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Molecular cloning, sequence analysis, and expression of a cDNA encoding the luciferase from the glow-worm, *Lampyris turkestanicus*☆

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Abstract

The first cDNA from lampyridae encoding a glow-worm luciferase from lantern mRNA of *Lampyris turkestanicus* has been cloned, sequenced, the amino acid sequence predicted, and the sequence reported to GenBank. The cDNA was 1644 base pairs in length and coding a 547-residue protein. The deduced amino acid sequence of the luciferase gene of *L. turkestanicus* showed 98.7% and 95.8% identity to *Lampyris noctiluca* and *Pyrocoelia rufa*, respectively. Phylogenetic analysis further confirmed that the deduced amino acid sequences of *L. turkestanicus* luciferase gene belong to the same subfamily, Lampyrinae. The cDNA encoding the luciferase of *L. turkestanicus* was expressed as a 62 kDa band in recombinant *Escherichia coli* and showed green luminescence in the presence of luciferin. Amongst amino acid differences of *L. turkestanicus* and *L. noctiluca* (its clade) there are two important substitutions. Signature amino-acid sequences and motifs found in the deduced sequence are CK2-phospho site, ASN-glycosylation, myristoylation site, PKC-phospho site, microbodies C-terminal targeting signal, and AMP-binding domain.

Keywords: Luciferase; *Lampyris turkestanicus*; Bioluminescence; Glow-worm; Firefly; AMP-binding domain

Luciferase is a generic term describing any enzyme that catalyzes a reaction yielding visible light. Light emission is a consequence of the formation of a product or intermediate in an electronically excited state; return to the ground state occurs via emission of a photon light. Luciferases are highly diverse, catalyzing a great variety of reactions and using widely different substrates. Firefly luciferase generates bioluminescence by catalyzing the oxidation of luciferin in the presence of ATP, Mg^{2+}, and oxygen [1]. Chemical modification and mutagenesis studies have failed to identify any amino acid residue involved in substrate binding or enzyme catalysis [2]. The crystal structure of *Photinus pyralis* luciferase was published earlier [3], but without bound substrates or other ligands as noted by Baldwin [4]. However, some amino acids are suspected to be important for catalysis, one of the active site residues for the color determination (green to yellow) must be located in the fragment between 208 and 318 [5]. Along with extensive commercial interest, the firefly luciferase genes are increasingly used as a reporter gene in molecular biology experiments; enzymatic assay of the luciferase is mainly preferred due to several merits, such as

☆ *Abbreviations*: AMIDATION, amidation site; ASN_GLYCOSYLATION, N-glycosylation site; CK2_PHOSPHO_SITE, casein kinase II phosphorylation site; MICROBODIES_CTER, microbodies C-terminal targeting signal; MYRISTYL, N-myristoylation site; AMP_BINDING, putative AMP-binding domain signature; PKC_PHOSPHO_SITE, protein kinase phosphorylation site; SERPIN, serpins (serine protease inhibitors) signature.

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sensitivity, rapidity, and the non-invasive method of quantification.

The bioluminescent reaction has application in a wide range of analytical techniques [6]. The scope of its application includes: the ultra-sensitive detection of ATP [7]; the detection of phosphatase activity [8]; use in DNA sequencing [9]; and as a tool for monitoring in vivo protein folding and chaperonin activity [10]. The gene for firefly luciferase of the North American firefly, *P. pyralis*, was cloned and sequenced by DeLuca and colleagues [11]. Since those studies, the luciferase genes have been isolated from several species of fireflies [12–18]. However, the luciferase gene has been isolated from four other species of lampyrinae [19], although at least five species of the genus occur only from Middle East to Europe. Studies have shown that two species of firefly exist in the north of Iran. One of these, which is more available, has the scientific name *Lampyris turkestanicus* and its luciferin has only been characterized [20], whereas the other one's (rare one) name is *Lampyroidea maculata* [21].

This paper reports the cloning and sequencing of a cDNA for luciferase from *L. turkestanicus*; this is the first species from Lampyridae subfamily, in Iran, and its luciferase sequence was determined and reported to GenBank. The overall goal of this study was to increase the knowledge concerning amino acids that might function in catalyzing light production by luciferin oxidation and obtaining a unique sequence from another subfamily of fireflies. Comparison of the amino acid sequences of several firefly luciferases may indicate which amino acids are functionally important since these should be conserved among the various species. The sequence of *L. turkestanicus* luciferase has been deposited in the GenBank as entry AY742225.

Materials and methods

Glow-worm collection. The glow-worm *L. turkestanicus* was collected from the Amol forest, Mazandaran province, Northern Iran, and stored in jars until the evening's was completed. The live fireflies were taken to the laboratory and immediately frozen in liquid nitrogen. The frozen fireflies were stored in a −80 °C freezer.

Extraction of total RNA. The lanterns of frozen glow-worm *L. turkestanicus* were removed under liquid nitrogen and then 1.0 g of lantern was pulverized under liquid nitrogen with a mortar and pestle. RNA extraction kit was used for total RNA isolation (CinnaGen, Cat. No. RN7712C). The RNA was analyzed by 1.0% agarose gel electrophoresis. The concentration of extracted RNA was determined by UV absorption spectroscopy at 260 nm.

RT-PCR of L. turkestanicus luciferase gene. The specific primers used for synthesis and amplification of cDNA encoding the luciferase were 5′-ATGGAAGACGCAAAAAATATTATGCACG-3′ for the translational start-sequence region and 5′-TTACAATTTGGATTTTTTTCCCATCATAAGG-3′ for the 3′ coding region (reverse primer), based on the luciferase gene of *Lampyris noctiluca* (GenBank Accession No. X89479). The first strand of cDNA was synthesized at 42 °C for 60 min in the presence of 200 U/μL M-MuLV Reverse transcriptase (Fermentas, #EP0441), 20 U RNase inhibitor, dNTPmix (final concentration each at 1 mM), and reverse primer. The PCR amplification of cDNA was carried out by use of first cDNA strand and under the following condition: initial denaturation at 94 °C for 5 min, a 35-cycle amplification (94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min), and a final extension for 5 min at 72 °C. The cloning primers 5′-TTCGATGGATCCATGGAAGATGCAAAAAAT-3′ for the translational start-sequence region and 5′-CTAAGCAAGCTTTTAC AATTTGGATTTTTT-3′ for the 3′ coding region were designed based on luciferase gene of *L. noctiluca* (GenBank Accession No: X89479) at *Bam*HI/*Hin*dIII restriction sites. The cDNA fragments coding for *L. turkestanicus* luciferase that were digested by *Bam*HI/*Hin*dIII were inserted into the *Bam*HI/*Hin*dIII restriction sites of digested/dephosphorylated PQE30 high expression vector and ligated mixtures were transformed into competent cells of *Escherichia coli* XL1-Blue (CinnaGen, Cat. No. BA7605C) by electroporation.

Expression. Expression was achieved by following a procedure similar to that of Devine et al. [15]. Two LB-ampicillin/tetracycline plates were used for the first screening and incubated at 37 °C overnight. The master plates were sprayed with 1 mM luciferin (in 0.1 M Tris acetate) for 5 min, wrapped with Saran Wrap, and exposed to X-ray film for 1 h. After film development, the positive colonies (bioluminescent) were identified (they also could be observed by eye after dark-adaptation). Three colonies were picked from master plates, and streaked onto fresh LB-ampicillin/tetracycline. Positive colonies were purified by plating and screening. Plasmid containing luciferase gene was purified by a Geno Pure Plasmid Midi Kit (Cat. No. 3143414, Roche)

DNA sequencing and data analysis. The cDNA was sequenced using an automatic sequencer (MWG, Germany). With the 17 GenBank-registered amino acid sequences of luciferase genes, phylogenetic analysis was performed using PUAP version 4.0b10a and alignments were carried out using sequence navigator and adjusted by eye. All sequences used in the phylogenetic analysis were obtained from the GenBank and accession numbers are as follows: *L. turkestanicus* (AY742225, this study); *Pyrocoelia rufa* (AF328553); *Pyrocoelia miyako* (L39928); *L. noctiluca* (X89479); *P. pyralis* (M15077); *Photuris pennsylvanica* (D245415); *P. pennsylvanica* (D245416); *Luciola lateralis* (U51019); *L. lateralis* (Q01158); *Luciola cruciata* (M26194); *Luciola mingrelica* (S61961); *Hotaria parvula* (L39929); *Hotaria unmunsana* (AF420006); *Phrixothrix viviannii* (AF139644); *Phrixothrix hirtus* (AF139645); and *Pyrophorus plagiophthalamus* (S29353 and S29354).

Computer analysis of data. The following programs and databases were used: GenBank, NCBI; ProDom at the ExPASy Server [22].

Results

The cDNA library was prepared from lanterns of locally collected glow-worm *L. turkestanicus*, expressed in *E. coli*, and screened for light production after luciferin

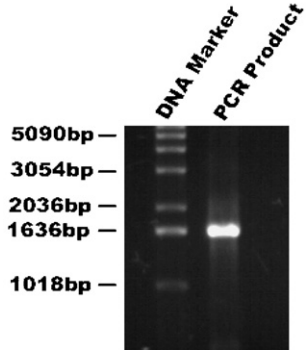

Fig. 1. RT-PCR product of luciferase gene on 1% agarose gel. Lane 1, size marker; lane 2, luciferase RT-PCR product, 1644 bp in length. For further details please see Materials and methods.

addition. Since the screening detected expressed bioluminescence, only functional cDNA sequences were identified. The PCR product on 1% agarose gel electrophoresis was about 1.7 kb which is sufficient to code for the entire luciferase polypeptide of approx. 547 amino acid residues.

Cloning, sequence analysis, and characterization of the cDNA encoding L. turkestanicus luciferase

To identify a cDNA encoding the luciferase of the glow-worm, *L. turkestanicus*, we designed a RT-PCR primer set based on the sequences of the *L. noctiluca*

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1     ATG GAA GAT GCA AAA AAT ATT ATG CAC GGT CCA CCG CCA TTC TAT CCT TTG GAG GAT GGA
1     M   E   D   A   K   N   I   M   H   G   P   P   P   F   Y   P   L   E   D   G

61    ACT GCT GGA GAA CAA TTG CAC AAA GCA ATG AAG AGG TAT GCA CAG GTT CCA GGG ACA ATT
21    T   A   G   E   Q   L   H   K   A   M   K   R   Y   A   Q   V   P   G   T   I

121   GCT TTT ACC GAT GCA CAT GCA GAG GTA  AAT ATT ACA TAT TCC GAA TAT TTT GAA ATG GCT
41    A   F   T   D   A   H   A   E   V   N   I   T   Y   S   E   Y   F   E   M   A

181   TGC CGG TTA GCC GAA ACT ATG AAG AGG TAC GGA CTT GGT TTA CAA CAC CAC ATT GCT GTT
61    C   R   L   A   E   T   M   K   R   Y   G   L   G   L   Q   H   H   I   A   V

241   TGC AGC GAA AAT TCT CTT CAG TTT TTT ATG CCT GTA TGC GGT GCT CTA TTT ATT GGA GTT
81    C   S   E   N   S   L   Q   F   F   M   P   V   C   G   A   L   F   I   G   V

301   GGA GTT GCA CCA ACA AAC GAT ATT TAC AAT GAA CGT GAA TTA TAC AAC AGT TTG TCC ATA
101   G   V   A   P   T   N   D   I   Y   N   E   R   E   L   Y   N   S   L   S   I

361   TCA CAA CCT ACA ATA GTA TTC TGT TCC AAA AGA GCG CTG CAA AAA ATC CTA GGG GTA CAA
121   S   Q   P   T   I   V   F   C   S   K   R   A   L   Q   K   I   L   G   V   Q

421   AAG AAA TTA CCT ATA ATT CAG AAA ATT GTT ATT CTG GAT TCT CGA GAG GAT TAT ATG GGG
141   K   K   L   P   I   I   Q   K   I   V   I   L   D   S   R   E   D   Y   M   G

481   AAA CAA TCT ATG TAC TCG TTC ATT GAA TCT CAT TTA CCT GCA GGT TTT AAT GAA TAT GAT
161   K   Q   S   M   Y   S   F   I   E   S   H   L   P   A   G   F   N   E   Y   D

541   TAC ATA CCG GAT TCA TTT GAC CGC GAA ACA GCA ACA GCA CTT ATA ATG AAT TCA TCG GGA
181   Y   I   P   D   S   F   D   R   E   T   A   T   A   L   I   M   N   S   S   G

601   TCT ACC GGA TTA CCC AAG GGA GTT GAG CTT ACT CAC AAA AAC ATT TGT GTT AGA TTT TCT
201   S   T   G   L   P   K   G   V   E   L   T   H   K   N   I   C   V   R   F   S

661   CAC TGC AGA GAT CCT GTG TTT GGT AAT CAA ATT ATT CCC GAT ACT GCG ATT TTA ACG GTT
221   H   C   R   D   P   V   F   G   N   Q   I   I   P   D   T   A   I   L   T   V

721   ATA CCA TTT CAT CAT GGT TTT GGA ATG TTT ACA ACA CTA GGA TAT TTA ACG TGT GGA TTT
241   I   P   F   H   H   G   F   G   M   F   T   T   L   G   Y   L   T   C   G   F

781   CGT ATT GTG CTT ATG TAT AGA TGT GAA GAG GAA TTA TTT TTA CGA TCA CTT CAA GAT TAT
261   R   I   V   L   M   Y   R   C   E   E   E   L   F   L   R   S   L   Q   D   Y

841   AAA ATT CAA AGT GCG TTG CTG GTA CCT ACC CTA TTT TCA TTC TTT GCC AAA AGC ACC TTA
281   K   I   Q   S   A   L   L   V   P   T   L   F   S   F   F   A   K   S   T   L

901   GTC GAC AAA TAC GAT TTA TCG AAC TTA CAT GAA ATT GCT TCT GGT GGA GCT CCC CTC GCG
301   V   D   K   Y   D   L   S   N   L   H   E   I   A   S   G   G   A   P   L   A

961   AAA GAA GTT GGA GAA GCT GTA GCA AAA CGT TTT AAG CTG CCG GGC ATA CGA CAA GGG TAT
321   K   E   V   G   E   A   V   A   K   R   F   K   L   P   G   I   R   Q   G   Y

1021  GGA CTT ACT GAG ACT ACC TCA GCA ATT ATA ATT ACA CCC GAA GGG GAT GAT AAA CCA GGA
341   G   L   T   E   T   T   S   A   I   I   I   T   P   E   G   D   D   K   P   G

1081  GCA TGT GGT AAA GTT GTT CCA TTC TTT TCT GCC AAA ATT GTT GAT CTG GAT ACG GGA AAA
361   A   C   G   K   V   V   P   F   F   S   A   K   I   V   D   L   D   T   G   K

1141  ACT TTG GGT GTT AAT CAG AGG GGG GAA TTA TGT GTG AAA GGC CCA ATG ATA ATG AAG GGT
381   T   L   G   V   N   Q   R   G   E   L   C   V   K   G   P   M   I   M   K   G

1201  TAC GTA AAC AAC CCA GAA GCA ACA AGT GCA TTG ATA GAC AAA GAC GGA TGG TTA CAC TCT
401   Y   V   N   N   P   E   A   T   S   A   L   I   D   K   D   G   W   L   H   S

1261  GGT GAC ATA GCT TAC TAC GAC AAA GAT GGT CAC TTC TTC ATA GTA GAT CGT TTG AAA TCG
421   G   D   I   A   Y   Y   D   K   D   G   H   F   F   I   V   D   R   L   K   S

1321  TTA ATT AAA TAC AAA GGT TAT CAG GTA CCG CCT GCC GAA TTA GAA TCG ATA TTG CTG CAA
441   L   I   K   Y   K   G   Y   Q   V   P   P   A   E   L   E   S   I   L   L   Q

1381  CAT CCG TTC ATA TTT GAT GCA GGT GTT GCA GGA ATT CCC GAC CCA GAT GCC GGT GAA CTT
461   H   P   F   I   F   D   A   G   V   A   G   I   P   D   P   D   A   G   E   L

1441  CCT GCA GCC GTT GTT GTC TTA GAG GAA GGC AAA ACG ATG ACT GAA CAA GAA GTG ATG GAT
481   P   A   A   V   V   V   L   E   E   G   K   T   M   T   E   Q   E   V   M   D

1501  TAT GTT GCG GGA CAA GTA ACT GCT TCT AAG CGT TTA CGT GGA GGA GTT AAG TTT GTG GAC
501   Y   V   A   G   Q   V   T   A   S   K   R   L   R   G   G   V   K   F   V   D

1561  GAA GTA CCT AAA GGT CTA ACT GGG AAG ATT GAT GCA AGA AAA ATC AGG GAG ATC CTT ATG
521   E   V   P   K   G   L   T   G   K   I   D   A   R   K   I   R   E   I   L   M

1621  ATG GGA AAA AAA TCC AAA TTG TAA
541   M   G   K   K   S   K   L   *
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Fig. 2. The nucleotide and deduced amino acid sequences of *Lampyris turkestanicus* luciferase gene. The start codon of ATG is boxed and the termination codon is asterisked. The GenBank Accession No. is AY742225.

Table 1
Pairwise comparisons among amino acid sequences of the *L. turkestanicus* luciferase gene and the known luciferase genes

Species	GenBank number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. *L. turkestanicus*	AY742225	—	0.012	0.045	0.043	0.153	0.290	0.283	0.379	0.333	0.335	0.329	0.312	0.319	0.511	0.509	0.466	0.504
2. *L. noctiluca*	X89475	7	—	0.045	0.043	0.155	0.294	0.286	0.385	0.337	0.338	0.333	0.312	0.319	0.513	0.511	0.466	0.506
3. *P. miyako*	L39928	25	25	—	0.010	0.175	0.306	0.302	0.398	0.351	0.352	0.347	0.326	0.333	0.525	0.523	0.469	0.507
4. *P. rufa*	AF328553	24	24	6	—	0.173	0.306	0.302	0.392	0.349	0.351	0.345	0.324	0.332	0.521	0.519	0.469	0.507
5. *P. pyralis*	M19077	84	85	96	95	—	0.303	0.297	0.400	0.320	0.322	0.318	0.318	0.318	0.527	0.524	0.444	0.497
6. *P. pennsylvanica*	D25415	58	160	167	167	166	—	0.023	0.432	0.386	0.386	0.386	0.378	0.382	0.519	0.517	0.476	0.506
7. *P. pennsylvanica*	D25416	154	156	165	165	163	13	—	0.430	0.384	0.384	0.384	0.376	0.376	0.517	0.515	0.479	0.506
8. *P. pennsylvanica*	U31240	207	210	217	214	218	235	234	—	0.450	0.450	0.459	0.436	0.440	0.514	0.511	0.500	0.524
9. *H. parvula*	L39929	181	183	191	190	174	210	209	244	—	0.020	0.020	0.182	0.186	0.511	0.516	0.476	0.520
10. *H. unmunsana*	AF420006	182	184	192	191	175	210	209	244	11	—	0.032	0.190	0.190	0.509	0.513	0.476	0.520
11. *L. mingrelica*	S61961	179	181	189	188	173	210	209	249	11	18	—	0.184	0.190	0.513	0.518	0.476	0.520
12. *L. lateralis*	U51019	170	170	178	177	173	206	205	237	100	104	101	—	0.065	0.515	0.521	0.483	0.515
13. *L. cruciata*	M26194	174	174	182	181	173	208	205	239	102	104	104	36	—	0.502	0.508	0.466	0.510
14. *P. plagiophthalamus*	S29353	275	276	283	281	284	280	279	276	274	273	275	277	270	—	0.020	0.521	0.535
15. *P. plagiophthalamus*	S29354	274	275	282	280	282	279	278	274	277	275	278	280	273	11	—	0.512	0.528
16. *P. vivianii*	AF139644	253	253	255	255	241	258	260	271	258	258	258	262	253	280	275	—	0.267
17. *P. hirtus*	AF139655	270	271	272	272	266	271	271	280	278	278	278	276	273	285	281	144	—

Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute distance values.

luciferase gene already known [23]. To assess the *L. turkestanicus* luciferase gene, the 1644-bp luciferase cDNA was inserted to a PQE30 vector. The molecular sizes of the amplified PCR products were identical to that expected (Fig. 1). The nucleotide sequence of PCR products was analyzed and its amino acid sequence was deduced. As the result of the complete nucleotide and amino acid sequences shown in Fig. 2, the 1644-bp luciferase gene has an open reading frame of 547 amino acid residues. The nucleotide and amino acid sequences were compared with those of known luciferase genes.

The deduced amino acid sequence of the luciferase gene of *L. turkestanicus* showed 98.7% and 95.8% identity to *L. noctiluca* and *P. rufa*, respectively, while the lowest identity was found with *P. plagiophthalamus* (Table 1). Phylogenetic analysis using amino acid sequence data showed that *L. turkestanicus* is a sister taxon to *L. noctiluca* within the Lampyrinae subfamily (90% bootstrap value) (Fig. 3). These two are, in turn, allied with a clade of two another species of Lampyrinae namely; *P. rufa* and *P. miyako* (Fig. 3 and Table 1).

Expression of L. turkestanicus luciferase in E. coli XL1-Blue

We constructed the expression plasmid PQE30-Luc to confirm that the cDNA cloned in PQE30 was the luciferase cDNA. The PQE30-Luc vector was used to generate recombinant *E. coli* expressing luciferase as described in Materials and methods. Transfer vector PQE30-Luc was constructed by digestion of PQE30 with *Bam*HI and *Hin*dIII, and ligation with *L. turkestanicus* luciferase gene under the control of T_5 promoter *E. coli* XL1-Blue was transformed by recombinant vector PQE30-Luc with electroporation. To verify the luciferase expressed in *E. coli* cells, the transformed cell extracts were assayed for luciferase activity by checking of light emission in the dark (Fig. 4). To examine the expression of luciferase gene by recombinant cells, the protein synthesis in XL1-Blue cells that were transformed by recombinant vector was analyzed by SDS–PAGE. The luciferase protein expressed by the *L. turkestanicus* gene was present as a band of about 62 kDa.

Discussion

We have cloned cDNA encoding the luciferase in *L. turkestanicus* and expressed it functionally in *E. coli* (Fig. 4). The complete sequences of this cDNA comprised a 1644 bp encoding the luciferase of 547 amino acid residues. The results reported here show that the luciferase responsible for catalyzing the light-emitting reaction in the glow-worm *L. turkestanicus* has 98.7% sequence similarity with that of the glow-worm *L. noctiluca* and 85.3% with firefly *P. pyralis*, although three

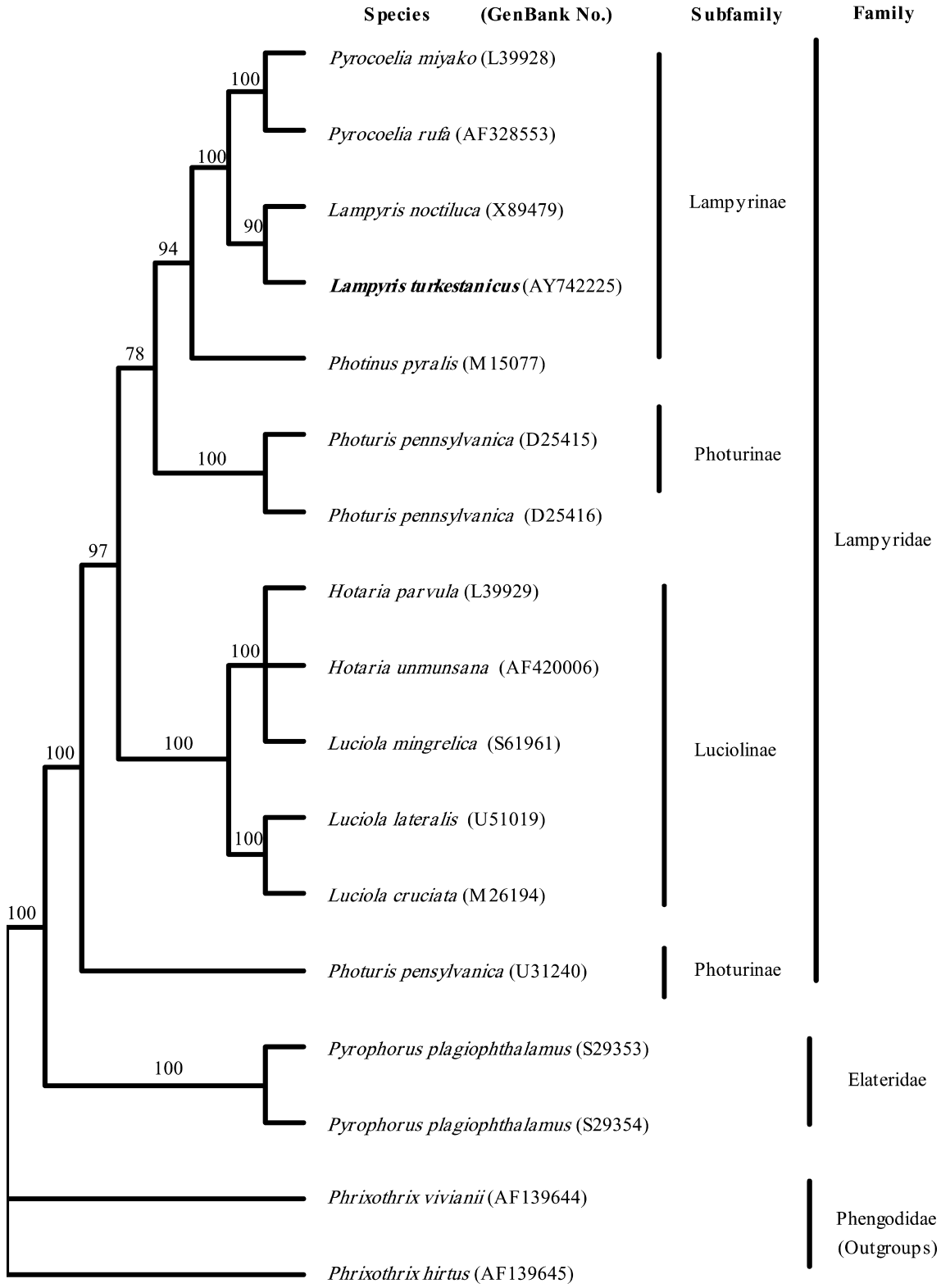

Fig. 3. A phylogenetic tree for aligned amino acid sequences of the *Lampyris turkestanicus* luciferase and the known luciferases. The sequences were extracted from: AY742225, *Lampyris turkestanicus* (this study); AF420006, *Hotaria unmunsana*; U51019, *Luciola lateralis*; M26194, *Luciola cruciata*; AF328553, *Pyrocoelia rufa*; L39928, *Pyrocoelia miyako*; X89479, *L. noctiluca*; M15077, *Photinus pyralis*; U31240, *Photuris pennsylvanica*; AF139644, *Phrixothrix vivianii*; AF139645, *Phrixothrix hirtus*; D25415, firefly mRNA for *P. pennsylvanica*; D25416, firefly mRNA for *Photuris pennsylvanica*; S61961, *Luciola mingrelica*; L39929, *Hotaria parvula*; S29354, *Pyrophorus plagiophthalamus*; and S29353, *P. plagiophthalamus*. The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1000 replicates. The outgroup was chosen as *Phrixothrix hirtus* on the basis of the sequence homology by pairwise comparison.

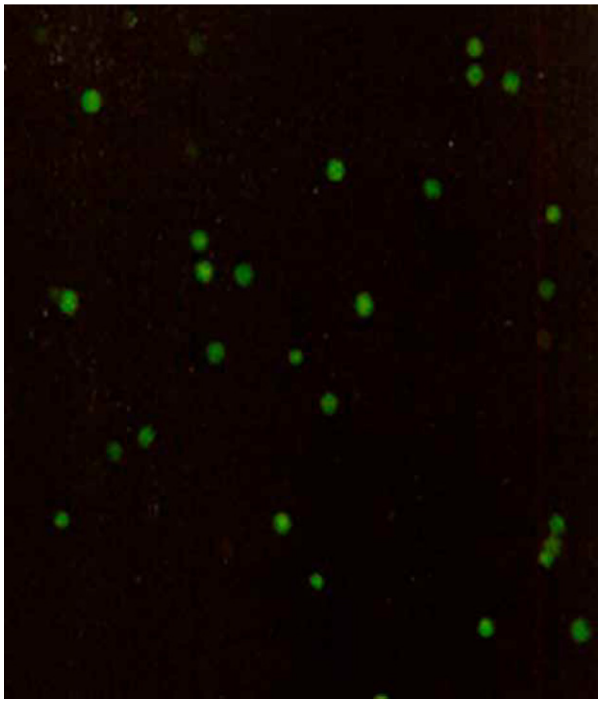

Fig. 4. Pictures of glowing colonies were taken with a camera by long exposure of Konica 400 ASA film. For further details please see Materials and methods.

residues shorter than it. The phylogenetic analysis of the *L. turkestanicus* luciferase gene with that of other light-emitting beetles showed a close relationship among the species of lampyrinae, including *L. noctiluca*, forming a monophyletic group. In contrast, relatively low genetic homologies are determined between the *L. turkestanicus* luciferase gene and those genes of the Luciolinae group.

Glow-worm *L. turkestanicus* luciferase has a C-terminus that showed the same key characteristics as those of *P. pyralis* and *L. noctiluca*. There are two important substitutions in *L. turkestanicus* gene compared with *L. noctiluca*, in a region that cannot be mutated without having a major deleterious effect on luciferase activity [24,25]. Lys213 in *L. turkestanicus* is replaced by Gln and Arg in *L. noctiluca* and *P. pyralis*, respectively. It should be noted; these residues with amino side chains (in Lampyrinae sub family) are converted to Glu with negative carboxylate side chain in Luciolinae. Two other important differences are conversion of Ala12 and Ser104 in *L. noctiluca* to Pro in *L. turkestanicus*. It may be suggested these residues have a key role in the control of luminescence emission. Similar critical residues have been found for control of luminescence in other luciferases [26]. In *L. turkestanicus*, the peroxisomal targeting tripeptide SKL was found as well as most firefly and glow-worm luciferases, and in all railroad-worm luciferases [27].

Related sequence and domain structure

When predicted amino-acid sequence was used as the input sequence for computer-based searches for similarity, high scoring related sequences were found between *L. turkestanicus* luciferase, and 4-coumarate CoA ligase, long chain CoA ligase, and some other proteins. These relationships have been reported for the other firefly luciferases.

Using ProSite [22] a domain structure map for the predicted amino acid sequence of *L. turkestanicus* glow-worm luciferase is developed. Fig. 5 illustrates these results and Table 2 defines the sites, different

Table 2
Different motifs and sites in *Lampyris turkestanicus* luciferase

Amino acid position	Motif information
541 MGKK 544	AMIDATION
21 TAGE24 52 TYSE 55 154 SRED157 166 SFIE 169 276 SLQD 279 299 TLVD 302 492 TMTE 495	CK2_PHOSPHO_SITE
50 NITY 53 197 NSSG 200	ASN_GLYCOSYLATION
101 GVAPTN 106 203 GLPKGV 208 248 GMFTTL 253 316 GAPLAK 321 335 GIRQGY 3407 341 GLTETT 346 504 AGQVTAS 509	MYRISTYL
66 TMK 68 129SKR131 211 THK 213 370 SAK 372 378 TGK 380 509 SKR511 527 TGK 529	PKC_PHOSPHO_SITE
545 SKL 547	Microbodies C-terminal targeting signal
195 IMNSSGSTGLPK 206	AMP_BINDING
457 ILLHPFIFDA 467	SERPIN

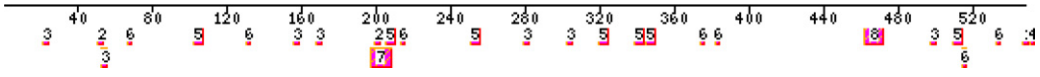

Fig. 5. Putative functional domains of *Lampyris turkestanicus* glow-worm luciferase. 1, Amidation; 2, ASN-Glycosylation; 3, CK2_PHOSPHO_SITE; 4, MICROBODIES_CTER; 5, MYRISTYL; 6, PKC_PHOSPHO_SITE; 7, AMP_BINDING; 8, SERPIN.

Table 3
Signature of putative AMP-binding domain in different beetle luciferases

Accession numbers/species	Putative AMP-binding domain signature
AF420006/*Hotaria unmunsana*	197–208: LMNSSGSTGLPK
L39929/*Hotaria parvula*	197–208: LMNSSGSTGLPK
S61961/*Luciola mingrelica*	197–208: LMNSSGSTGLPK
U51019/*Luciola lateralis*	197–208: IMNSSGSTGLPK
OO1158/*Luciola lateralis*	197–208: IMNSSGSTGLPK
M26194/*Luciola cruciata*	197–208: IMNSSGSTGLPK
AF328553/*Pyrocoelia rufa*	196–207: IMNSSGSTGLPK
L39928/*Pyrocoelia mivako*	196–207: IMNSSGSTGLPK
AY742225/*Lampyris turkestanicus*	195–206: IMNSSGSTGLPK
X89479/*Lampyris noctiluca*	195–206: IMNSSGSTGLPK
Ml5077/*Photinus pyralis*	195–206: IMNSSGSTGLPK
D25415/*Photuris pennsylvanica*	194–205: IMNSSGSTGLPK
D25416/*Photuris pennsylvanica*	194–205: IMNSSGSTGLPK
S29353/*Pyrophorus plagiophthalamus*	192–203: ILCSSGTTGLPK
S29354/*Pyrophorus plagiophthalamus*	192–203: ILCSSGTTGLPK
S29355/*Pyrophorus plagiophthalamus*	192–203: ILCSSGTTGLPK
AF139644/*Phrixothrix vivianii*	192–203: IMSSSGTTGLPK
AF13 9645/*Phrixothrix hirtus*	192–203: IMTSSGTTGLPK

motifs, and their functions in glow-worm *L. turkestanicus* luciferase. Due to the importance of AMP-binding domain, its putative signature among the various firefly species is compared in Table 3. The putative AMP-binding domain signature of *L. turkestanicus* was found as 195-IMNSSGSTGLPK-206 and is highly conserved among the various firefly species. Minor deviation of this motif was found for click beetle luciferases which are red light emitters. The domains that can be recognized involve ATP (AMP)-binding sites, regions that interact with ATP, regions that are involved in reactions leading to the formation of adenylate intermediates, and function in peptide synthetases. As expected, there are several regions that are found in other firefly luciferases.

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