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Genomic Structure of the Luciferase Gene of the Firefly, *Lampyris noctiluca*

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We describe here the complete nucleotide sequence and the exon-intron structure of the luciferase gene of the firefly, *Lampyris noctiluca*. The luciferase gene of the *L. noctiluca* firefly consisted of six introns and seven exons coding for 547 amino acid residues. From the translational start site to the end of last exon, the genomic DNA length of the *L. noctiluca* luciferase gene spans 1,976 bp.

Key words: Firefly, *Lampyris noctiluca*, Luciferase gene, Genomic structure

Introduction

The firefly is widely distributed throughout worldwide, except for the South Pole and the North Pole (Minami, 1983). Approximately 2,000 firefly species are found in worldwide. In Korea, eight firefly species have been reported (The Entomological society of Korea and Korean Society of Applied Entomology, 1994): *Drilaster unicolor* belonging to Dirlastinae, *Luciola lateralis*, *Hotaria unmunsana* and *H. papariensis* belonging to Luciolinae, and *Lampyris noctiluca*, *Lucidina accensa*, *Lucidina biplaiata* and *Pyrocoelia rufa* belonging to Lampyrinae.

The European firefly, *L. noctiluca*, is known to occur mainly in Europe. In the case of Korea, the *L. noctiluca* is known to occur mainly in north part of Korea. *Lampyris* can be found glowing throughout Europe, from May to August, although July is the favoured month. Green light ($\lambda_{max} \approx 550$ nm) is emitted by the firefly at all stages of the life cycle (Sala-Newby *et al.*, 1996). The luminescent signal of the firefly has been recognized as an important method of sexual communication. The mating protocol of Lloyds signal system I is represented by the *L. noctiluca* (Lloyd, 1971, 1978, 1983). In this species, a female broadcasts a continuous signal and a flying male recognize it. The sequences of a cDNA encoding the luciferase of *L. noctiluca* have been reported (Sala-Newby *et al.*, 1996).

In this study, we analyzed the genomic structure of the luciferase from *L. noctiluca* collected at Bekdu Mt., Korea (or Jangbek Mt. for China), and compared the sequence with the luciferase cDNA sequence of *L. noctiluca* found in United Kingdom, Europe (Sala-Newby *et al.*, 1996).

Materials and Methods

Insects

The firefly, *Lampyris noctiluca* used in this study was collected at Bekdu Mt., Korea (or Jangbek Mt. for China) from July to August 2001.

PCR of *L. noctiluca* luciferase genomic DNA

Genomic DNA was extracted from the larvae of *L. noctiluca* by Wizard™ Genomic DNA Purification Kit, according to the manufacturer's instructions (Promega). The primers used for amplification of the genomic DNA of the luciferase from the *L. noctiluca* were 5-ATGGAAGATGATAGTAAACATATTATGCAT-3' for the translational start sequence region and 5'-TTACAATTTGGATTTTTGTCCCATTGTAAGG-3' for the 3 coding region, based on the luciferase cDNA of *L. noctiluca* (Sala-Newby *et al.*, 1996). After a 35-cycles amplification (94°C for 1 min; 55°C for 1 min; 72°C for 1 min), PCR products

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were ethanol precipitated, centrifugated at 10,000 × g for 15 min, and rinsed with 70% ethanol. These DNAs were analyzed 1.0% agarose gel electrophoresis. The PCR products for sequencing were cloned into pGem-T vector (Promega, Madison, WI).

DNA sequencing and data analysis

DNA sequencing was performed using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). With the twelve GenBank-registered amino acid sequences of luciferase genes, phylogenetic analysis was performed using PAUP (Phylogenetic Analysis using Parsimony) ver. 4.0b8 (Swofford, 2000). The accession numbers of the sequences in the GenBank are as follows: *Lampyris noctiluca* BD (this study), *Lampyris noctiluca* (X89479), *Pyrocoelia rufa* (AF328553), *Pyrocoelia miyako* (L39928), *Photinus pyralis* (M15077), *Luciola lateralis* (U51019), *Luciola cruciata* (M26194), *Hotaria parvula* (L39929), *Hotaria unmunsana* (AF420006), *Luciola mingrelica* (S61961), *Photuris pennsylvanica* (U31240), *Phrixothrix hirtus* (AF139645), and *Phrixothrix vivianii* (AF139644).

Results and Discussion

The firefly, *L. noctiluca* was collected at Bekdu Mt., Korea (or Jangbek Mt. for China) during summer season. To identify the genomic DNA of the luciferase gene of the firefly, we have designed the PCR primer set based on the sequences of the luciferase cDNA of *L. noctiluca* already known (Sala-Newby *et al.*, 1996). The genomic DNA of the luciferase gene of the *L. noctiluca* firefly was cloned and sequenced (Fig. 1).

Comparison of the genomic sequence with the sequence of cloned luciferase cDNA in the *L. noctiluca* revealed the presence of seven exons (Fig. 1 and 2). From the known cDNA 5 site to the end of exon 7, the gene is 1,976 bp long and consisted of 547 amino acid residues. Although the genomic DNA length is different, the genomic structure of the luciferase gene in various firefly species consisted of six introns and seven exons. The genomic structure of *L. noctiluca* luciferase gene revealed that the six introns separate the seven exons. This is the same as that of luciferases from other fireflies, such as *L. lateralis* (Kim *et al.*, 2004), *Hotaria*-group fireflies (Choi *et al.*, 2003), and *P. rufa* (Li *et al.*, 2003).

The intron boundaries are listed in Fig. 2. The consensus sequences, including an invariant GT at the intron 5 boundary and an invariant AG at its 3 boundary were very well conserved in the *L. noctiluca* genomic sequence, as is true for other firefly luciferase genes, *L. lateralis* (Kim *et*

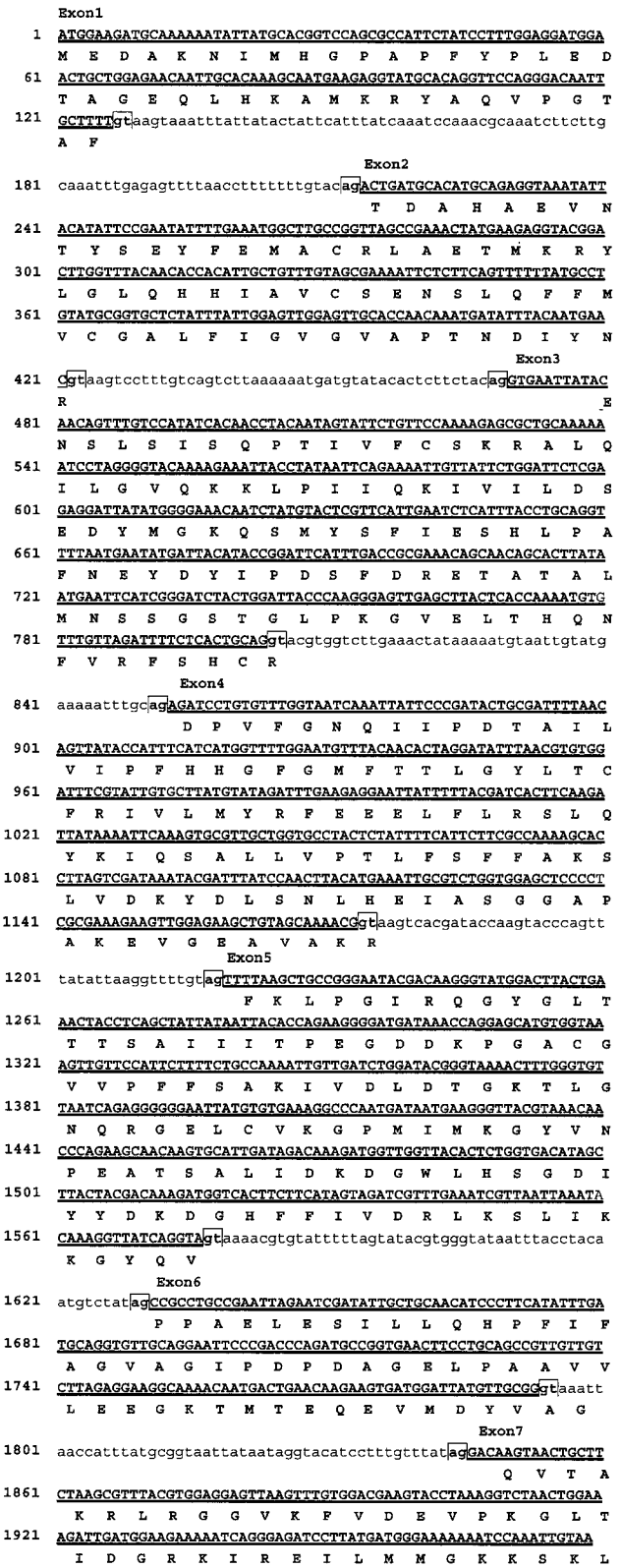
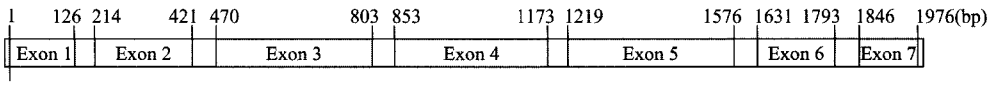


Fig. 1. The nucleotide sequence and genomic organization of *L. noctiluca* luciferase gene. Nucleotide numbers are presented on the left, and the first base of initiation codon of the ORF is defined as +1. Exons are labeled with bold-lines.

A



B

Exon	Length of exon(bp)	Position in gene close	Sequence at exon-intron junction		
			M E D A	.....	I A F
1	126	1-126	ATGGAAGATGCA	.....	ATTGCTTTTgtaagtaaat
			T D A H	.....	Y N E
2	208	214-421	tgtagACTGATGCACAT	.....	TACAATGAACgtaagtcc
			R E L Y	.....	S H C R
3	334	470-803	tctacagGTGAATTATAC	.....	TCTCACTGCAGgtacgt
			D P V	.....	V A K R
4	321	853-1173	atttgcagAGATCCTGTG	.....	TAGCAAAACGgtaagtc
			F K L	.....	G Y Q V
5	358	1219-1576	ggttttgtagTTTTAATTTA	.....	GGTTATCAGGTAgtaaa
			P P A	.....	Y V A
6	163	1631-1793	atgtctatagCCGCCTGCC	.....	TATGTTGCGGgtaaatat
			G Q V T	.....	K L Stop
7	131	1846-1976	tttatagGACAAGTAAC	.....	AATTGTtaa

Fig. 2. Genomic organization of the luciferase gene of the *L. noctiluca* firefly. (A) Exon/intron structures. Numbers indicate the length (bp) of exons and introns. (B) Lengths of exons and exon/intron boundaries.

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                                          30                                  60
L. noctiluca (X89479)  MEDAKNIMHG PAPFYPLEDG TAGEQLHKAM KRYAQVPGTI AFTDAHAEVN ITYSEYFEMA
L. noctiluca BD        .......... .......... .......... .......... .......... ..........
                                          90                                 120
L. noctiluca (X89479)  CRLAETMKRY GLGLQHHIAV CSENSLQFFM PVCGALFIGV GVASTNDIYN ERELYNSLSI
L. noctiluca BD        .......... .......... .......... .......... ...P...... ..........
                                         150                                 180
L. noctiluca (X89479)  SQPTIVSCSK RALQKILGVQ KKLPIIQKIV ILDSREDYMG KQSMYSFIES HLPAGFNEYD
L. noctiluca BD        ......F... .......... .......... .......... .......... ..........
                                         210                                 240
L. noctiluca (X89479)  YIPDSFDRET ATALIMNSSG STGLPKGVEL THQNVCVRFS HCRDPVFGNQ IIPDTAILTV
L. noctiluca BD        .......... .......... .......... .....F.... .......... ..........
                                         270                                 300
L. noctiluca (X89479)  IPFHHGFGMF TTLGYLTCGF RIVLMYRFEE ELFLRSLQDY KIQSALLVPT LFSFFAKSTL
L. noctiluca BD        .......... .......... .......... .......... .......... ..........
                                         330                                 360
L. noctiluca (X89479)  VDKYDLSNLH EIASGGAPLA KEVGEAVAKR FKLPGIRQGY GLTETTSAII ITPEGDDKPG
L. noctiluca BD        .......... .......... .......... .......... .......... ..........
                                         390                                 420
L. noctiluca (X89479)  ACGKVVPFFS AKIVDLDTGK TLGVNQRGEL CVKGPMIMKG YVNNPEATSA LIDKDGWLHS
L. noctiluca BD        .......... .......... .......... .......... .......... ..........
                                         450                                 480
L. noctiluca (X89479)  GDIAYYDKDG HFFIVDRLKS LIKYKGYQVP PAELESILLQ HPFIFDAGVA GIPDPDAGEL
L. noctiluca BD        .......... .......... .......... .......... .......... ..........
                                         510                                 540
L. noctiluca (X89479)  PAAVVVLEEG KTMTEQEVMD YVAGQVTASK RLRGGVKFVD EVPKGLTGKI DGRKIREILM
L. noctiluca BD        .......... .......... .......... .......... .......... ..........
                     547
L. noctiluca (X89479)  MGKKSKL
L. noctiluca BD        .......
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Fig. 3. The deduced amino acid sequence of the luciferase gene of the *L. noctiluca* firefly. Residues are numbered according to the *L. noctiluca* luciferase cDNA sequences previously known (Sala-Newby *et al.*, 1996), and identical residues are dotted.

Table 1. Pairwise identities and similarities of the deduced amino acid sequences among *L. noctiluca* luciferase and other luciferase genes

Animal	Percent Similarity 1	2	3	4	5	6	7	8	9	10	11	12	13
1. *L. noctiluca* BD		99	96	96	92	81	80	81	80	80	76	72	69
2. *L. noctiluca* (X89479)	99		96	95	92	81	80	80	80	80	76	73	69
3. *P. rufa* (AF139645)	95	95		98	89	78	78	79	79	78	74	72	68
4. *P. miyako* (L39928)	95	94	98		89	78	78	79	78	78	74	72	70
5. *P. pyralis* (M15077)	84	84	81	81		80	80	80	79	80	75	72	70
6. *L. lateralis* (U51019)	68	68	66	66	67		97	89	90	90	71	69	66
7. *L. cruciata* (M26194)	67	67	65	65	67	93		89	89	89	71	70	67
8. *L. mingrelica* (S61961)	66	65	64	64	66	80	80		97	97	71	69	67
9. *H. parvula* (L39929)	65	65	64	63	66	81	80	97		98	72	70	67
10. *H. unmunsana* (AF420006)	65	65	63	63	66	80	80	96	97		72	70	67
11. *P. pennsylvanica* (U31240)	61	61	59	59	59	55	55	53	54	54		70	67
12. *P. hirtus* (AF139644)	53	53	52	52	54	51	52	51	51	51	49		85
13. *P. vivianii* (AF139645)	47	47	46	48	48	46	47	46	46	46	45	70	

Percent Identity

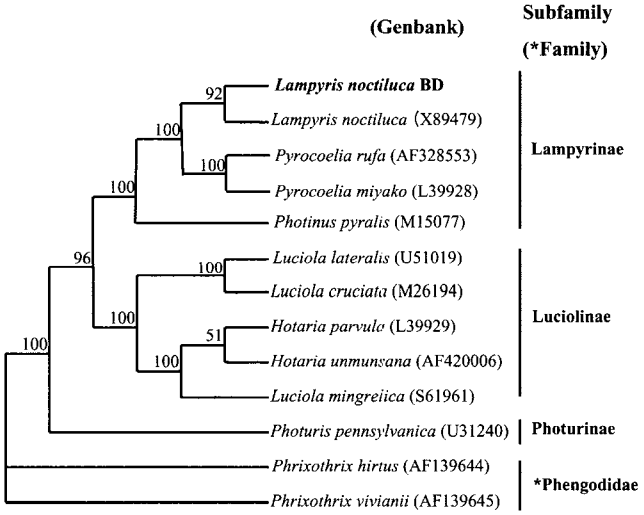

Fig. 4. A phylogenetic tree for aligned amino acid sequences of the *L. noctiluca* luciferase and the known luciferases. The sequences were extracted from; *L. noctiluca* BD (this study), *L. noctiluca* (Sala-Newby *et al.*, 1996), *P. rufa* (Lee *et al.*, 2002), *P. miyako* (Ohmiya *et al.*, 1995), *P. pyralis* (De Wet *et al.*, 1987), *L. lateralis* (Tatsumi *et al.*, 1992), *L. cruciata* (Masuda *et al.*, 1989), *H. parvula* (Ohmiya *et al.*, 1995), *H. unmunsana* (Choi *et al.*, 2002), *L. mingrelica* (Devine *et al.*, 1993), *P. pennsylvanica* (Ye *et al.*, 1997), *P. hirtus* (Viviani *et al.*, 1999), and *P. vivianii* (Viviani *et al.*, 1999). The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1,000 replicates.

al., 2004), *Hotaria*-group fireflies (Choi *et al.*, 2003), and *P. rufa* (Li *et al.*, 2003).

The deduced amino acid sequence of the luciferase gene of the Korean *L. noctiluca* sample differed from amino acid residues at three positions 104, 127 and 216 in the previous known *L. noctiluca* (Fig. 3). The deduced amino acid sequences were compared with those of known luciferase genes. The deduced amino acid sequence of the luciferase gene of the Korean *L. noctiluca* showed 99.0% protein identity to the previous known *L. noctiluca* and 95% – 84% protein identity to other species within the Lampyrinae subfamily, while the lowest identity was found with *P. vivianii* (Table 1). A phylogenetic analysis using the amino acid sequences revealed that the deduced amino acid sequences of the *L. noctiluca* luciferase gene formed a subgroup with *P. rufa*, *P. miyako* and *P. pyralis* sequences within the Lampyrinae subfamily (100% of bootstrap value) (Fig. 4).

Acknowledgments

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