6				1 1

18 black setae on dorsal portion of coxa and 18 setae scattered on ventral portion. No setae on central area of inner trochanter. On outer trochanter 6 black and 1 yellow setae on ventral edge; Gistally the row curves to dorsal side on anterolateral face; with 4 black dorsal setae and 3 ventral, outer (posterior) surface with 2 small accessory setae, inner (anterior) surface with 2–3 small setae. Tibia with a row of small yellow setae on ventral edge; distally, 2 yellow tibial spurs ventrally, and 4 black setae dorsally. Tarsus with a row of small, yellow setae on ventral edge; distally 2 dorsal and 2 ventrolateral setae. Basal seta on claw much shorter than claw.

Abdomen. Dorsal and lateral humps present on segment I, dorsally 3 setae in *sa 1* position, 3 *sa 2*, and 3 at *sa 3* (at lateral humps); on ventral side 6 setae at *sa 1* position; the *sa 2* on each half are fused together and contain 3 setae, and at base of each upper seta surrounded with light brown circle; *sa 3* includes 3 setae dorsally of hump and 2 setae ventrally on hump; the microsculpture of the lateral hump surface is tiny beaded.

Dorsally segments II-VII with a pair mediolateral long thin black setae, and a pair short lateral setae. Gills present on abdominal segments II-VI. Numbers of single gill filaments and their position are given in Tabl. 1. Lateral fringe present on segments III-VIII. Lateral tubercles present on segments III-VIII. Chloride epithelia present ventrally as wide and long plates occupying almost all ventral portion of segments; dorsally and laterally absent. Posteriorly and dorsally on segment VIII a transverse band of setae with 4 present on each side of longitudinal centre line of abdomen, 4 setae ventrally (Fig. 8). Dorsal sclerite (Fig. 7) on segment IX with 4 major setae and 8 smaller setae in between, and with 4 setae (2 short and 2 longer) between central major setae. Lateral sclerites of anal prolegs bear 4 long setae each at the distal edge, 5 short setae scattered on dorsal portion of each lateral sclerite; one short seta laterally at the base of each ventral sole plate; each sole plates with 1 seta; anal claw with 3 black setae dorsally, and 2 yellow setae ventrolaterally; each claw with 1 accessory hook.

*Case.* Length 6.8 mm, straight, round in cross section, made of plant pieces connected by a silk secret (Fig. 2).

#### Discussion

Wrangel Island is located on 71 °N off the coast of northeastern Eurasia, it is bounded by the Arctic Ocean, the East Siberian Sea and the Chukchi Sea. Its total area is about 7 600 km<sup>2</sup>. The dominating landscape is Arctic tundra. It is included in a Zone B of the Circumpolar Arctic Region Bioclimatic Zones, which is characterized by the average July temperature 3-5 °C [Walker et al., 2002]. There are many lakes, ponds and swamps, especially in the north-eastern part the western part are mountainous with numerous streams and rivers. The aquatic ecosystems are home to many aquatic invertebrates; however, caddisflies are extremely rare. The severe conditions of survival were a natural boundary for the penetration of species not adapted to life in the High Arctic environment; also, the island position played a role of natural boundaries. Nevertheless, several tens of aquatic invertebrates (especially dipterans of Chironomidae) have recorded on the island, with a few very rare caddisflies belonging to two families: Brachycentridae and Limnephilidae.

The family Limnephilidae is widely distributed in northern latitudes and therefore named Northern Casemaker Caddisflies. The genera of tribe Limnephilini to which definitely belong the larvae are shredders; they use plant material, in some cases rocks, for case building and inhabit lentic habitats [Wiggins, 1996].

The identification of many limnephilid larvae is difficult, especially of a tribe Limnephilini. These difficulties caused the lack of descriptions of larval stages of many species, the uncertainty of the diagnosis of genera of the Limnephilini tribe, especially Limnephilus, which is considered polytypical and includes a number of species that apparently do not belong to it [Vshivkova, 2003; Vshivkova et al., 2007]. There are some keys adapted to the fauna of large regions or local faunas that allow the basic differentiation of limnephilid larvae to genera or species [Hiley, 1976; Wiggins, 1996; Ivanov et al., 2001; Wallace et al., 2003; Ruiter et al., 2013, Waringer, Graf, 2013; Rinne, Wiberg-Larsen, 2017, and others]. However, the general reliable key to larvae, which includes all the world genera of Limnephilidae, is absent, that often limits the definition of immature stages.

The head pattern of limnephilid larvae is often used as an important diagnostic feature. The head pattern of larvae we studied are similar to the heads of genera Anabolia, Asynarchus, Limnephilus (part), Philarctus, and Clistoronia, and can help to distinguish this complex closely related genera. However, as G.B. Wiggins [1996] pointed out, this character working not well to separate the larvae of the mentioned genera. We have made attempts to link the studied larvae with the genera of the specified complex, gradually excluding genera with obviously unsuitable characters. We excluded *Clistoronia* by the character of presence of accessory setae on the mesofemur lateral surfaces (they lacking in Clistoronia). Anabolia and Asynarchus were excluded by the character of lacking in studied larvae of dorsal chloride epithelia (present in both noted genera). They can be separated from true Limnephilus (s.str.) based on possessing in studied larvae the mesofemur accessory setae (absent in true Limnephilus) which similarly present in Philarctus; and by the presence of accessory setae on the basal

mesotrochanter segment (absent in true *Limnephilus*), but similarly present in *Philarctus*. Thus, the studied larvae could be associated with *Philarctus* or some *«Limnephilus» sensu lato* for which larvae are not known. In *Philarctus*, as noted G.B. Wiggins [1963, 1996], the case is often constructed of mollusks, although, immature specimens may use small vegetation pieces, switching to mineral or other material prior to pupation [Ruiter et al., 2013]. The body of the studied larvae is completely made of pieces of plants connected by a silk secret. We must take into account that the case of a larva V instar may differ from the case of the previous stage. Also, it should be borne in mind that on Wrangel Island freshwater mollusks are practically absent [Vinarski et al., 2015].

Comparison of our IV instar larvae with the description of *Ph. bergrothi* of V instar larva made by W. Mey [1982] (larvae collected in Mongolia, Bajan-Ölgij Aimal: 49°N, 89°E, 15.VII.1975), in general, coincide. The difference is noted in the number of gill filaments on the abdominal segments, which, however, may be due to the younger age of the larvae from Wrangel Island.

This species has been recorded in areas north of the Arctic Circle (runs 66°33'49" north of the Equator). It was recently observed also at latitude 69° N, on the island of Vaigach, Khalamerto Lake (collected in the shallow lake with silty-sand and gravel bottom substrates vegetated by mosses and higher macrophytes; the average depth of sampling was  $0.28 \pm 0.17$  m) [Bespalaya et al., 2021]. The Ph. bergrothi (= Ph. quaerius (Milne, 1935) [Morse, 2009] is widely distributed in East Palaearctic and Nearctic [Levanidova et al., 1995; Wiggins, 1963; Wiggins, Parker 1997; Ivanov, 2011; Rinella et al., 2012; Kendrick, Huryn, 2014; Zasypkina, 2016; Morse, 2009, and others]. In the Western Palearctic, the species is limited in the continental part of temperate latitudes [Malicky, 2005], and recently recorded on the arctic island of the West Russia [Bespalaya et al., 2021]. Thus, the findings of Ph. bergrothi from Wrangel Island are obviously the northernmost finds of this species.

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