

**Supplementary material for the manuscript:**

**Comparative analysis of mitochondrial genomes of geographic variants of the gypsy moth,  
*Lymantria dispar*, reveals a previously undescribed genotypic entity**

by

Abdelmadjid Djoumad, Audrey Nisole, Reza Zahiri, Luca Freschi, Sandrine Picq,  
Dawn E. Gundersen-Rindal, Michael E. Sparks, Ken Dewar, Don Stewart, Halim Maaroufi,  
Roger C. Levesque, Richard C. Hamelin, & Michel Cusson

*Scientific Reports*

**Table S1.** List of primers used to amplify and sequence the mitochondrial genomes.

Name	Sequence	Position on Ldd_NJ	Length	Direction	Fragment
P1577_16S-F	GGTATTTTAAAGGAATTCGGC	633	21	F	2
Alb_16SrRNA	TGATTATGCTACCTTTGTACAGTCA	753	25	R	2
P1525inv	CTGCCAAAATAGTAAATCCAT	1,359	21	R	2
P1525_ND1	ATGGATTACTATTTTGGCAG	1,359	21	F	2
P1519inv	CTCCTGTATTAGCTTTTGCT	1,677	20	F	2
P1519_ND1	AGCAAAAGCTAATACAGGAG	1,677	20	R	2
P1554_Post_ND1	TACTCTACCTCGTTATCGTTAT	2,260	22	F	2
P1524_Cytb	TGATCCAGTTTGATGAAGAA	2,999	20	F	2
P1533inv	CATATTGGACGAGGAATTTATTATGAATC	3,297	29	R	2
P1533_ND6*	GATTCATAATAAATTCCTCGTCCAATATG	3,297	29	F	2,1
P1553_Post_Cytb	GGTTTGATTTTCGTATAAGAATGAAT	3,589	26	F	2,1
P1552_Post_Cytb	ATTCATTCTTATACGAAAATCAAACC	3,589	26	R	2,1
P1556_Alb_Cytb	ATATTGGTAATTTGACTACTGC	3,735	23	F	2,1
P1532_ND6*	GGTCTTTTAGTTTTATTTATTTATGTTTCAAGAATTGC	4,023	38	R	2,1
P1532inv	GCAATTCCTGAAACATAAAATAAACTAAAAGACC	4,023	38	F	1
P1522inv	AATCCTATAGAAAGGGGGTT	4,158	20	F	1
P1522_ND6	AACCCCTTTCTATAGGATT	4,158	20	R	1
P1545_ND4	AATTAAGTAGGAATTAAC	5,052	20	R	1
P1544_ND4	CATCTAATATGGCTGCYCCTC	5,751	21	F	1
P1528inv	TACCTGAATAACTTTCTCCATG	5,906	22	R	1
P1528_ND4	CATGGAGAAAGTTATTCAGGTA	5,906	22	F	1
P1527_ND5	TTCTTCAGTGGTTATTTAT	6,314	19	F	1
P1521_ND5	AACATCCCGGCATTATATGA	6,513	20	R	1
P1530_ND5	CACCTACTCCAGTTTCTGCT	6,742	20	F	1
P1535_ND5	CACCTGCTGTAATAAGTAGAAG	6,772	24	R	1
P1536_ND5	CAAATATAGCTTTATGTGGRATTCC	7,150	25	F	1
P1529_ND5	CCTCACTTCATCTATATCAAT	7,680	22	R	1
P1531inv	TCGAGCTGAAGATTTTGGATCA	8,547	22	F	1
P1531_ND3	TGATCCAAAATCTTCAGCTCGA	8,547	22	R	1
Ldja_COIII-R	TGAAGCCGCAGCATGATATTG	8,825	21	F	1
Ldja_COIII-F	CAATATCATGCTGCGGCTCA	8,825	21	R	1
KazUmbI_COIII-R	ATTTGAAATGGRTTAAAGGGGATA	9,164	24	R	1
KazUmbI_COIII-F	TATCCCCTTTAAYCCATTTCAAAT	9,164	24	F	1
P1523inv	CAGTTGCGGTTATCCAATCA	9,612	20	R	1
P1523_ATP6	TGATTGGATAACCGCAACTG	9,612	20	F	1
P1557_Alb_ATP6	GAACCTCTCTGCTTTAATA	9,822	20	R	1
R-COII	TGACTGAAAGTAAGTAATGGTCTC	10,526	27	F	1
P1559_Xyl_COII-F	GAGACCATTACTTGCTTTTCAGTCATCT	10,526	24	R	1
2Alb_COII-R	AGACCATTACTTACTTTTCAGCCA	10,527	23	F	1

P1518_COII	GCGGAACTAACCACAGATTT	10,621	20	R	1
Alb_COII	TGATGCTAACCAGGACGAT	10,704	20	R	1
P1547_Post_COII	TGATGCCAACCCAGGACGAT	10,704	20	R	1
P1558_Alb_COI	TAGGTGTTAAAATTGATGCTAAC	10,714	23	R	1
P1562_Alb_COII	CCTCTCTCCGACTTCTATAT	10,984	20	R	1
P1549_Post_COII	TGTTCTATTAGAGGTGATGCTC	11,183	22	F	1
P1550_Post_COII	AGGTGATGCTCTATTTTGGAGAT	11,194	23	F	1
P1365*	CCTGTTTTAGCAGGTGCTATTAC	12,226	23	R	1
P1364 *	GATCATTTCCGATTAATGATCCAGG	12,692	25	F	2
Alb_COI	TACCGCTTAAACTCAGCCAT	12,853	20	R	2
Xyl_COI	TACAATTTATCGCTTAAACTCAGCCA	12,854	26	R	2
P1526_ND2	ATTGCAAATTTTAAGGAGTATTT	12,943	23	F	2
P1379_ND2	AATCCAATAAATGGGGTAATCCTCCT	13,291	27	F	2
P1378inv	ATCCTTCAACAATATTGGGGAATCAA	13,694	26	F	2
P1378_ND2	TTGATCCCCAATATTGTTGAAGGAT	13,694	26	R	2
ND2-F	TGGATGTTGAATTGGGTTAGA	13,945	21	F	2
P1520_ND2	TGGATGTTGAATTGGRTTAGA	13,945	21	R	2
P1536inv_ND2	CAAATATAGCTTTATGTGGRATTCC	13,945	21	R	2
Xyl_16SrRNA	AGAATAATCCTTTAATCAGGCACT	14,213	24	F	2
Alb_ND2tRNA	GATTCCTTTATAGTTGGGGTATG	14,254	24	F	2
P1576_16S-R	ATATGAAAGCGACGGGCAATATGTAC	15,505	26	R	2

\*Primers used to amplify the two main (initial) mitochondrial fragments

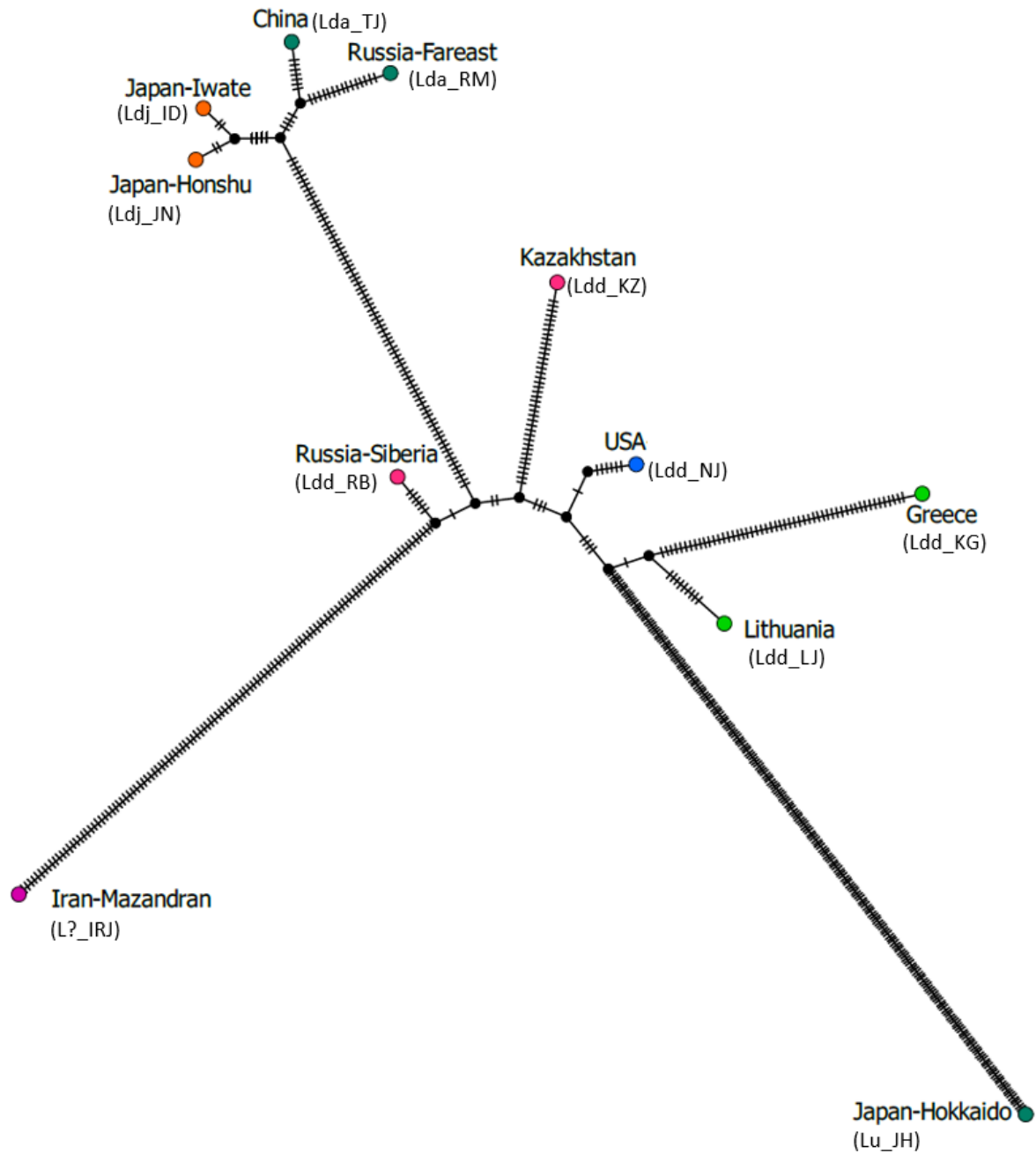
**Table S2.** Pair-wise percent identity among the 11 mitochondrial genomes sequenced. Values provided for the entire genome (bottom half, black letters) and for the protein coding genes (PCGs) only (upper half, red letters).

		<b>PCGs</b>										
		<i>L. d. dispar</i>					<i>L. d. asiatica</i>		<i>L. d. japonica</i>			
		Ldd_NJ	Ldd_KG	Ldd_LJ	Ldd_KZ	Ldd_RB	Lda_TJ	Lda_RM	Ldj_JN	Ldj_ID	L?_IR	Lu_JH
<b>Complete mitogenome</b>	<b>Ldd_NJ</b>	-	99.7	99.8	99.7	99.8	99.2	99.2	99.3	99.3	98.9	97.9
	<b>Ldd_KG</b>	99.0	-	99.6	99.6	99.6	99.2	99.2	99.3	99.3	98.9	97.8
	<b>Ldd_LJ</b>	99.4	99.3	-	99.6	99.8	99.1	99.1	99.2	99.2	98.8	97.9
	<b>Ldd_KZ</b>	99.4	99.3	99.4	-	99.6	99.2	99.2	99.2	99.2	98.8	97.9
	<b>Ldd_RB</b>	99.0	98.6	99.0	99.1	-	99.2	99.2	99.3	99.3	98.9	97.9
	<b>Lda_TJ</b>	98.6	98.4	98.4	98.7	98.9	-	99.8	99.9	99.9	98.8	97.6
	<b>Lda_RM</b>	98.6	98.3	98.4	98.7	98.9	99.6	-	99.8	99.8	98.8	97.6
	<b>Ldj_JN</b>	98.7	98.4	98.5	98.8	98.9	99.6	99.5	-	99.8	98.8	97.6
	<b>Ldj_ID</b>	98.8	98.5	98.6	98.9	98.9	99.6	99.5	99.6	-	98.8	97.6
	<b>L?_IR</b>	98.4	98.3	98.5	98.6	98.4	98.4	98.3	98.5	98.5	-	97.9
	<b>Lu_JH</b>	97.7	97.7	97.9	97.8	97.5	97.3	97.3	97.4	97.4	97.7	-

**Table S3.** Assessment of whether each of the 13 mitochondrial protein-coding genes are under purifying or positive selection. At a significance level of 0.05, all but two genes were observed to be under purifying selection (blue highlighting), and none under positive selection.

Gene	Purifying (p-value)	Positive (p-value)
<i>COI</i>	<0.001	1
<i>COII</i>	0.017	1
<i>COII</i>	<0.001	1
<i>ATP6</i>	0.001	1
<i>ATP8</i>	0.016	1
<i>Cytb</i>	<0.001	1
<i>ND1</i>	<0.001	1
<i>ND2</i>	<0.001	1
<i>ND3</i>	0.065	1
<i>ND4</i>	<0.001	1
<i>ND4L</i>	0.302	1
<i>ND5</i>	<0.001	1
<i>ND6</i>	0.006	1

Figure S1



**Figure S1.** Haplotype network construction for the 11 mitochondrial genomes examined in the present study, carried out using POPART<sup>35</sup>. Each hatch mark represents one substitution.

Figure S2

A

Lep0813_Iran	gcatgagctgtaacaatagtagtattataaaatttgatcatttccgattaatgatccaggattt
Lep0816_Iran	gcatgagctgtaacaatagtagtattataaaatttgatcatttccgattaatgatccaggattt
Lep0941_Russia	gcatgagctgtaacaatagtagtattataaaatttgatcatttccgattaatgatccaggattt
Lep0944_Russia	gcatgagctgtaacaatagtagtattataaaatttgatcatttccgattaatgatccaggattt
Lep0963_Russia	gcatgagctgtaacaatagtagtattataaaatttgatcatttccgattaatgatccaggattt
	*****
Lep0813_Iran	cctaattcagctcgaattagtagtaaaacttagagatggtccactatttctgatcaaatacca
Lep0816_Iran	cctaattcagctcgaattagtagtaaaacttagagatggtccactatttctgatcaaatacca
Lep0941_Russia	cctaattcagctcgaattagtagtaaaacttagagatggtccactatttctgatcaaatacca
Lep0944_Russia	cctaattcagctcgaattagtagtaaaacttagagatggtccactatttctgatcaaatacca
Lep0963_Russia	cctaattcagctcgaattagtagtaaaacttagagatggtccactatttctgatcaaatacca
	*****
Lep0813_Iran	aaaataaaatataagggtccaatatccttatgatttggtgaatataatcattttcgctta
Lep0816_Iran	aaaataaaatataagggtccaatatccttatgatttggtgaatataatcattttcgctta
Lep0941_Russia	aaaataaaatataagggtccaatatccttatgatttggtgaatataatcattttcgctta
Lep0944_Russia	aaaataaaatataagggtccaatatccttatgatttggtgaatataatcattttcgctta
Lep0963_Russia	aaaataaaatataagggtccaatatccttatgatttggtgaatataatcattttcgctta
	*****
Lep0813_Iran	ttttttaataaaaatggctgagtttaagcggtaaaattgtaaatttattaacgagaaatctc
Lep0816_Iran	tttttttaataaaaatggctgagtttaagcggtaaaattgtaaatttattaacgagaaatctc
Lep0941_Russia	tttttttaataaaaatggctgagtttaagcggtaaaattgtaaatttattaacgagaaatctc
Lep0944_Russia	tttttttaataaaaatggctgagtttaagcggtaaaattgtaaatttattaacgagaaatctc
Lep0963_Russia	tttttttaataaaaatggctgagtttaagcggtaaaattgtaaatttattaacgagaaatctc
	*****
Lep0813_Iran	tctttttattatattaagggtcttatattcaataatgatgtaaaattgcaaattttaaggag
Lep0816_Iran	tctttttattatattaagggtcttatattcaataatgatgtaaaattgcaaattttaaggag
Lep0941_Russia	tctttttattatattaagggtcttatattcaataatgatgtaaaattgcaaattttaaggag
Lep0944_Russia	tctttttattatattaagggtcttatattcaataatgatgtaaaattgcaaattttaaggag
Lep0963_Russia	tctttttattatattaagggtcttatattcaataatgatgtaaaattgcaaattttaaggag
	*****
Lep0813_Iran	tattttaaattataactaaggcttaaaagactatttctttataaataaattttgaagattatta
Lep0816_Iran	tattttaaattataactaaggcttaaaagactatttctttataaataaattttgaagattatta
Lep0941_Russia	tattttaaattataactaaggcttaaaagactatttctttataaataaattttgaagattatta
Lep0944_Russia	tattttaaattataactaaggcttaaaagactatttctttataaataaattttgaagattatta
Lep0963_Russia	tattttaaattataactaaggcttaaaagactatttctttataaataaattttgaagattatta
	*****
Lep0813_Iran	gtttaccttaacttaaaaccttaataaaaaaaaaagggttctaataattataactaaaaata
Lep0816_Iran	gtttaccttaacttaaaaccttaataaaaaaaaaagggttctaataattataactaaaaata
Lep0941_Russia	gtttaccttaacttaaaaccttaataaaaaaaaaagggttctaataattataactaaaaata
Lep0944_Russia	gtttaccttaacttaaaaccttaataaaaaaaaaagggttctaataattataactaaaaata
Lep0963_Russia	gtttaccttaacttaaaaccttaataaaaaaaaaagggttctaataattataactaaaaata
	*****

Lep0813\_Iran g  
Lep0816\_Iran g  
Lep0941\_Russia  
Lep0944\_Russia  
Lep0963\_Russia

Lep0813\_Iran a  
Lep0816\_Iran a  
Lep0941\_Russia  
Lep0944\_Russia  
Lep0963\_Russia

Lep0813\_Iran  
Lep0816\_Iran  
Lep0941\_Russia  
Lep0944\_Russia  
Lep0963\_Russia

Lep0813\_Iran  
Lep0816\_Iran  
Lep0941\_Russia  
Lep0944\_Russia  
Lep0963\_Russia

Lep0813\_Iran a  
Lep0816\_Iran a  
Lep0941\_Russia  
Lep0944\_Russia  
Lep0963\_Russia

Lep0813\_Iran  
Lep0816\_Iran  
Lep0941\_Russia  
Lep0944\_Russia  
Lep0963\_Russia

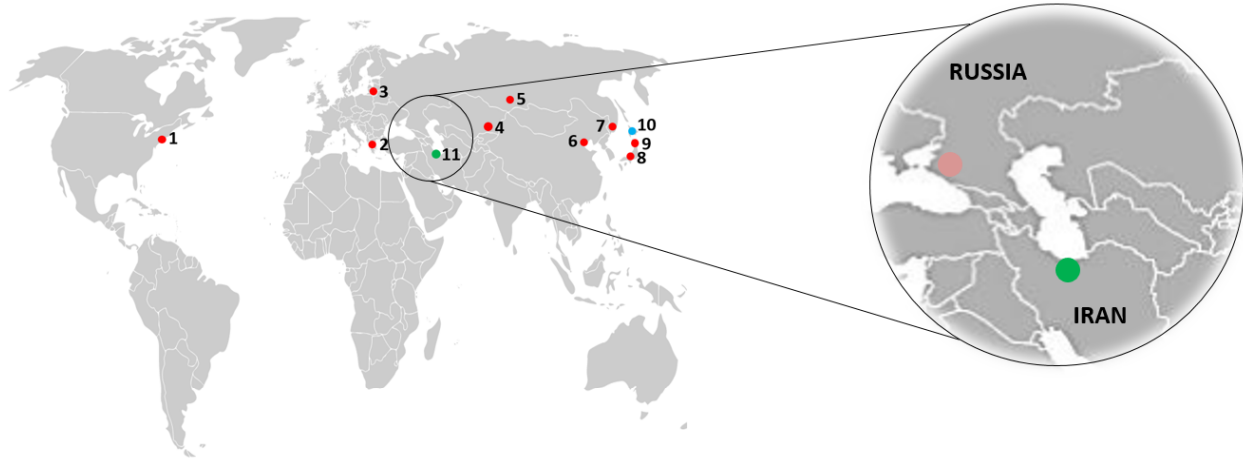
Lep0813\_Iran  
Lep0816\_Iran  
Lep0941\_Russia  
Lep0944\_Russia  
Lep0963\_Russia

Lep0813\_Iran  
Lep0816\_Iran  
Lep0941\_Russia  
Lep0944\_Russia  
Lep0963\_Russia



**Lep0813\_Iran** cctcctaaagctccaatgattacattttattgagataattaaaataataaaaattttttattt  
**Lep0816\_Iran** cctcctaaagctccaatgattacattttattgagataattaaaataataaaaattttttattt  
**Lep0941\_Russia** cctcctaaagctccaatgattacattttattgagataattaaaataataaaaattttttattt  
**Lep0944\_Russia** cctcctaaagctccaatgattacattttattgagataattaaaataataaaaattttttattt  
**Lep0963\_Russia** cctcctaaagctccaatgattacattttattgagataattaaaataataaaaattttttattt  
 \*\*\*\*\*

**B**



Name	Origin	Region	Supplier
Lep0813	Iran	Noor, Mazandaran	H. Rajaei
Lep0816	Iran	Noor, Mazandaran	H. Rajaei
Lep0941	Russia	Krasnodar Reg.	R.V. Yakovlev
Lep0944	Russia	Krasnodar Reg.	R.V. Yakovlev
Lep0963	Russia	Krasnodar Reg.	R.V. Yakovlev

**Figure S2.** A) Nucleotide alignment (MAFFT) of the mitochondrial *COI-ND2* region for five specimens from the Caucasus (Caspian Sea region), including L?\_IR. The alignment shows that all five specimens appear to share the same mitochondrial haplotype. In red, sequence from Lep0816-Iran, corresponding to L?\_IR. In blue, SNPs unique to the Caucasian specimens, relative to the other *Lymantria dispar* haplotypes (in grey). B) Map showing the sampling locations of the specimens. Background map is a cropped version of the one available at: [https://commons.wikimedia.org/wiki/Maps\\_of\\_the\\_world#/media/File:BlankMap-World-v2.png](https://commons.wikimedia.org/wiki/Maps_of_the_world#/media/File:BlankMap-World-v2.png) (for information about license, follow the same link).