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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 Khruliova, 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* Hübner, 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 Khruliova, 1989) are known to be high arctic species inhabiting tundra biotopes (KOZHANCHIKOV 1950; LUKHTANOV & KHRULIOVA 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) (KOZHANCHIKOV 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. groenlandic*a cannot be excluded.

Here we use analysis of mitochondrial DNA barcodes in combination with published data on morphology (KOZHANCHIKOV 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 (Somnitelnaya, 70°58'N, 179°36'W, 25 June 2006: CCDB-17968_A01, CCDB-17968_A02, CCDB-17968_A03, CCDB-17968_A04; Mamontovaya, 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; ZAHIRI *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; PRZYBYŁOWICZ *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 invariable 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. qinghaiensis* 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. groenlandica* 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

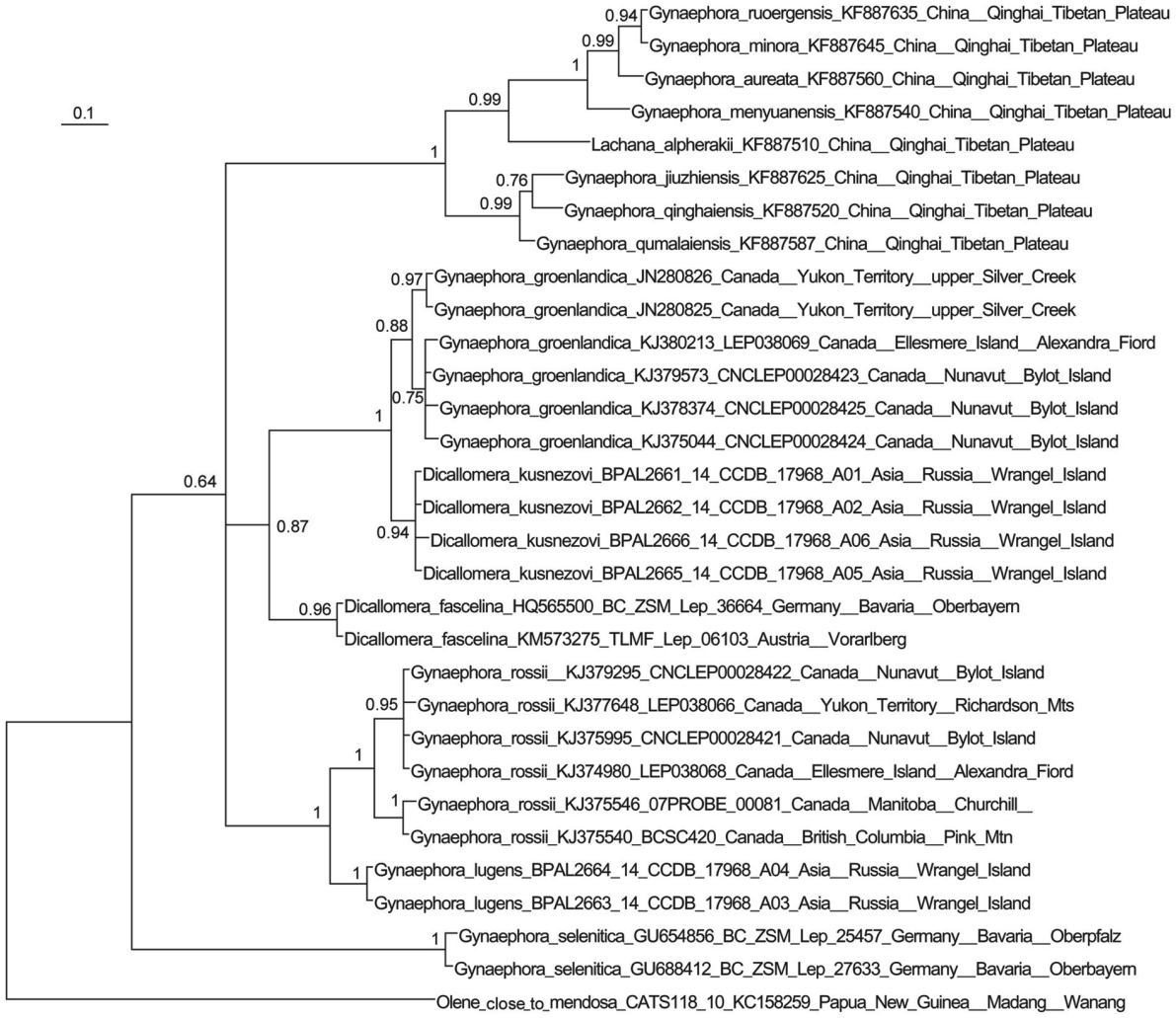

Fig. 1. Bayesian tree of *Gynaephora* and *Dicallomera* taxa based on analysis of *COI* DNA barcodes. Numbers at nodes indicate Bayesian posterior probability values. The samples JN280825 and JN280826 represent the subspecies *G. groenlandica beringiana* Schmidt et Cannings, 2013. The samples KJ380213, KJ 379573, KJ378374 and KJ 375044 represent the subspecies *G. groenlandica groenlandica* (Wocke, 1874). Scale bar = 0.1 substitutions per position.

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/Taxbrowser_Taxonpage?taxid=646969; http://www.boldsystems.org/index.php/Taxbrowser_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 Nearctic 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 conspecifity or species non-conspecifity 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

BARRIO I.C., SCHMIDT B.C., CANNINGS S., HIK D.S. 2013. First records of the Arctic moth *Gynaephora groenlandica* (Wocke) south of the arctic circle: a new alpine subspecies. Arctic **66**: 429-434

BARRIO I.C., HIK D.S., LIU J.Y. 2015. Diet breadth of *Gynaephora groenlandica* (Lepidoptera: Erebidae): is polyphagy greater in alpine versus Arctic populations? Can. Entomol. **147**: 215-221.

BENNETT V.A., KUKAL O., LEE R E. 1999. Metabolic opportunists: feeding and temperature influence the rate and pattern of respiration in the high arctic woollybear caterpillar *Gynaephora groenlandica* (Lymantriidae). J. Exp. Biol. **202**: 47-53.

BENNETT V.A., LEE R.E., NAUMAN J.S., KUKAL O. 2003. Selection of overwintering microhabitats used by the Arctic woollybear caterpillar, *Gynaephora groenlandica*. Cryoletters **24**: 191-200.

DANKS H.V. 2004. Seasonal adaptations in arctic insects. Integr. Comp. Biol. **44**: 85-94.

DEWAARD J.R., IVANOVA N.V., HAJIBABAEI M., HEBERT P.D.N. 2008. Assembling DNA barcodes: analytical protocols. (In: Environmental Genomics, Methods in Molecular Biology. Vol. 410. C.C. MARTIN ed. Humana Press, Totowa, New Jersey): 275-283.

FERGUSON D.C. 1978. Lymantriidae. (In: The Moths of America North of Mexico, Vol. 22(2). R. B. DOMINICK et al. ed. E. W. Classey Ltd.): 1-110.

HALL T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analyses program for Windows 95/98/NT. Nucl. Acid. Symp. S. **41**: 95-98.

HAUSMANN A., HASZPRUNAR G., SEGERER A.H., SPEIDEL W., BEHOUNEK G., HEBERT P.D.N. 2011. Now DNA-barcoded: the butterflies and larger moths of Germany (Lepidoptera: Rhopalocera, Macroheterocera). Spixiana **34**: 47-58.

HUEMER P., MUTANEN M., SEFC K.M., HEBERT P.D.N. 2014.Testing DNA barcode performance in 1000 species of European Lepidoptera: large geographic distances have small genetic impacts. PLoS One **9**: E115774.

IVANOVA N.V., DEWAARD J.R., HEBERT P.D.N. 2006. An inexpensive, automation friendly protocol for recovering high quality DNA. Mol. Ecol. Resour. **6**: 998-1002.

KOZHANCHIKOV I.V. 1950. Volnyanki (Orgyidae). Fauna SSSR, **12**: 1-581 pp, Moskva-Leningrad.

LAMBERT D.M., BAKER A., HUYNEN L., HADDRATH O., HEBERT P.D.N., MILLAR C.D. 2005. Is a large-scale DNA-based inventory of ancient life possible? J. Heredity **96**: 279-284.

LEVIN D.B, DANKS H.V., BARBER S.A. 2003. Variations in mitochondrial DNA and gene transcription in freezing-tolerant larvae of *Eurosta solidaginis* (Diptera: Tephritidae) and *Gynaephora groenlandica* (Lepidoptera: Lymantriidae). Insect Mol. Biol. **12**: 281-289.

LUKHTANOV V.A., KHRULIOVA O.A. 1989. Morphological and karyological evidence of the species independence of *Dicallomera kuznezovi* sp. n. (Lepidoptera, Lymantriidae) from Wrangel Island. Zool. Zhurn. **68**: 41-48.

LUKHTANOV V.A., TIKHONOV V.V. 2015. Chromosomal and molecular evidence for presence of *Polyommatus* (*Agrodiaetus*) *poseidon* (Lepidoptera, Lycaenidae) in Caucasus region. Comp. Cytogenet. **9**: 249-255.

LUKHTANOV V.A., SHAPOVAL N.A., DANTCHENKO A.V. 2008. *Agrodiaetus shahkuhensis* sp. n. (Lepidoptera, Lycaenidae), a cryptic species from Iran discovered by using molecular and chromosomal markers. Comp. Cytogenet. **2**: 99-114.

LUKHTANOV V.A., SHAPOVAL N.A., DANTCHENKO A.V. 2014. Taxonomic position of several enigmatic *Polyommatus* (*Agrodiaetus*) species (Lepidoptera, Lycaenidae) from Central and Eastern Iran: insights from molecular and chromosomal data. Comp. Cytogenet. **8**: 313-322.

LUKHTANOV V.A., SHAPOVAL N.A., ANOKHIN B.A., SAIFITDINOVA A.F., KUZNETSOVA V.G. 2015a. Homoploid hybrid speciation and genome evolution via chromosome sorting. Proc. Roy. Soc. B **282**: 20150157. doi:10.1098/rspb.2015.0157

LUKHTANOV V.A., DANTCHENKO A.V., VISHNEVSKAYA M.S., SAIFITDINOVA A.F. 2015b. Detecting cryptic species in sympatry and allopatry: analysis of hidden diversity in *Polyommatus* (*Agrodiaetus*) butterflies (Lepidoptera: Lycaenidae). Biol. J. Linn. Soc. **116**: 468-485.doi: 10.1111/bij.12596

MILLER S.E., HRCEK J., NOVOTNY V., WEIBLEN G.D., HEBERT P.D.N. 2013. DNA barcodes of caterpillars (Lepidoptera) from Papua New Guinea. Proc. Entomol. Soc. Wash. **115**: 107-109.

NICHOLS R. 2001. Gene trees and species trees are not the same. Trends Ecol. Evol. **16**: 358-364.

POSADA D. 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. **25**: 1253-1256.

PRZYBYŁOWICZ Ł., LUKHTANOV V., LACHOWSKA-CIERLIK D. 2014. Towards the understanding of the origin of the Polish remote population of *Polyommatus* (*Agrodiaetus*) *ripartii* (Lepidoptera: Lycaenidae) based on karyology and molecular phylogeny. J. Zool. Syst. Evol. Res. **52**: 44-51.

RITTER S., MICHALSKI S.G., SETTELE J., WIEMERS M., FRIC Z.F., SIELEZNIEW M., ŠAŠIĆ M., ROZIER Y., DURKA W. 2013. *Wolbachia* infections mimic cryptic speciation in two parasitic butterfly species, *Phengaris teleius* and *P. nausithous* (Lepidoptera: Lycaenidae). PLoS One **8**: 1-13.

RONQUIST F., TESLENKO P., VAN DER MARK D., AYRES A., DARLING S.H., HÖHNA B., LARGET L., LIU M., SUCHARD A., HUELSENBECK J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. **61**: 539-542.

RYDELL J., ROININEN H., PHILIP K.W. 2000. Persistence of bat defence reactions in high Arctic moths (Lepidoptera). Proc. Roy. Soc. B **267**: 553-557.

SPITZER K. 1984. Notes on taxonomy and distribution of the genus *Gynaephora* Hübner, 1819 (Lymantriidae). Nota Lepidopterologica **7**: 180-183.

STRATHDEE A.T., BALE J.S. 1998. Life on the edge: Insect ecology in arctic environments. Ann. Rev. Entomol. **43**: 85-106.

TALAVERA G., LUKHTANOV V., RIEPPEL L., PIERCE N.E., VILA R. 2013. In the shadow of phylogenetic uncertainty: the recent diversification of *Lysandra* butterflies through chromosomal change. Mol. Phylogenet. Evol. **69**: 46-478.

TROFIMOVA T.A. 2008. Systematic notes on *Dasorgyia* Staudinger, 1881, *Dicallomera* Butler, 1881, and *Lachana* Moore, 1888 (Lymantriidae). Nota Lepidopterologica **31**: 273-291.

YUAN M.L., ZHANG Q.L., WANg Z.F., GUO Z.L., BAO G.S. 2015. Molecular phylogeny of grassland caterpillars (Lepidoptera: Lymantriinae: *Gynaephora*) endemic to the Qinghai-Tibetan plateau. PLoS One **10**: E0127257.

ZAHIRI R., HOLLOWAY J.D., KITCHING I.J., LAFONTAINE J.D., MUTANEN M., WAHLBERG **N.** 2012. Molecular phylogenetics of Erebidae (Lepidoptera, Noctuoidea). Syst. Entomol. **37**: 102-124. doi:10.1111/j.1365-3113.2011.00607.x.

ZAHIRI R., LAFONTAINE J.D., SCHMIDT B.C., DEWAARD J.R., ZAKHAROV E.V., HEBERT P.D.N. 2014. A transcontinental challenge – a test of DNA barcode performance for 1,541 species of Canadian Noctuoidea (Lepidoptera). PLoS One **9**: E92797.

ZAKHAROV E.V., LOBO N.F., NOWAK C., HELLMA J.J. 2009. Introgression as a likely cause of mtDNA paraphyly in two allopatric skippers (Lepidoptera: Hesperiidae). Heredity **102**: 590-599.