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Complete Mitochondrial Genome of *Mythimna loreyi* (Duponchel, 1827) (Lepidoptera: Noctuidae) in South Korea

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국내 뒷흰가는줄무늬밤나방의 미토콘드리아 게놈(mitochondrial genome) 분석

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ABSTRACT: *Mythimna loreyi* (Duponchel, 1827) (Lepidoptera: Noctuidae) is a pest that damages agricultural plants, such as rice, wheat, and maize. We sequenced the entire 15,314-bp mitochondrial genome of this species. It has a typical set of genes (13 protein-coding genes, two ribosomal RNA genes, and 22 transfer RNA genes) as well as one major non-coding A+T-rich region. Using concatenated sequences of 13 protein-coding genes and two rRNAs (13,376 bp, including gaps), phylogenetic analysis demonstrated that the sister relationship between *M. loreyi* and *M. separata* had the highest nodal support. The monophyly of each family (Noctuidae, Euteliidae, Nolidae, Erebidae, and Notodontidae) of the superfamily Noctuoidea was supported by the highest nodal support.

Key words: Mitochondrial genome, *Mythimna loreyi*, Noctuidae, Rice armyworm, South Korea

초 록: 뒷흰가는줄무늬밤나방은 쌀, 밀, 옥수수와 같은 농작물에 피해를 주는 해충이다. 본 연구에서는 국내 뒷흰가는줄무늬밤나방의 미토콘드리아 게놈(15,314b)을 분석하였다. 13개의PCG와2개의 rRNA (13,376bp)를 연결한 서열을 사용한 계통발생 분석 결과, 뒷흰가는줄무늬밤나방과 멸강나방 사이의 가장 높은 노드 수치로 자매분류군을 형성하였다. 밤나방상과(Noctuoidea)의 각 과(Noctuidae, Euteliidae, Nolidae, Erebidae 및 Notodontidae)들은 가장 높은 노드수치로 단계통을 형성하였다.

검색어: 미토콘드리아게놈, 뒷흰가는줄무늬밤나방, 밤나방과, 한국

Mythimna loreyi (Duponchel, 1827) (Lepidoptera: Noctuidae) is distributed in Asia, Africa, Australia, and the Middle East (CABI, 2023) and is known to cause damage to Poaceae species such as rice, wheat, maize, sugarcane, barley, and sorghum (El-Sherif, 1972; Harai, 1975). In South Korea, *M. loreyi* was first collected at an adult stage in 1982 (Ahn et al., 1994) and has recently been shown to damage maize in South Korea (Nam et al., 2020).

M. separata is a well-known migratory insect in the genus *Mythimna*. *M. separata* can fly 12 km in 12 h in the laboratory (Li et al., 1964). *M. loreyi* has also been described as a migratory insect in Europe (Sparks et al., 2007), with a flight range of 32 km in 12 h. *M. loreyi* has been found in South Korea since 1982, but the economic impact and distribution have yet to be calculated. In South Korea, *M. loreyi* is frequently found in the same field as *M. sepatata.* However,

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these two species cannot be distinguished based on their morphological characteristics at the larval stage (Kim et al., 2020). This suggests that *M. loreyi* could be confused with *M. separata* (Nam et al., 2020).

The insect mitochondrial genome (mitogenome) is a circular molecule, 14−19 kb in size, and contains 22 transfer RNA (tRNA) genes and two ribosomal RNA genes encoding small and large subunit rRNAs (rrnS and rrnL), which are involved in the translation of 13 protein-coding genes (PCGs), and a large non-coding element termed the A + T-rich region, which contains both the origin for replication and transcription (Moritz et al., 1987; Wolstenholme, 1992; Cameron, 2014). Owing to their unique features, including the conservation of coding content, maternal inheritance, and rapid evolution, mitogenome sequences have been widely used as informative molecular markers for diverse evolutionary studies among species in the fields of molecular evolution, phylogenetics, population genetics, and comparative and evolutionary genomics (Harrison, 1989; Boore, 1999; Whinnett et al., 2005; Lopez-Vaamonde et al., 2012; Timmermans et al., 2014). To date, two *M. loreyi* mitogenomes have been reported in GenBank. However, one (MZ853169) has unpublished data, while another (MT506351) was used in the development of loop-mediated isothermal amplification (LAMP) (Nam et al., 2020). As yet, there have been no reports on its genome structure and features.

To better understand the insect mitogenome, taxon and genome sampling must be expanded. The analysis of the *M. loreyi* mitogenome has increased our understanding of insect mitogenomes and provided new insights into the mechanisms underpinning mitochondrial DNA evolution, particularly gene rearrangements. In this study, the entire mitogenome of *M. loreyi* was newly sequenced, and the mitogenome was then submitted to phylogenetic studies involving comparison with *M. separata,* which has a similar larval morphology.

Materials and Methods

Genomic DNA extraction

One wild adult was collected in a pheromone trap in Goheung, Jeollanam Province, South Korea (34° 38' 28.8" N, 127° 24' 39.6" E) for the study. Following the manufacturer's instructions, genomic DNA was extracted from the hind legs using an AccuPrep® Genomic DNA Extraction Kit, and 100% ethanol (Bioneer, Daejeon, Korea). Excess DNA and specimens were deposited at the Gyeongsang National University, Jinju, South Korea.

Mitochondrial genome sequencing

Using the extracted DNA, three long overlapping fragments (LFs; COI-ND5, ND5-lrRNA, and ND1-COI) were amplified using three sets of primers developed based on previously reported *M. loreyi* data. The developed primers were used to amplify 25 overlapping short fragments (SF) using the LFs as templates. The following settings were used for the LF polymerase chain reaction (PCR): 4 min initial denaturation at 94°C, followed by 30 cycles of 10 sec at 98°C, 15 min at 50°C, and a final 10-min extension at 72°C. SF PCR was performed as follows: initial denaturation for 4 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 48°C, and 1 min at 72°C, with a final 7 min extension at 72°C. AccuPower PCR PreMix (Bioneer, Daejeon, Korea) was used for PCR. To validate effective DNA amplification, electrophoresis was performed on a 1.5% agarose gel using 0.5 × TAE buffer. All products were sequenced bidirectionally.

Because clear direct sequences of *M. loreyi* SF13 and SF22 could not be obtained, they were sequenced after cloning, whereas the remaining SFs were sequenced directly after PCR purification. *M. loreyi* SFs were sequenced directly after purification using AccuPrep PCR Purification Kit reagents (Bioneer, Daejeon, Korea). The pGEM-T Easy vector (Promega, Madison, WI, USA) and HIT DH5a High 108 competent cells (Real Biotech Co., Banqiao City, Taiwan) were used for cloning. The resulting plasmid DNA was isolated using a Plasmid Mini Extraction Kit (Bioneer, Daejeon, Korea). All products were sequenced bidirectionally.

Boundary delimitation and annotation

Individual SF sequences were manually assembled into complete mitogenomes using SeqMan software (DNASTAR, Madison, WI, USA). The MITOS WebServer (http://mitos.

bioinf.uni-leipzig.de/index.py) was used for tRNA identification, boundary delimitation, and secondary structure folding with the search mode set to default, Mito/Chloroplast as the searching source, and the genetic code of invertebrate mitogenomes for tRNA isotype prediction (Lowe and Chan, 2016). To identify individual PCGs, the blastn program in BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used with the default settings. Gene delimitation and confirmation of start and stop codons were performed using MAFFT ver. 7 (Katoh and Standley, 2013) to align available PCGs with those of other Noctuoidea species. The two rRNAs and the A + T-rich region were identified and delimited using nucleotide blastn in BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and validated by the alignment of other Noctuoidea rRNA and A + T-rich region sequences using MAFFT ver. 7 (Katoh and Standley, 2013). *M. loreyi* sequence data have been deposited in the GenBank database under the accession number OQ536314.

Genomic comparison between *M. loreyi* and *M. separata*

Two *M. loreyi* mitogenome sequences and three *M. separata* sequences of were obtained from GenBank and compared with *M. loreyi* mitogenome sequences obtained in this investigation. Pairwise comparisons were used to evaluate the degree of sequence divergence of each of the 13 PCGs of *M. loreyi* and *M. separata.* When there were multiple mitogenome sequences from the same species, the average divergence of the species was used to calculate the sequence divergence of each gene within the genus. These values were then plotted using the boxplot in JMP software ver. 15.0.0 (SAS Institute, Cary, NC, USA) to show the minimum, maximum, and median values.

Phylogenetic analysis

Phylogenetic analysis was performed using 18 mitogenomes from the Noctuoidea, including those obtained in the present study (Fig. 1). The nucleotide sequence of each PCG was aligned based on codons using RevTrans ver. 2.0 (Wernersson and Pedersen, 2003). Each of the two rRNA genes was individually aligned using MAFFT ver. 7 (Katoh and Standley, 2013). The nucleotide sequences of all protein-coding genes (PCGs) and rRNAs were aligned, and well-aligned blocks were selected using GBlocks 0.91b software (Castresana, 2000), with the maximum number of contiguous non-conserved positions set to 15 and gap positions allowed. Subsequently,

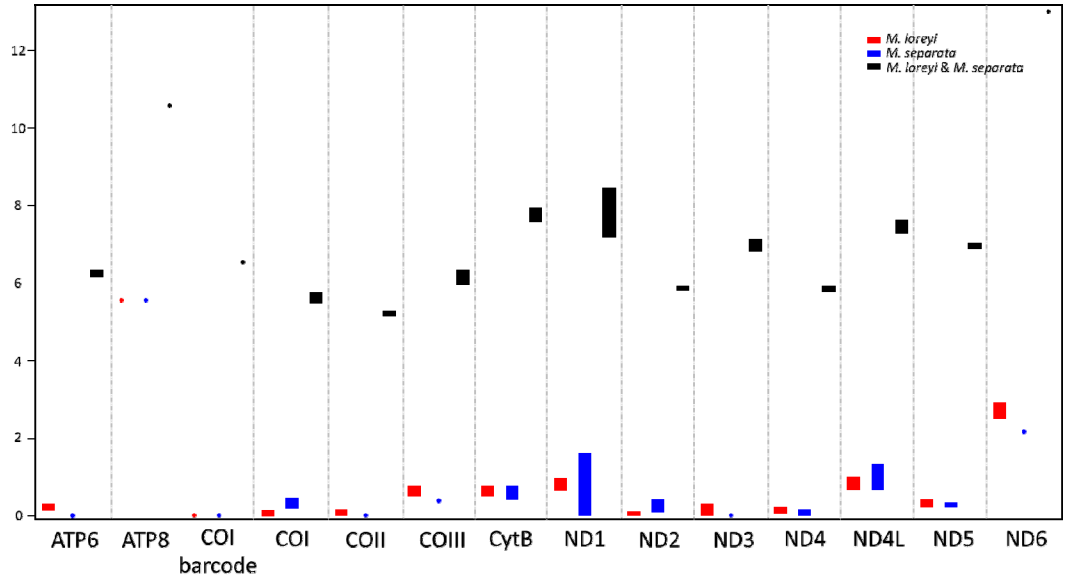

Fig. 1. Boxplot distribution of interspecific and intraspecific genetic divergence for 13 protein coding genes in *Mythimna loreyi* and *M. separata*. Dots indicate the maximum and minimum divergence (%), respectively. Boxplots indicate range of the maximum and minimum divergence.

13 PCGs and two rRNAs were concatenated in the alignment (13,376 bp, including gaps).

Bayesian inference (BI) and maximum likelihood (ML) methods were applied using MrBayes version 3.2.6 (Ronquist et al., 2012) and RAxML-HPC2 version 8.0.24 (Stamatakis, 2014), respectively, and were incorporated into the CIPRES Portal version 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 every 100 generations. The first 25% of the trees were rejected as burn-ins. Partitioned analyses were performed, with each partition unlinked to each parameter (state freq, revmat, shape, pinvar, or tratio). To depict the convergence of two concurrent runs, an average split frequency of less than 0.01 was chosen. For ML analysis, we used the RAxML algorithm, which employs a "rapid" bootstrapping approach to find the best-scoring tree. The confidence values for the BI and ML trees were calculated using Bayesian posterior probabilities (BPP) and 1,000 bootstrap (BS) iterations. PartitionFinder 2 with a greedy algorithm was used to discover an optimal partitioning scheme (six partitions) and substitution model (GTR + Gamma + I) (Lanfear et al., 2014; 2016). *Conogethes pinicolalis* (Jeong et al., 2021), *Chilo suppressalis* (Park et al., 2019), and *Ostrinia kasmirica* (Luo et al., 2021) which belong to the family Crambidae of the superfamily Pyraloidea, were used as outgroups. Phylogenetic trees were visualized using FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Genome structure, organization, and composition

The *M. loreyi* mitogenome was 15,314 bp long (Table 1), with typical gene sets (2 rRNAs, 22 tRNAs, and 13 PCGs) and a major non-coding A + T-rich region of 332 bp long (GenBank accession number OQ536314). Previous research has found that the shortest mitogenome is 15,312 bp long (MZ853169; unpublished) and the longest is 15,320 bp (MT506351; Nam et al., 2020). The length of *M. loreyi* was comparable to that of *M. separata*, ranging from 15,329 bp in KM099034 (unpublished data) to 15,332 bp in HG793808 (Yang and Kong, 2016) and KF730242 (Li et al., 2015). Each *M. loreyi* and *M. separata* mitogenome comprised 37 genes, including 13 PCGs, 22 tRNA genes, and two rRNA genes (Table 1), all of which are typically found in animals (Boore, 1999).

The orientation and gene order of the *M. loreyi* and *M. separata* mitogenomes were identical to those of many other lepidopteran groups. They do, however, differ from the most common type (A + T-rich region-*trn*I-*trn*Q-*trn*M-ND2), which is thought to be ancestral to insects (Boore, 1999), as well as from the lepidopteran superfamilies Hepialoidea and Nepticuloidea. The only difference was that *trn*M was moved upstream of *trn*I, giving rise to the order *trn*M-*trn*I-*trn*Q in the A+T-rich region and ND2 junction (Table 1).

Protein-coding genes

Except for COI and ND1, *M. loreyi* and *M. separata* exhibited typical ATN codons that were identical in the remaining 11 PCGs. However, the start codons for ATP8, ND6, and ND1 differed between the two species, with ATA and ATT for ATP8 in *M. loreyi* and *M. separata*, ATT and ATC for ND3 in *M. loreyi* and *M. separata*, and ATA and TTG for ND1 in both species (Table 1). The ATN, GTG, TTG, and GTT start codons were those previously identified in animal mtDNA (Wolstenholme, 1992). Ten of the 13 PCGs in both *M. loreyi* and *M. separata* mitogenomes had a complete termination codon, TAA, while the remaining three PCGs (COI, COII, and ND4) had an incomplete termination codon, T (Table 1).

Individual gene divergence

The sequence divergence among the three *M. loreyi* mitogenomes ranged from 0% (COI, COII, ND2, and ND3) to 5.556% (ATP8), with 0% divergence in the DNA barcoding region (Fig. 1, Table 2). The sequence divergence among the three *M. separata* mitogenomes ranged from 0% (ATP6, COII, ND1, ND3, and ND4) to 5.556% (ATP8), with 0% divergence in the DNA barcoding region, indicating relatively low sequence divergence between the intra-specific genomic sequences (Fig. 1, Table 2).

Table 1. Summaries of six mitochondrial genomes of *Mythimna*

Gene	Anticodon	Start codon	Stop codon	*Mythimna loreyi* (This study)	*Mythimna loreyi* (MZ853169)	*Mythimna loreyi* (MT506351)	*Mythimna separata* (HG793808)	*Mythimna separata* (KF730242)	*Mythimna separata* (KM099034)
trnM	CAT			1-68 (68)	1-68 (68)	1-68 (68)	1-68 (68)	1-68 (68)	1-68 (68)
trnI	GAT			69-133 (65)	69-133 (65)	69-133 (65)	69-133 (65)	69-133 (65)	69-133 (65)
trnQ	TTG			131-199 (69)	131-199 (69)	131-199 (69)	131-199 (69)	131-199 (69)	131-199 (69)
ND2		ATT	TAA	250-1263 (1014)	250-1263 (1014)	250-1263 (1014)	250-1260 (1011)	250-1260 (1011)	250-1260 (1011)
trnW	TCA			1262-1330 (69)	1262-1330 (69)	1262-1330 (69)	1259-1328 (70)	1259-1328 (70)	1259-1328 (70)
trnC	GCA			1323-1388 (66)	1323-1388 (66)	1323-1388 (66)	1321-1385 (65)	1321-1385 (65)	1321-1385 (65)
trnY	GTA			1395-1460 (66)	1395-1460 (66)	1395-1460 (66)	1391-1455 (65)	1391-1455 (65)	1391-1455 (65)
COI		CGA	T-tRNA	1473-3006 (1534)	1473-3006 (1534)	1473-3006 (1534)	1458-2988 (1531)	1458-2991 (1534)	1458-2991 (1534)
trnL$_2$	TAA			3007-3073 (67)	3007-3073 (67)	3007-3073 (67)	2992-3058 (67)	2992-3058 (67)	2992-3058 (67)
COII		ATT	T-tRNA	3074-3755 (682)	3074-3755 (682)	3074-3755 (682)	3059-3740 (682)	3059-3740 (682)	3059-3740 (682)
trnK	CTT			3756-3826 (71)	3756-3826 (71)	3756-3826 (71)	3741-3811 (71)	3741-3811 (71)	3741-3811 (71)
trnD	GTC			3837-3905 (69)	3837-3905 (69)	3837-3905 (69)	3832-3898 (67)	3832-3898 (67)	3832-3898 (67)
ATP8		ATA†, ATT‡	TAA	3906-4064† (159)	3906-4064† (159)	3906-4064† (159)	3899-4057‡ (159)	3899-4057‡ (159)	3899-4057‡ (159)
ATP6		ATG	TAA	4058-4735 (678)	4058-4735 (678)	4058-4735 (678)	4051-4728 (678)	4051-4728 (678)	4051-4728 (678)
COIII		ATG	TAA	4735-5523 (789)	4735-5523 (789)	4735-5523 (789)	4728-5516 (789)	4728-5516 (789)	4728-5516 (789)
trnG	TCC			5526-5594 (69)	5526-5594 (65)	5526-5594 (69)	5519-5583 (65)	5519-5583 (65)	5519-5583 (65)
ND3		ATT	TAA	5595-5948 (354)	5595-5948 (354)	5595-5948 (354)	5584-5937 (354)	5584-5937 (354)	5584-5937 (354)
trnA	TGC			5952-6017 (66)	5952-6018 (67)	5952-6018 (67)	5947-6013 (65)	5947-6013 (65)	5947-6013 (65)
trnR	TCG			6018-6083 (65)	6018-6082 (64)	6018-6083 (65)	6013-6077 (65)	6013-6077 (65)	6013-6077 (65)
trnN	GTT			6084-6149 (66)	6083-6148 (66)	6084-6149 (66)	6083-6149 (67)	6083-6149 (67)	6083-6149 (67)
trnS$_1$	GCT			6153-6218 (66)	6152-6217 (66)	6152-6220 (69)	6147-6217 (71)	6147-6217 (71)	6147-6217 (71)
trnE	TTC			6220-6286 (67)	6219-6285 (67)	6220-6286 (67)	6219-6284 (66)	6219-6284 (66)	6219-6285 (67)
trnF	GAA			6305-6372 (68)	6304-6371 (68)	6305-6372 (68)	6293-6359 (67)	6293-6359 (67)	6294-6360 (67)
ND5		ATT	TAA	6372-8117 (1746)	6371-8116 (1746)	6372-8117 (1746)	6364-8109 (1746)	6364-8109 (1746)	6365-8110 (1746)
trnH	GTG			8118-8186 (69)	8117-8185 (69)	8118-8186 (69)	8110-8175 (66)	8110-8175 (66)	8111-8176 (66)
ND4		ATG	T-tRNA	8187-9525 (1339)	8186-9524 (1339)	8187-9525 (1339)	8176-9514 (1339)	8176-9514 (1339)	8177-9515 (1339)
ND4L		ATG	TAA	9587-9877 (291)	9586-9876 (291)	9595-9885 (291)	9539-9829 (291)	9539-9829 (291)	9540-9830 (291)
trnT	TGT			9980-9944 (65)	9879-9944 (66)	9888-9953 (66)	9832-9896 (65)	9832-9896 (65)	9833-9897 (65)
trnP	TGG			9945-10009 (66)	9945-10009 (66)	9954-10018 (65)	9897-9962 (66)	9897-9962 (66)	9898-9962 (65)
ND6		ATT†, ATC‡	TAA	10017-10547† (531)	10017-10547†(531)	10026-10556† (531)	9970-10503‡ (534)	9970-10503‡ (534)	9970-10503‡ (534)
CytB		ATG	TAA	10557-11705 (1149)	10557-11705 (1149)	10566-11714 (1149)	10506-11654 (1149)	10506-11654 (1149)	10506-11654 (1149)
trnS$_2$	TGA			11705-11770 (66)	11705-11770 (66)	11714-11779 (66)	11658-11724 (67)	11658-11724 (67)	11658-11724 (67)
ND1		ATA†, TTG‡	TAA	11790-12722† (933)	11789-12721† (933)	11798-12736† (939)	11744-12682‡ (939)	11744-12682‡ (939)	11743-12681‡ (939)
trnL$_1$	TAG			12729-12796 (68)	12728-12795 (68)	12737-12804 (68)	12683-12750 (68)	12683-12750 (68)	12682-12749 (68)
lrRNA				12797-14136 (1340)	12819-14106 (1289)	12805-14143 (1339)	12751-14108 (1358)	12751-14108 (1358)	12750-14108 (1359)
trnV	TAC			14137-14203 (67)	14136-14202 (67)	14144-14210 (67)	14109-14174 (66)	14109-14174 (66)	14109-14174 (66)
srRNA				14204-14982 (779)	14203-14971 (769)	14211-14992 (782)	14175-14958 (784)	14175-14958 (784)	14175-14957 (783)
A+T−rich region				14983-15314 (332)	15022-15297 (276)	14993-15320 (328)	14959-15332 (374)	14959-15332 (374)	14958-15329 (372)

Superscripts indicate identical start and stop codons among *Mythimna* species. Values in parentheses indicate gene size (bp).

The divergence between *M. loreyi* and *M. separata* in the 658 bp DNA barcoding region was 6.535%, which was lower than the divergence in ATP8 (10.556%), CytB (7.928%), ND1 (8.733%), ND3 (7.123%), ND4L (7.616%), ND5 (7.009%), and ND6 (12.996%) (Fig. 1, Table 2). These findings suggest that genes with higher sequence divergence, rather than the DNA barcoding region, may be more useful for population-level studies, possibly by providing more variable nucleotide

Table 2. Estimates of intraspecific and interspecific genetic divergence for mitochondrial protein coding genes of *Mythimna loreyi* and *M. separata*

Taxon		ATP6	ATP8	COI barcode	COI	COII	COIII	CytB	ND1	ND2	ND3	ND4	ND4L	ND5	ND6
Mythimna loreyi	Min.	0.147	5.556	0	0	0	0.507	0.512	0.639	0	0	0.072	0.662	0.228	2.527
	Max.	0.294	5.556	0	0.13	0.147	0.760	0.767	0.958	0.099	0.285	0.217	0.993	0.399	2.888
	Aver.	0.196	5.556	0	0.09	0.098	0.634	0.654	0.816	0.066	0.190	0.145	0.883	0.342	2.768
Mythimna separata	Min.	0	5.556	0	0.196	0	0.380	0.426	0	0.099	0	0	0.662	0.228	2.166
	Max.	0	5.556	0	0.457	0	0.380	0.767	1.597	0.395	0	0.145	1.325	0.342	2.166
	Aver.	0	5.556	0	0.305	0	0.380	0.653	1.065	0.296	0	0.097	1.104	0.304	2.166
M. loreyi & *M. separata*	Min.	6.167	10.556	6.535	5.479	5.140	5.957	7.502	6.922	5.824	6.838	5.725	7.285	6.895	12.996
	Max.	6.314	10.556	6.535	5.740	5.286	6.337	7.928	8.733	5.923	7.123	5.942	7.616	7.009	12.996
	Aver.	6.265	10.556	6.535	5.610	5.237	6.168	7.786	7.703	5.857	7.028	5.846	7.395	6.971	12.996

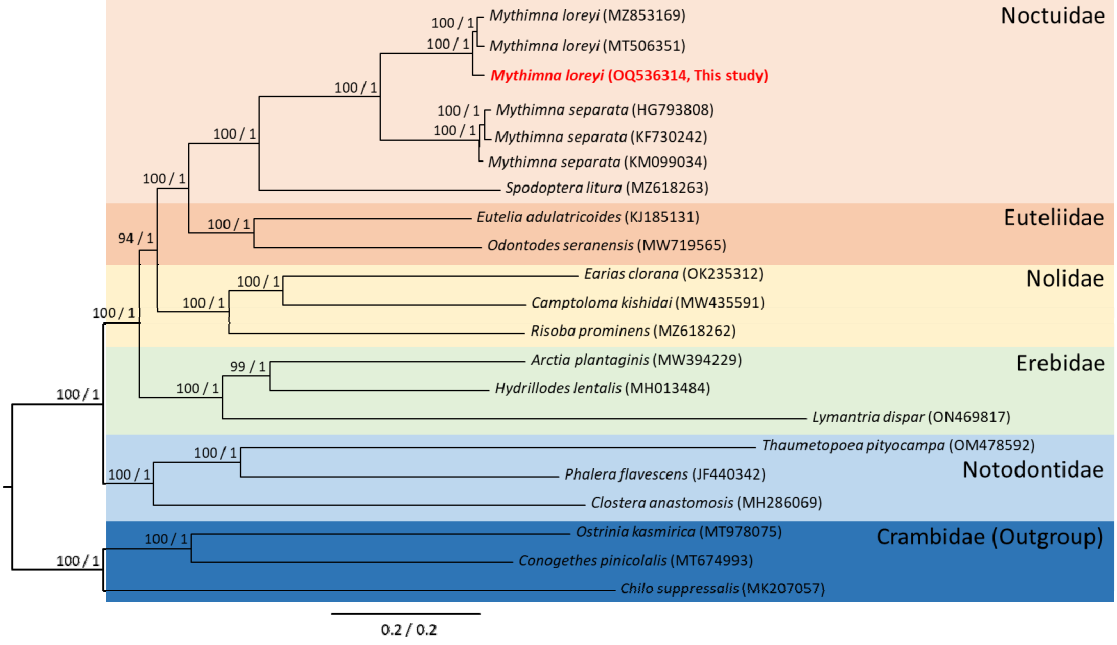

Fig. 2. Phylogenetic tree for the superfamily Noctuoidea. The tree was constructed using the concatenated 13 PCGs and 2 rRNAs via the maximum-likelihood (ML) and Bayesian Inference (BI) methods. The numbers at each node specify bootstrap percentages of 1000 pseudo-replicates by ML analysis and Bayesian posterior probabilities in percent by 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: *Mythimna loreyi*, MZ853169 (Unpublished); *Mythimna loreyi*, MT506351 (Nam et al., 2020); *Mythimna separata*, HG793808 (Yang and Kong, 2016); *Mythimna separata*, KF730242 (Li et al., 2015); *Mythimna separata*, KM099034 (Unpublished); *Spodoptera litura*, MZ618263 (Unpublished); *Eutelia adulatricoides*, KJ185131 (Yang et al., 2015); *Odontodes seranensis*, MW719565 (Unpublished); *Camptoloma kishidai*, MW435591 (Unpublished); *Earias clorana*, OK235312 (Unpublished); *Risoba prominens*, MZ618262 (Unpublished); *Hydrillodes lentalis*, MH013483 (Yang et al., 2019); *Arctia plantagninis*, MW394229 (Galarza and Mappes, 2021); *Lymantria dispar*, ON469817 (Jeong et al., 2021); *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).

positions required for a comprehensive examination of the evolutionary history of the given populations.

Phylogenetic analysis

Phylogenetic analysis was performed on 18 mitogenome sequences, including the mitogenome of *M. loreyi* analyzed in this study, using concatenated 13 PCGs and two rRNAs. These were composed of five Noctuoidea families (Noctuidae, Euteliidae, Nolidae, Erebidae, and Notodontidae) (Fig. 2). The topologies of the PCG and rRNA datasets analyzed using the ML and BI algorithms were identical. The monophyly of each family was supported by the highest nodal support, revealing familial relationships of (((Noctuidae + Euteliidae) + Nolidae) + Erebidae)) + Nodontidae) in the superfamily Noctuoidea (Fig. 2). Furthermore, the greatest nodal support (BS = 94-100%, BPP = 1.0; Fig. 2) often confirmed the monophyly of each family. In both analyses, the sister relationship between *M. loreyi* and *M. separata* had the highest nodal support (BS = 100%, BPP = 1.0; Fig. 2).

Discussion

The current study indicates that *M. loreyi* and *M. separata*, which is morphologically difficult to distinguish are molecularly distinct by analyzing the newly entire mitogenomes of *M. loreyi*. *M. loreyi* and *M. separata* are distinct at the start codons of ATP8, ND6, and ND1.

In addition, Individual gene divergence analyses clearly evidenced substantial divergence between these two species. Phylogenetic analysis showed a distinct separation between these two species. Collectively, our results confirmed that two species are molecularly distinct and mitochondrial genes with higher sequence divergence will be useful for population-level studies.

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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

Jeong, D.: National Institute of Agricultural Sciences, Researcher; Collected and examined specimens

Lee, G.-S.: National Institute of Agricultural Sciences, Researcher; Collected and examined specimens

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

All authors read and approved the manuscript.

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