

Complete Mitochondrial Genome of the Gypsy Moth, *Lymantria dispar* (Lepidoptera: Erebidae)

Na Ra Jeong, Youngwoo Nam¹ and Wonhoon Lee^{2*}

Department of Plant Medicine and Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju 52828, Korea

¹Forest Entomology and Pathology Division, National Institute of Forest Science, Seoul 02455, Korea

²Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Korea

매미나방의 미토콘드리아 게놈 분석

정나라 · 남영우¹ · 이원훈^{2*}

경상대학교 식물외과, ¹국립산림과학원 산림병해충연구과, ²경상대학교 농생명과학연구소

ABSTRACT: The Gypsy moth, *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae) is a serious pest that attacks forest as well as fruit trees. We sequenced the 15,548 bp long complete mitochondrial genome (mitogenome) of this species. It consists of a typical set of genes (13 protein-coding genes, 2 rRNA genes, and 22 tRNA genes) and one major non-coding A + T-rich region. The orientation and gene order of the *L. dispar* mitogenome are identical to that of the ancestral type found in majority of the insects. Phylogenetic analyses using concatenated sequences of 13 PCGs and 2 rRNAs (13,568 bp including gaps) revealed that the *L. dispar* examined in our study, together with other geographical samples of *L. dispar* in a group forming the family Erebidae and consistently supported the monophyly of each family (Erebidae, Euteliidae, Noctuidae, Nolidae and Notodontidae), generally with the highest nodal supports.

Key words: Mitochondrial genome, *Lymantria dispar*, Erebidae, Republic of Korea

초록: 매미나방은 산림과 과수에 심각한 피해를 입히는 해충이다. 본 연구에서는 국내 매미나방의 미토콘드리아 게놈(15,548 bp)을 분석하였다. 13개의 PCG와 2개의 rRNA를 연결한 서열(13,568 bp)을 사용한 23개의 미토콘드리아 게놈의 계통분석 결과, 분석한 매미나방은 다른 지역의 매미나방과 같은 과에 속하며 각각의 과(Erebidae, Euteliidae, Noctuidae, Nolidae, Notodontidae)들은 높은 노드수치로 단계를 형성하였다.

검색어: 미토콘드리아게놈, 매미나방, 태극나방과, 한국

The Gypsy moth, *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae), is distributed throughout most of the northern hemisphere (Bogdanowicz et al., 1997; 2000). This species damages a wide variety of 400 species of plants (Lowe et al., 2000; Pogue and Schaefer, 2007). *L. dispar* is composed of three subspecies based on morphological characteristics, geographic location, and flight capable females: *Lymantria dispar dispar*, *Lymantria dispar asiatica*, *Lymantria dispar*

japonica (Pogue and Schaefer, 2007). Particularly, *Lymantria* species (*L. d. asiatica*, *L. d. japonica*, *L. albescens*, *L. umbrosa*, *L. postalba*), called AGMs, are considered a serious threat to North America because females can fly (USDA, 2016). So far, 11 gypsy moth complete mitochondrial genomes, including 3 subspecies, have been reported, but none have been reported in the Republic of Korea. Here, we analyzed the complete mitochondrial genome of *L. dispar* in this study.

*Corresponding author: wonhoon@gnu.ac.kr

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Materials and Methods

Eggs were collected in Chungju-si, Republic of Korea (36° 58'43.9"N 127°59'43.1"E) and its DNA was extracted from one egg. Leftover DNA and the specimen were deposited at the Gyeongsang National University, Jinju, Republic of Korea. Using the extracted DNA, three long overlapping fragments (LFs: COI-ND5, ND5-IrRNA, and ND1-COI) were amplified using four sets of primers designed using data regarding the previously published species of *L. dispar* published in earlier studies (Djoudmad et al., 2017). Using the LFs as templates, 25 overlapping short fragments (SF) were amplified using the afore mentioned primers.

Phylogenetic analysis was performed using 23 available

mitogenomes from the superfamily Noctuoidea, including the one obtained in the current study (Fig. 1). The nucleotide sequence of each PCG was aligned based on the codons using RevTrans ver. 2.0 (Wernersson and Pedersen, 2003). Each of the two rRNA genes was individually aligned using MAFFT ver. 7 (Kato and Standley, 2013) and well-aligned blocks were selected using GBlocks 0.91b software (Castresana, 2000) with gap positions allowed. Subsequently, 13 PCGs and 2 rRNAs were concatenated in an alignment (13,568 bp including gaps).

An optimal partitioning scheme (five partitions) and substitution model (GTR + Gamma + I) were determined using PartitionFinder 2 with the Greedy algorithm (Lanfear et al., 2014; 2016). The five partition schemes for PCG+rRNA data matrix were obtained. These partition schemes and substitution

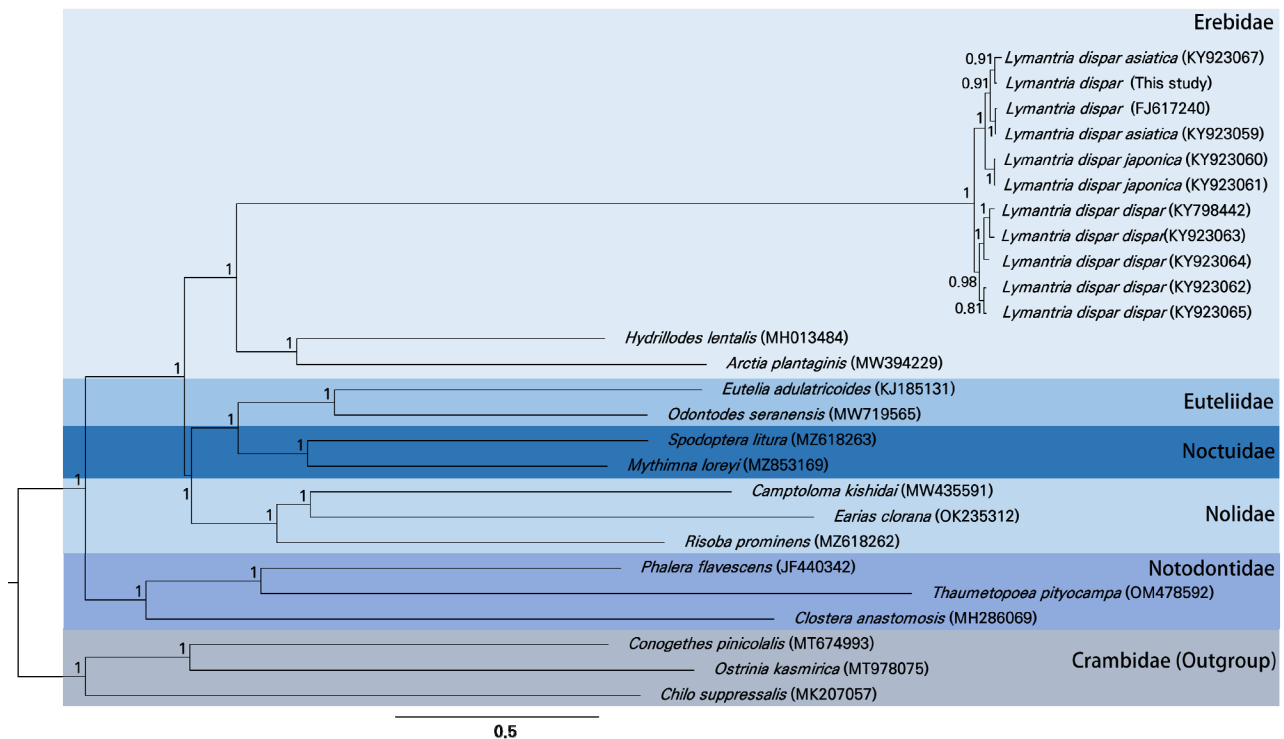


Fig. 1. Phylogeny of lepidopteran Noctuoidea. Bayesian inference (BI) tree using 13 protein-coding genes and 2 rRNA genes including gaps. The numbers at each node specify Bayesian posterior probabilities for BI analysis. The scale bar indicates the number of substitutions per site. Three species of Crambidae in Pyraloidea were utilized as outgroups. GenBank accession numbers are as follows: *Lymantria dispar asiatica*, KY923067 (Djoudmad et al. 2017); *Lymantria dispar*, FJ617240 (Unpublished); *Lymantria dispar asiatica*, KY923059 (Djoudmad et al. 2017); *Lymantria dispar japonica*, KY923060 (Djoudmad et al. 2017); *Lymantria dispar japonica*, KY923061 (Djoudmad et al. 2017); *Lymantria dispar dispar*, KY798442 (Djoudmad et al. 2017); *Lymantria dispar dispar*, KY923063 (Djoudmad et al. 2017); *Lymantria dispar dispar*, KY923064 (Djoudmad et al. 2017); *Lymantria dispar dispar*, KY923062 (Djoudmad et al. 2017); *Lymantria dispar dispar*, KY923065 (Djoudmad et al. 2017); *Hydrillodes lentalis*, MH013483 (Yang et al., 2019); *Arctia plantaginis*, MW394229 (Galarza and Mappes, 2021); *Eutelia adalatricoides*, KJ185131 (Yang et al., 2015); *Odontodes seranensis*, MW719565 (Unpublished); *Spodoptera litura*, MZ618263 (Unpublished); *Mythimna loreyi*, MZ853169 (Unpublished); *Camptoloma kishidai*, MW435591 (Unpublished); *Earias clorana*, OK235312 (Unpublished); *Risoba prominens*, MZ618262 (Unpublished); *Phalera flavescens*, JF440342 (Sun et al., 2012); *Thaumetopoea pityocampa*, OM478592 (Unpublished); *Clostera anastomosis*, MH286069 (Zhu et al., 2018); *Conogethes pinicolalis*, MT674993 (Jeong et al., 2021); *Ostrinia kasmirica*, MT978075 (Park et al., 2019); *Chilo suppressalis*, MK207057 (Luo et al., 2021).

models were applied for each phylogenetic analysis.

Bayesian inference (BI) methods were applied using Mr-Bayes ver. 3.2.7 (Ronquist et al., 2012), which was incorporated into the CIPRES Portal ver. 3.1 (Miller et al., 2010). For the BI analysis, two independent runs of four incrementally heated Markov and Monte Carlo chains (one cold chain and three hot chains) were simultaneously run for 10 million generations, with tree sampling conducted at every 100 generations. The first 25% of the sampled trees were discarded as burn-in. Partitioned analyses were conducted with each partition unlinked in each parameter (statefreq, revmat, shape, pinvar, and tratio). An average split frequency of less than 0.01 was used to represent the convergence of the two simultaneous runs. The confidence values for BI tree were determined with the Bayesian posterior probabilities (BPP). *Conogethes pinicolalis* (Jeong et al., 2021), *Chilo suppressalis* (Park et al., 2019), and *Ostrinia kasmirica* (Luo et al., 2021) that belong to the family Crambidae of the superfamily Pyraloidea were used as outgroups. FigTree ver. 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) was used to visualize phylogenetic trees.

Results

The *L. dispar* mitogenome was found to be 15,548 bp in length, with typical gene sets - 2 rRNAs, 22 tRNAs, and 13

PCGs - and a major non-coding A + T-rich region of 393 bp length (GenBank accession number ON469817). The previous studies showed that the shortest mitogenome was 15,569 bp (FJ617240; Unpublished) and the longest 15,698 bp (Djoumad et al., 2017). The largest size variation was detected in the A + T-rich region (393 bp in this study, the shortest 368 bp in Djoumad et al. (2017), and longest 461 bp in Djoumad et al. (2017)) (Table 1).

A phylogenetic analysis was performed using 23 mitogenome sequences, including the mitogenome of *Lymantria dispar* analyzed in this study, using concatenated 13 PCGs and 2 rRNAs. These were composed of 5 families (Erebidae, Euteliidae, Noctuidae, Nolidae and Notodontidae) in Noctuoidea (Fig. 1). Monophyly of each family was supported with the highest nodal supports, presenting the familial relationships of ((Erebidae + ((Euteliidae + Noctuidae) + Nolidae)) + Notodontidae) in Noctuoidea (Fig. 1). Furthermore, the monophyly of each family were also supported generally with the highest nodal supports (BPP = 1.0; Fig. 1). The 11 *Lymantria dispar* mitogenomes formed a strong group with the higher nodal supports from 0.81 to 1 (Fig. 1). *L. dispar* analyzed in this study was confirmed to *L. d. asiatica* by supporting the monophyly with the highest node value of *L. d. asiatica* (KY923067) (Djoumad et al., 2017).

Table 1. Summaries of 11 mitochondrial genomes of *Lymantria dispar*

Gene	Anti-codon	Start codon	Stop codon	<i>Lymantria dispar</i> (This study)	<i>Lymantria dispar</i> (FJ617240)	<i>Lymantria dispar asiatica</i> (KY923067)	<i>Lymantria dispar asiatica</i> (KY923059)	<i>Lymantria dispar japonica</i> (KY923060)
<i>trnM</i>	CAT			1-67 (67)	1-67 (67)	14228-14294 (67)	14220-14286 (67)	14228-14294 (67)
<i>trnI</i>	GAT			68-134 (67)	68-134 (67)	14161-14227 (67)	14153-14219 (66)	14161-14227 (67)
<i>trnQ</i>	TTG			139-207 (69)	130-198 (69)	14088-14156 (69)	14080-14148 (69)	14088-14156 (68)
<i>ND2</i>		ATT	TAA	255-1268 (1014)	[†] 255-1268 (1014)	13027-14040 (1014)	13019-14032 (1014)	13027-14040 (1014)
<i>trnW</i>	TCA			1267-1334 (68)	1267-1334 (68)	12961-13028 (68)	12953-13020 (68)	12961-13028 (68)
<i>trnC</i>	GCA			1327-1392 (66)	1327-1392 (66)	12903-12968 (66)	12895-12960 (66)	12903-12968 (66)
<i>trnY</i>	GTA			1399-1462 (64)	1399-1462 (64)	12833-12896 (64)	12825-12888 (64)	12833-12896 (64)
<i>COI</i>		CGA	T-tRNA	1473-3003 (1531)	1473-3003 (1531)	11292-12822 (1531)	11284-12814 (1531)	11292-12822 (1531)
<i>trnL₂</i>	TAA			3004-3070 (67)	3004-3070 (67)	11224-11291 (68)	11217-11283 (67)	11225-11291 (67)
<i>COII</i>		ATA	T-tRNA	3071-3752 (682)	3071-3752 (682)	10542-11223 (682)	10535-11216 (682)	10543-11224 (682)
<i>trnK</i>	CTT			3753-3823 (71)	3753-3823 (71)	10471-10541 (71)	10464-10534 (71)	10472-10542 (71)
<i>trnD</i>	GTC			3823-3891 (69)	3823-3891 (69)	10403-10471 (69)	10396-10464 (69)	10404-10472 (69)
<i>ATP8</i>		ATT	TAA	3892-4074 (183)	3892-4074 (183)	10220-10402 (183)	10213-10395 (183)	10221-10403 (183)

Table 1. Continued

Gene	Anti-codon	Start codon	Stop codon	<i>Lymantria dispar</i> (This study)	<i>Lymantria dispar</i> (FJ617240)	<i>Lymantria dispar asiatica</i> (KY923067)	<i>Lymantria dispar asiatica</i> (KY923059)	<i>Lymantria dispar japonica</i> (KY923060)
<i>ATP6</i>		ATG	TAA	4068-4745 (678)	4068-4745 (678)	9549-10226 (678)	9542-10219 (678)	9550-10227 (678)
<i>COIII</i>		ATG	TAA	4757-5545 (789)	4757-5545 (789)	8749-9537 (789)	8742-9530 (789)	8750-9538 (789)
<i>trnG</i>	TCC			5548-5612 (65)	5548-5612 (65)	8682-8746 (65)	8675-8739 (65)	8683-8747 (65)
<i>ND3</i>		ATT	TAA	5613-5966 (354)	5613-5966 (354)	8328-8681 (354)	8321-8674 (354)	8329-8682 (354)
<i>trnA</i>	TGC			5971-6036 (66)	5971-6036 (66)	8258-8323 (66)	8251-8316 (66)	8259-8324 (66)
<i>trnR</i>	TCG			6055-6119 (65)	6055-6117 (63)	8174-8235 (62)	8167-8228 (62)	8175-8236 (62)
<i>trnN</i>	GTT			6131-6195 (65)	6131-6195 (65)	8099-8163 (65)	8092-8156 (65)	8100-8164 (65)
<i>trnS1</i>	GCT			6196-6261 (66)	6195-6262 (68)	8032-8099 (68)	8025-8092 (68)	8033-8100 (68)
<i>trnE</i>	TTC			6281-6346 (66)	6283-6349 (67)	7943-8007 (65)	7934-7998 (65)	7943-8008 (66)
<i>trnF</i>	GAA			6349-6414 (66)	6352-6417 (66)	7875-7940 (66)	7866-7931 (66)	7875-7940 (66)
<i>ND5</i>		ATT	TAA	6442-8190 (1749)	6439-8187 (1749)	6095-7843 (1749)	6096-7844 (1749)	6101-7849 (1749)
<i>trnH</i>	GTG			8191-8256 (66)	8188-8253 (66)	6030-6094 (65)	6031-6095 (65)	6036-6100 (65)
<i>ND4</i>		ATG	T-tRNA ¹ , TA ²	8257-9595 (1339) ¹	8254-9592 (1339) ¹	4690-6029 (1340) ²	4691-6030 (1340) ²	4696-6035 (1340) ²
<i>ND4L</i>		ATG	TAA	9618-9902 (285)	9615-9899 (285)	4383-4667 (285)	4384-4668 (285)	4389-4673 (285)
<i>trnT</i>	TGT			9915-9980 (66)	9912-9977 (66)	4306-4370 (65)	4306-4370 (65)	4312-4376 (65)
<i>trnP</i>	TGG			9981-10045 (65)	9978-10042 (65)	4241-4305 (65)	4241-4305 (65)	4247-4311 (65)
<i>ND6</i>		ATA	TAA	10056-10592 (537)	10053-10589 (537)	3694-4230 (537)	3694-4230 (537)	3700-4236 (537)
<i>CytB</i>		ATG	TAA	10653-11813 (1161)	[†] 10636-11796 (1161)	2471-3631 (1161)	2471-3631 (1161)	2475-3635 (1161)
<i>trnS₂</i>	TGA			11816-11883 (68)	11799-11867 (69)	2400-2468 (69)	2400-2468 (69)	2404-2472 (69)
<i>ND1</i>		ATA	TAA	11913-12851 (939)	11896-12834 (939)	1433-2371 (939)	1433-2371 (939)	1437-2375 (939)
<i>trnL₁</i>	TAG			12852-12920 (69)	12835-12903 (69)	1364-1432 (69)	1364-1432 (69)	1368-1436 (69)
<i>lrRNA</i>				12921-14293 (1373)	12904-14254 (1351)	12-1363 (1352)	12-1363 (1352)	12-1367 (1356)
<i>trnV</i>	TAC			14294-14359 (66)	14270-14335 (66)	15522-15587 (66)	15523-15588 (66)	15546-15611 (66)
<i>srRNA</i>				14360-15155 (796)	14336-15134 (799)	14732-15515 (784)	14730-15516 (787)	14754-15539 (786)
A+T-rich region				15156-15548 (393)	15135-15569 (435)	14356-14730 (375)	14347-14728 (382)	14355-14752 (398)
Gene	<i>Lymantria dispar japonica</i> (KY923061)	<i>Lymantria dispar dispar</i> (KY798442)	<i>Lymantria dispar dispar</i> (KY923062)	<i>Lymantria dispar dispar</i> (KY923063)	<i>Lymantria dispar dispar</i> (KY923064)	<i>Lymantria dispar dispar</i> (KY923065)		
<i>trnM</i>	14217-14283 (67)	14242-14308 (67)	14248-14314 (67)	14251-14317 (67)	14235-14301 (67)	14240-14306 (67)		
<i>trnI</i>	14150-14216 (67)	14175-14241 (67)	14181-14247 (67)	14184-14250 (67)	14168-14234 (67)	14173-14239 (67)		
<i>trnQ</i>	14077-14145 (69)	14102-14170 (69)	14108-14176 (69)	14111-14179 (69)	14095-14163 (69)	14100-14168 (69)		
<i>ND2</i>	13016-14029 (1014)	13041-14054 (1014)	13047-14060 (1014)	13050-14063 (1014)	13034-14047 (1014)	13039-14052 (1014)		
<i>trnW</i>	12950-13017 (68)	12975-13042 (68)	12981-13048 (68)	12984-13051 (68)	12968-13035 (68)	12973-13040 (68)		
<i>trnC</i>	12892-12957 (66)	12917-12982 (66)	12923-12988 (66)	12926-12991 (66)	12910-12975 (66)	12915-12980 (66)		
<i>trnY</i>	12822-12885 (64)	12847-12910 (64)	12853-12916 (64)	12856-12919 (64)	12840-12903 (64)	12845-12908 (64)		
<i>COI</i>	11281-12811 (1531)	11306-12836 (1531)	11312-12842 (1531)	11315-12845 (1531)	11299-12829 (1531)	11304-12834 (1531)		
<i>trnL₂</i>	11214-11280 (67)	11239-11305 (67)	11245-11311 (67)	11248-11314 (67)	11232-11298 (67)	11237-11303 (67)		
<i>COII</i>	10532-11213 (682)	10557-11238 (682)	10563-11244 (682)	10566-11247 (682)	10550-11231 (682)	10555-11236 (682)		
<i>trnK</i>	10461-10531 (71)	10486-10556 (71)	10492-10562 (71)	10495-10565 (71)	10479-10549 (71)	10484-10554 (71)		
<i>trnD</i>	10393-10461 (69)	10418-10486 (69)	10424-10492 (69)	10427-10495 (69)	10411-10479 (69)	10416-10484 (69)		
<i>ATP8</i>	10210-10392 (183)	10235-10417 (183)	10241-10423 (183)	10244-10426 (183)	10228-10410 (183)	10233-10415 (183)		
<i>ATP6</i>	9539-10216 (678)	9564-10241 (678)	9570-10247 (678)	9573-10250 (678)	9557-10234 (678)	9562-10239 (678)		

Table 1. Continued

Gene	<i>Lymantria dispar japonica</i> (KY923061)	<i>Lymantria dispar dispar</i> (KY798442)	<i>Lymantria dispar dispar</i> (KY923062)	<i>Lymantria dispar dispar</i> (KY923063)	<i>Lymantria dispar dispar</i> (KY923064)	<i>Lymantria dispar dispar</i> (KY923065)
<i>COIII</i>	8739-9527 (789)	8764-9552 (789)	8770-9558 (789)	8773-9561 (789)	8757-9545 (789)	8762-9550 (789)
<i>trnG</i>	8672-8736 (65)	8697-8761 (65)	8703-8767 (65)	8706-8770 (65)	8690-8754 (65)	8695-8759 (65)
<i>ND3</i>	8318-8671 (354)	8343-8696 (354)	8349-8702 (354)	8352-8705 (354)	8336-8689 (354)	8341-8694 (354)
<i>trnA</i>	8248-8313 (66)	8273-8338 (66)	8279-8344 (66)	8282-8347 (66)	8266-8331 (66)	8271-8336 (66)
<i>trnR</i>	8164-8225 (62)	8189-8250 (62)	8195-8256 (62)	8198-8259 (62)	8182-8243 (62)	8187-8248 (62)
<i>trnN</i>	8089-8153 (65)	8114-8178 (65)	8120-8184 (65)	8123-8187 (65)	8107-8171 (65)	8112-8176 (65)
<i>trnS1</i>	8022-8089 (68)	8047-8114 (68)	8053-8120 (68)	8056-8123 (67)	8040-8107 (68)	8045-8112 (68)
<i>trnE</i>	7937-8003 (67)	7944-8010 (67)	7960-8026 (67)	7962-8027 (66)	7950-8015 (66)	7953-8018 (66)
<i>trnF</i>	7869-7934 (66)	7876-7941 (66)	7892-7957 (66)	7894-7959 (66)	7882-7947 (66)	7885-7950 (66)
<i>ND5</i>	6093-7841 (1749)	6102-7850 (1749)	6102-7850 (1749)	6108-7856 (1749)	6106-7854 (1749)	6102-7850 (1749)
<i>trnH</i>	6028-6092 (65)	6037-6101 (65)	6037-6101 (65)	6043-6107 (65)	6041-6105 (65)	6037-6101 (65)
<i>ND4</i>	4688-6027 (1340) ²	4697-6036 (1340) ²	4697-6036 (1340) ²	4703-6042 (1340) ²	4701-6040 (1340) ²	4697-6036 (1340) ²
<i>ND4L</i>	4381-4665 (285)	4390-4674 (285)	4390-4674 (285)	4396-4680 (285)	4394-4678 (285)	4390-4674 (285)
<i>trnT</i>	4304-4368 (65)	4313-4377 (65)	4312-4377 (66)	4318-4383 (66)	4317-4381 (65)	4312-4377 (66)
<i>trnP</i>	4239-4303 (65)	4248-4312 (65)	4247-4311 (65)	4253-4317 (65)	4252-4316 (65)	4247-4311 (65)
<i>ND6</i>	3692-4228 (537)	3701-4237 (537)	3700-4236 (537)	3706-4242 (537)	3705-4241 (537)	3700-4236 (537)
<i>CytB</i>	2471-3631 (1161)	2474-3634 (1161)	2479-3639 (1161)	2483-3643 (1161)	2486-3646 (1161)	2478-3638 (1161)
<i>trnS₂</i>	2400-2468 (69)	2403-2471 (69)	2408-2476 (69)	2412-2480 (69)	2415-2483 (69)	2407-2475 (69)
<i>ND1</i>	1433-2371 (939)	1436-2374 (939)	1441-2379 (939)	1445-2383 (939)	1445-2383 (939)	1440-2378 (939)
<i>trnL₁</i>	1364-1432 (69)	1367-1435 (69)	1372-1440 (69)	1376-1444 (69)	1376-1444 (69)	1371-1439 (69)
<i>lrRNA</i>	12-1363 (1352)	12-1366 (1355)	12-1371 (1360)	12-1375 (1364)	12-1375 (1364)	12-1370 (1359)
<i>trnV</i>	15535-15600 (66)	15609-15674 (66)	15628-15693 (66)	15618-15683 (66)	15521-15586 (66)	15572-15637 (66)
<i>srRNA</i>	14743-15528 (786)	14817-15608 (792)	14837-15621 (785)	14826-15611 (786)	14730-15514 (785)	14779-15565 (787)
A+T-rich region	14344-14741 (398)	14369-14816 (448)	14375-14835 (461)	14378-14824 (447)	14361-14728 (368)	14367-14777 (411)

Superscripts indicate identical start and stop codons among the gypsy moth species. Values in parentheses indicate gene size (bp). †, re-annotated in this study.

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Lee, W.: Gyeongsang National University, Professor, Ph.D.; Examined specimens and designed the research

All authors read and approved the manuscript.

Statements for Authorship Position & Contribution

Jeong, N.R.: Gyeongsang National University, Student in Ph.D; Designed the research, wrote the manuscript, and conducted the experiments

Nam, Y.: National Institute of Forest Science, Researcher; Collected and examined specimens

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