New records of moths (Lepidoptera) from Novaya Zemlya, Arctic Russia, with a supplement of DNA barcoding data

Новые находки чешуекрылых (Lepidoptera) с архипелага Новая Земля с данными ДНК-баркодинга

Vitaly M. Spitsyn*, Alexander V. Kondakov, Alena A. Tomilova, Elizaveta A. Spitsyna, Grigory S. Potapov

В.М. Спицын, А.В. Кондаков, А.А. Томилова, Е.А. Спицына, Г.С. Потапов

ABSTRACT. This paper reports the first records of four moth species from Novaya Zemlya: Eupithecia gelidata, Rheumaptera subhastata, Entephria byssata (Geometridae), and Udea cf. cacuminicola (Crambidae). The COI barcode data is provided for each species. Molecular sequences suggest that the Lepidoptera fauna of Novaya Zemlya was originated recently via long-distance dispersal of widespread lineages from the mainland.

Introduction

Novaya Zemlya is one of the most inaccessible archipelagoes of the High Arctic [Potapov et al., 2018]. As a result, its fauna remains poorly known. The Lepidoptera fauna of Novaya Zemlya includes 30 species [Jacobson, 1898; Rebel, 1923; Karsholt et al., 2013; Kullberg et al., 2018], whereas 4–5 species are recorded from neighboring areas, i.e. Yugorsky Peninsula and Kolguev Island [Kullberg et al., 2013, 2018].

In this paper, we present the first records of four moth species from Novaya Zemlya and discuss the putative origin of this insular fauna by means of a molecular approach.

Material and Methods

Moths were collected with an entomological net near the Bezymyannaya Bay (Novaya Zemlya, Yuzhny Island) from July 19 to July 26, 2017. Specimens were prepared using standard methods [Schauff, 2001]. They are deposited in the Russian Museum of Biodiversity Hotspots (RMBH), N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Arkhangelsk, Russia).

Total DNA was extracted from a single leg of each dry specimen according to standard phenol/chloroform procedures [Sambrook et al., 1989]. The mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified and sequenced using primers LCO1490 [Folmer et al., 1994] and LepR [Hajibabaei et al., 2006]. The PCR mix contained approximately 200 ng of total cell DNA, 10 pmol of each primer, 200 nmol of each dNTP, 2.5 l of PCR buffer (with 20 mmol MgCl₂), 0.8 units Taq DNA polymerase (SibEnzyme Ltd., Russia), and H₂O was added for a final volume of 25 l. Temperature cycling
was as follows: 95°C (5 min), 30–33 cycles of 95°C (50 sec), 48°C (50 sec), 72°C (50 sec) and a final extension at 72°C (5 min). The sequencing was carried out at the facilities of the Inter-Institution Center of Group Use (Genom) (Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences, Moscow) using the ABI PRISM® BigDye™ Terminator v. 3.1 reagents kit. Reaction products were analyzed using an automatic sequencer ABI PRISM® 3730 (Applied Biosystems).

We obtained new COI sequences from 12 specimens (Table 1). The resulting sequences were checked manually using a sequence alignment editor BioEdit v. 7.2.5 [Hall, 1999].

Results

Here we present the first records of four species of Lepidoptera for the Novaya Zemlya Archipelago with a supplement of the DNA barcoding data. In summary, 34 Lepidoptera species are recorded from Novaya Zemlya [Kullberg et al., 2018; this study].

Crambidae

*Udea cf. cacuminicola* Munroe, 1966

**Fig. 1.**

**MATERIAL EXAMINED.** Russia, Novaya Zemlya, Yuzhny Island, near the Bezymyannaya Bay, 72°49’09”N, 53°47’33”E, 21.XII.2017, Spitsyn leg. — 1 ex.

**DNA BARCODING.** We sequenced a specimen from Novaya Zemlya having a widespread haplotype that is known from Greenland to Norway and Italy.

**Rheumaptera subhastata** (Nolcken, 1870)

**Fig. 3.**

**MATERIAL EXAMINED.** Russia, Novaya Zemlya, Yuzhny Island, near the Bezymyannaya Bay, 72°49’09”N, 53°47’33”E, 21.XII.2017, Spitsyn leg. — 1 ex.

**DNA BARCODING.** A sequence from Novaya Zemlya differs by one substitution from the COI haplotype common in Canada and by 2–3 substitutions from the series of the nearest haplotypes from Norway, Finland, Sweden, Austria, Germany, Canada, and the USA.

**Entephria byssata** (Aurivillius, 1891)

**Fig. 4.**

**MATERIAL EXAMINED.** Russia, Novaya Zemlya, Yuzhny Island, near the Bezymyannaya Bay, 72°49’09”N, 53°47’33”E, 21.XII.2017, Spitsyn leg. — 7 ex; tundra with *Astragalus alpinus*, 72°51’10”N, 53°42’48”E, 23.XII.2017, Spitsyn leg. — 3 ex; tundra with *Hedysarum arcticum*, 72°52’41”N, 53°37’49”E, 23.XII.2017, Spitsyn leg. — 7 ex; associations with *Astragalus alpinus* on the

| Table 1. List of new COI sequences for moths obtained under this study. |
|-------------------------------------------------|
| **Species** | **NCBI's GenBank acc. no.** | **Voucher no.** | **Specimen locality** |
| *Udea cf. cacuminicola* | MN700905 | Sph 705 | Russia: Novaya Zemlya |
| *Udea cf. cacuminicola* | MN700906 | Sph 706 | Russia: Novaya Zemlya |
| *Eupithecia gelidata* | MN700907 | Sph 707 | Russia: Novaya Zemlya |
| *Rheumaptera subhastata* | MN700908 | Sph 708 | Russia: Novaya Zemlya |
| *Entephria byssata* | MN700909 | Sph 709 | Russia: Novaya Zemlya |
| *E. byssata* | MN700910 | Sph 710 | Russia: Novaya Zemlya |
| *E. byssata* | MN700911 | Sph 711 | Russia: Novaya Zemlya |
| *E. byssata* | MN700912 | Sph 712 | Russia: Novaya Zemlya |
| *E. byssata* | MN700913 | Sph 713 | Russia: Novaya Zemlya |
| *E. byssata* | MN700914 | Sph 714 | Russia: Novaya Zemlya |
| *E. byssata* | MN700915 | Sph 715 | Russia: Novaya Zemlya |
| *E. byssata* | MN700916 | Sph 716 | Russia: Novaya Zemlya |
canyon slope, 72°50’15’’N, 53°22’41’’E, 23.XII.2017, Spitsyn leg. — 1 ex; tundra with *Hedysarum arcticum*, 72°48’36’’N, 53°50’24’’E, 23.XII.2017, Spitsyn leg. — 1 ex.

**DNA BARCODING.** We identified three haplotypes of this species on Novaya Zemlya, two of which are found in Scandinavia.

**REMARK.** Previously, this species (as *Entephria punctipes*) was listed in the fauna of the Novaya Zemlya Archipelago based on an incorrectly identified specimen of *Psychophora sabini* [Sumakow, 1912], and it was subsequently excluded from the list of the Lepidoptera species of Novaya Zemlya [Kullberg et al., 2018]. *Entephria punctipes* is considered as the Nearctic taxon at the present time [Aarvik et al., 2017]. Here, we present the first record of *Entephria byssata* from Novaya Zemlya that was confirmed by morphological characters and DNA barcoding.

**Discussion**

We can conclude that the studied species of Lepidoptera immigrated to Novaya Zemlya after the Last Glacial Maximum. Most of the species share widespread haplotypes or singletons. Similar biogeographic patterns are common for a number of other Lepidoptera in the Northern Palearctic [Bolotov et al., 2015; Spitsyn et al., 2020]. Studies of other animal groups of the fauna of Novaya Zemlya revealed that a number of taxa have endemic haplotypes and divergent lineages from this archipelago [Potapov et al., 2018; Makhrv et al., 2019]. Novaya Zemlya was suggested as a putative refugium for these groups [Potapov et al., 2018; Makhrv et al., 2019]. However, other taxa are represented by widespread haplotypes, e.g. reindeer [Kvie et al., 2016] and moths [this study].

**Acknowledgements.** The authors send their sincere gratitude to Dr. Elena Churakova for her help in preparing the paper. This study was partially supported by the Ministry of Science and Higher Education of Russia (Project No. AAAA-A17-11703301032-2) and by the Russian Foundation for Basic Research (Projects No. 19-34-50016 and No. 19-34-90012).

**Competing interests.** The authors declare no competing interests.

**References**

Aarvik L., Bengtsson B.A., Elven H., Ivinskis P., Jürivete U., Karsholt O., Mutanen M., Savenkov N. 2017. Nordic-Baltic Checklist of Lepidoptera // Norw. J. Entomol. Suppl.3. P.1–236.

Bolotov I.N., Tatarinov A.G., Filippov B.Y., Gofarov M.Y., Kondakov A.V., Kulakova O.I., Potapov G.S., Zubryi N.A., Spitsyn V.M. 2015. The distribution and biology of *Pararctia subnubulosa* (Dyar, 1899) (Lepidoptera: Erebidae: Arctiinae), the largest tiger moth species in the High Arctic // Polar Biol. Vol.38. P.905–911. doi: 10.1007/s00300-014-1643-2

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 // Mol. Marine Biol. Biotechnol. Vol.3. P.294–299.

Hajibabaei M., Janzen D.H., Burns J.M., Hallwachs W., Hebert P.D. 2006. DNA barcodes distinguish species of tropical Lepidoptera // Proc. Natl. Acad. Sci. USA. Vol.103. No.4. P.968–971.

**Fig. 1–4.** Moth species from Novaya Zemlya: 1 — *Udea cf. cacuminicola* Munroe, 1966; 2 — *Eupithecia gelidata* Möschler, 1860; 3 — *Rheumaptera subhastata* (Nolcken, 1870); 4 — *Entephria byssata* (Aurivillius, 1891). Scale bar = 10 mm. (Photos: Vitaly M. Spitsyn).
New records of Lepidoptera from Novaya Zemlya

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis. Department of Microbiology, North Carolina State University.

Jacobson G.G. 1898. [Zoological investigation on Novaya Zemlya in 1896. The insects of Novaya Zemlya] // Mémoires de l’Académie des sciences de St.-Pétersbourg. Classe des sciences physiques et mathématiques. VIIe série. Vol.8. P.171–244 [in Russian].

Karsholt O., Nieukerken E.J. van. 2013. Fauna Europaea: Lepidoptera, moths. Fauna Europaea version 2017.06, https://fauna-eu.org. Accessed 4 April 2018.

Kullberg J., Filipppov B.Y., Zubrii N.A., Kozlov M.V. 2018. Moths and butterflies (Insecta: Lepidoptera) of the Russian Arctic islands in the Barents Sea // Polar Biol. P.1–12. doi:10.1007/s00300-018-2425-z

Kullberg J., Filipppov B.Y., Zubrii N.A., Kozlov M.V. 2013. Faunistic notes on Lepidoptera collected from arctic tundra in European Russia // Nota lepid. Vol.36. No.2. P.127–136.

Kvie K.S., Heggenes J., Anderson D.G., Kholodova M.V., Sipko T., Mizin I., Roed K.H. 2016. Colonizing the High Arctic: Mitochondrial DNA Reveals Common Origin of Eurasian Archipelagic Reindeer (Rangifer tarandus) // PLoS ONE. Vol.11. No.11. P.1–15. doi:10.1371/journal.pone.0165237

Makhrov A.A., Bolotov I.N., Spitsyn V.M., Gofarov M.Yu., Artamonova V.S. 2019. Resident and Anadromous Forms of Arctic Charr (Salvelinus alpinus) from North-East Europe: An Example of High Ecological Variability without Speciation // Dokl. Biochem. Biophys. Vol.485. P.119–122.

Potapov G.S., Kondakov A.V., Spitsyn V.M., Filippov B.Yu., Kolesova Yu.S., Zubrii N.A., Bolotov I.N. 2018. An integrative taxonomic approach confirms the valid status of Bombus glacialis, an endemic bumblebee species of the High Arctic // Polar Biol. Vol.41. No.4. P.629–642. doi:10.1007/s00300-017-2224-y

Rebel H. 1923. Lepidoptera von Novaja Semlja // Report of the Scientific Results of the Norwegian Expedition to Novaya Zemlya. No.7. P.1–15.

Sambrook J., Fritsch E.F., Maniatis T. 1989. Molecular Cloning: A Laboratory Manual, (2nd ed.). (pp. 10.51–10.67). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Schauff M.E. (ed.). 2001. Collecting and preserving insects and mites: techniques and tools. Systematic Entomology Laboratory, National Museum of Natural History, USDA, Washington, DC, 68 pp.

Spitsyn V.M., Berezin M.V., Kondakov A.V., Khruleva O.A., Tomilova A.A., Bolotov I.N. 2020. [Assessment of endemism level of Papilionoidea from Wrangel Island] // Arkticheskie issledovaniya: ot ekstensivnogo osvoeniya k kompleksnomu razvitiyu. Materialy II mezhdunarodnoi nauchno-prakticheskoi konferentsii. Arkhangelsk. P.442–444 [in Russian].

Sumakov G.G. 1912. [Beiträge zur Fauna der Insecten von Nowaja Semlja] // Sitzungsberichte der Naturforscher-Gesellschaft bei der Universität Jurjew (Dorpat). Vol.21. P.98–102 [in Russian].