Taxonomic Position and Status of Arctic Gynaephora and Dicallomera Moths (Lepidoptera, Erebidae, Lymantriinae)*

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We use analysis of mitochondrial DNA barcodes in combination with published data on morphology to rearrange the taxonomy of two arctic species, Gynaephora groenlandica and G. rossii. We demonstrate that (1) the taxon lugens Kozhanchikov, 1948 originally described as a distinct species is a subspecies of Gynaephora rossii, and (2) the taxon kusnezovi Lukhtanov et Khruliiova, 1989 originally described as a distinct species in the genus Dicallomera is a subspecies of Gynaephora groenlandica. We also provide the first evidence for the occurrence of G. groenlandica in the Palearctic region (Wrangel Island).

Key words: COI, DNA barcode, Gynaephora, Dicallomera, Lymantriinae, polar environments.

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The genera Gynaephora Hübnner, 1819 and Dicallomera Butler, 1881 belong to the subfamily Lymantriinae of the family Erebidae (ZAHIRI et al. 2012). These genera are closely related to each other and are characterized by several similarities in wing venation and genitalia structure (TROFIMOVA 2008). The genus Gynaephora was revised by SPITZER (1984) and TROFIMOVA (2008). It includes several species distributed across the Holarctic region. The precise counting of the species number in this genus is complicated because of unclear status of some described taxa (TROFIMOVA 2008) and unclear position of Lachana Moore, 1888, a central Asian group which is considered as a part of Gynaephora (SPITZER 1984) or as a distinct genus (TROFIMOVA 2008). The genus Dicallomera was revised by TROFIMOVA (1984). It includes six species distributed only in the Palearctic region (TROFIMOVA 2008). Three representatives of Gynaephora, G. groenlandica (Wocke, 1874), G. rossii (Curtis, 1835) and G. lugens Kozhanchikov, 1948, and one representative of Dicallomera (D. kusnezovi Lukhtanov et Khruliiova, 1989) are known to be high arctic species inhabiting tundra biotopes (KOZHANCHIKOV 1950; LUKHTANOV et KHRULEVA 1989). Of these arctic taxa, two species (G. groenlandica and G. rossii) are relatively well studied with respect to taxonomy (FERGUSON 1978; BARRIO et al. 2013) and ecology (DANKS 2004). Currently, they became model systems in numerous studies of adaptations to polar environments (STRATHDEE & BALE 1998; BENNETT et al. 1999, 2003; RYDELL et al. 2000; LEVIN et al. 2003; DANKS 2004; BARRIO et al. 2015). Much less is known about two other arctic taxa, D. kusnezovi and G. lugens.

Gynaephora lugens differs from the morphologically very similar G. rossii by a more contrasting wing pattern (KOZHANCHIKOV 1950). These two taxa are allopatric in their distribution ranges (KOZHANCHIKOV 1950) and therefore, in our

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opinion, can be interpreted as subspecies or only local forms of the same species.

The nominal species *D. kusnezovi* possesses male genitalia structure very similar to the genitalia structure of *D. fascelina* (Linnaeus, 1758) (LUKHTANOV & KHRULIOVA 1989), the type species of the genus *Dicallomera*, but distinctly different from genitalia of *G. selenitica* (Esper, 1789) (KOZHANICHIKOV 1950), the type-species of the genus *Gynaephora*. Therefore, in the original description (LUKHTANOV & KHRULIOVA 1989) we compared *Dicallomera kusnezovi* with other taxa of the genus *Dicallomera*, but not with *Gynaephora*. Unfortunately, we did not recognize that the taxon *G. groenlandica* has male genitalia structure (FERGUSON 1978) typical for *Dicallomera* and that the conspecificity of *D. kusnezovi* and *G. groenlandica* cannot be excluded.

Here we use analysis of mitochondrial DNA barcodes in combination with published data on morphology (KOZHANICHIKOV 1950; FERGUSON 1978; LUKHTANOV & KHRULIOVA 1989) in order to test the hypotheses on the conspecificity of two pairs of taxa, *G. rossii* – *G. lugens* and *G. groenlandica* – *D. kusnezovi*.

### Material and Methods

The samples used for molecular analysis were collected in polar north-east Russia (Wrangel Island) by O.A.Khruleva (Somnitenaya, 70°58’N, 179°36’W, 25 June 2006: CCDB-17968_A01, CCDB-17968_A02, CCDB-17968_A03, CCDB-17968_A04; Mamon-tovaya, 71°10’N, 179°45’W, 7 August 2006: CCDB-17968_A05; 5 July 2006: CCDB-17968_A06).

We studied standard COI barcodes (658-bp 5’ segment of mitochondrial cytochrome oxidase subunit I). DNA was extracted from a single leg removed from voucher specimens (samples CCDB-17968_A01, CCDB-17968_A02, CCDB-17968_A03 and CCDB-17968_A04) or from total larvae (samples CCDB-17968_A05 and CCDB-17968_A06) employing a standard DNA barcode glass fibre protocol (IVANOVA et al. 2006). All polymerase chain reactions and DNA sequencing were carried out following standard DNA barcoding procedures for Lepidoptera as described previously (DEWAARD et al. 2008). Photographs of specimens used in the analysis and collecting data are available in the Barcode of Life Data System (BOLD) at http://www.barcodinglife.org/. All voucher specimens are deposited in the Zoological Institute of the Russian Academy of Sciences (St. Petersburg) and are identified with the corresponding unique BOLD Process IDs, which are automatically generated by BOLD at the time of the initial data submission.

For comparison we used published data on COI sequences of *Gynaephora*, *Dicallomera*, *Lachana* and *Olene* (HAUSMANN et al. 2011; MILLER et al. 2013; HUEMER et al. 2014; ZAHRI et al. 2014; YUAN et al. 2015).

The methods of phylogenetic inference were described in details previously (LUKHTANOV et al. 2008, 2014, 2015a; TALAVERA et al. 2013; PRZYBYLOWICZ et al. 2014; LUKHTANOV & TIKHONOV 2015). Briefly, sequences were aligned using BioEdit version 7.1.7 software (HALL 1999) and edited manually. Phylogenetic relationships were inferred using Bayesian Inference and the program MrBayes 3.2.2 (RONQUIST 2012). A GTR substitution model with gamma distributed rate variation across sites and a proportion of invariant sites was specified before running the program as suggested by jModelTest (POSADA 2008). Two runs of 10 000 000 generations with four chains (one cold and three heated) were performed. Chains were sampled every 1000 generations, and burn-in was determined based on inspection of log likelihood over time plots using TRACER, version 1.4 (available from http://beast.bio.ed.ac.uk/Tracer).

### Results and Discussion

The analysis revealed five major groups of the COI barcodes (Fig. 1). All these groups were strongly supported (posterior probability from 0.94 to 1.00). The first group included the species (*G. ruoergensis*, *G. aureata*, *G. minora*, *G. jiuzhiensis*, *G. qumalaiensis*, *G. menyuanensis*, *G. qinghaisiensis* and *Lachana alpherakii*) that have been sometimes (e.g. TROFIMOVA 2008) considered as members of the genus *Lachana*. The second group included barcodes of two nominal species, *G. enlandica* and *D. kusnezovi*. The third group included barcodes of *D. fascelina*. The fourth group included barcodes of *G. rossii* and *G. lugens*. The fifth group included barcodes of *G. selenitica*.

DNA barcode analysis demonstrated that the taxon previously described by us as *D. kusnezovi* (LUKHTANOV & KHRULIOVA 1989) constituted a separate, well supported cluster on the COI tree (Fig. 1). However, the uncorrected p-distance between individuals from Wrangel Island (*D. kusnezovi*) and America (*G. groenlandica*) was relatively small (p = 0.6%, 4 fixed nucleotide substitutions in 658 bp fragment), much lower than the ‘standard’ 2.7-3.0% DNA-barcoding threshold usually used for allopatric taxa as an indicator for their species distinctness (LAMBERT et al. 2005; LUKHTANOV et al. 2015b).

Morphologically, the moths of *D. kusnezovi* from Wrangle Island (Palearctic region) and
G. groenlandica (Nearctic region) are practically identical with respect to wing pattern and genitalia structure as already mentioned in the Introduction (see also figures of in public BOLD database: http://www.boldsystems.org/index.php/Tax-browse_taxonpage?taxid=646969; http://www.boldsystems.org/index.php/Tax-browse_taxonpage?taxon=Gynaephora_groenlandica&searchTax=). Therefore, here we downgrade the status of the taxon kusnezovi and consider it as a subspecies: Gynaephora groenlandica kusnezovi (Lukhtanov et Khruliova, 1989), comb. et stat. nov. Gynaephora groenlandica was known until now only from Neartic region where it was presented by two subspecies: G. g. groenlandica (Wocke, 1874) and G. g. beringiana Schmidt et Cannings, 2013 (BARRIO et al. 2013). The discovery of this species on Wrangel Island provides the first evidence for the occurrence of G. groenlandica in the Palearctic region.

Similarly, we use a comparison between the samples of the taxa of G. lugens from Wrangle Island (Palearctic region) and G. rossii (Nearctic region) (Fig. 1) and the same argumentation (relatively low genetic distance: p = 1.4%, 9 fixed nucleotide substitutions in 658 bp fragment, morphological similarity described in the Introduction and allopatry) in order to downgrade the status of the taxon lugens and consider it as a subspecies: Gynaephora rossii lugens Kozhanchikov, 1948), stat. nov.

It is remarkable that with respect to COI barcodes (Fig. 1), G. groenlandica is similar to D. fascelina (Linnaeus, 1758), the type-species of the genus Dicallomera. This finding is in good correspondence with the fact that G. groenlandica kus-
nezovi is similar to D. fascelina with respect to male genitalia structure (LUKHTANOV & KHRULIOVA 1989). In fact, this morphological similarity was the reason why the taxon kuznezovi was described earlier by us in the genus Dicallomera and not recognized as a possible conspecific with G. groenlandica.

It should be noted that COI barcodes alone can provide weak evidence for species distinctness, species conspecificity or species non-conspecificity since trees inferred from single markers sometimes display relationships that reflect the evolutionary histories of individual genes rather than the species being studied (NIChOLS 2001). Mitochondrial introgression (ZAKHAROV et al.; 2009) and Wolbachia infection (RITTER et al. 2013) can lead to additional bias in inferring taxonomic conclusions based on mitochondrial genes. However, in our case we have taxonomic hypotheses (formulated in the Introduction) based on morphology. We believe that congruence between morphological and molecular mitochondrial data represents better support for these hypotheses than morphological data alone.

Currently Dicallomera is considered a valid genus close to Gynaephora (TROFIMOVA 2008). Therefore, it would seem logical to transfer the species groenlandica from Gynaephora to Dicallomera. However, considering Dicallomera as a valid genus would result in Gynaephora as a paraphyletic taxon in our COI based tree (Fig. 1). Therefore, we prefer to treat both groenlandica and rossii as members of the genus Gynaephora sensu lato until a comprehensive revision of this group based on analysis of multiple genes and morphology reveals the real phylogenetic relationships and composition of the genera Gynaephora, Dicallomera and Lachana.

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References


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