The Characteristic of Luciferase cDNA of Lamprigera sp. (Lampyridae: Coleoptera)

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Abstract. Some organisms can emit light naturally. Fireflies are most popular organisms among them. The light is produced in all species of fireflies due to luciferase enzymes. Lamprigera is one of the fireflies genus. Its species Lamprigera sp. is interesting to be studied because it has big larvae dan easily be found on ground at night since it has strong light. Information about the luciferase gene in Lamprigera sp. is restricted. In this study we characterize the luciferase cDNA of Lamprigera sp. collected in Kayu Aro, Kerinci, Jambi. Amplification of Lamprigera’s luciferase cDNA using LF and LR primers produced 367 bp fragments. The similarity levels of Lamprigera’s luciferase cDNA sequences with published Lamprigera yunnana is 83%. This indicates that luciferase cDNA of Lamprigera sp. is different from the published Lamprigera yunnana. However, phylogenetic tree construction shows that Lamprigera sp. have a close relationship with Lamprigera yunnana.

1. Introduction

Some species of bacteria, fungi and animals can emit light naturally. Their light emissions are colorful and it is species specific. Several identified organisms among them are: Vibrio harveyi [1], V. fischeri [2], Vibrio cholera [3], Photobacterium phosphorum [4], Xenorhabdus luminescens [5], Armillaria meleae [6], Noctiluca scintillans [7], Odontosyllis enopla [8], and Photinus pyralis [9]. These organisms emit light for many purposes, like mating, self-defense, camouflage, aposematism, and predation [5,6,7,9]. The phenomenon of light emission by living things is known as bioluminescence [10]. Luminescence is a condition for somethings that emit light in the visible light range [11]. It is due to the electron transfer from the ground state to the excited state [12].

Fireflies are most popular organisms among light-emitted organisms. They are in the Lampyridae family [13] and belongs to Coleoptera ordo [14]. Other organisms in this ordo are coconut and rice beetles [14]. There are more than two thousand species of fireflies found in the subtropical and tropical countries around the world. However, most of them live in the tropical countries including Indonesia. Light emission of firefly is produced by specific organ on the special segment located at the end of its stomach. Light is emitted when luciferase enzyme in the specific organ oxidize luciferin. Interestingly, fireflies light emissions are different among species. For examples Asymmetraca circumadata emit yellow color [15] and Lamprigera yunnana emit yellow-green color [16]. Some studies described that different color of light emission is caused by different composition of luciferin.
However, there is restricted information about the involvement of luciferase enzyme in producing different color.

In this study we used larvae of Lamprigera sp as the samples to characterize its luciferase cDNA sequence and aligned it with published sequence of Lamprigera yunnana. Lamprigera sp larvae can be found easily on the moist ground or on the tree leaves at night because it is big larvae and emit light brightly. In west Sumatera and Jambi, Lamprigera sp found in Lembah Anai, Agam, Payakumbuh, Gunung Tujuh District, and Kayu Aro. Its habitat is the cold-tempered areas.

2. Materials and Methods
2.1. Samples collection
Samples were collected from the plantation area in Kayu Aro. Samples collection was carried out at night. Samples then morphologically characterized according to published data [18].

2.2. Primer Design
A pair of primer is designed in the conserved area of mRNA luciferase sequence of four fireflies species, i.e. Lamprigera yunnana (MK276878.1), Photuris pennsylvanica (U31240.1), Luciola cruciata (M26194.1), and Lampyris noctiluca (AY748894.1). The designed primers sequence are LF 5’-TGT CAA AGT GCG TTG CTT GTA C-3’ dan LR 5’-AAA TAG ATT CCA GTT CAG CAG GTG-3’. These primers amplified 520 bp of cDNA products.

2.3. Total RNA Isolation
Total RNA isolated according to manual book of Quick-RNA™ Minirep Plus Kit (Zymo Research).

2.4. cDNA Synthesis
cDNA is synthesized using Bioline Sensifast cDNA Synthesis Kit (Cat.No BIO-65053).

cDNA is amplified for 35 cycles as described in Table 1.

| Steps   | Temperature | Time |
|---------|-------------|------|
| Denaturation | 98°C         | 10 s |
| Annealing   | 55°C         | 10 s |
| Elongation  | 72°C         | 120 s|

2.5. Alignment and Sequence Analysis
Luciferase cDNA sequence of Lamprigera sp is aligned with Lamprigera yunnana using Nucleotide BLAST (Basic Local Alignment Search Tool) (https://blast.ncbi.nlm.nih.gov/)

2.6. Phylogenetic Analysis
Phylogenetic analysis is done by Geneious Prime software with Neighbor Joining method. We used some published species (Table 2) to construct phylogenetic tree.

| No | Species         | Accession Number |
|----|-----------------|------------------|
| 1  | Lamprigera yunnana | MK276878        |
| 2  | Luciola cruciata     | M26194          |
| 3  | Luciola lateralis    | X66919          |
| 4  | Luciola mingrelica   | S61961          |
| 5  | Hotaria parvula     | L39929          |
| 6  | Hotaria unmunsana    | AF420006        |
7. *Luciola italica* \( \text{DQ138966} \)
8. *Luciola terminalis* \( \text{EU302126} \)
9. *Lampyroidea maculata* \( \text{DQ137139} \)
10. *Photuris pennsylvanica* \( \text{U31240} \)

### 3. Results and Discussions

#### 3.1. Results

Total RNA isolation is conducted to provide cDNA through reverse transcribe PCR (RT-PCR). We got good purity and concentration of luciferase total RNA as described in Table 3.

| Parameters                          | Value   |
|-------------------------------------|---------|
| Concentration (ng/μl)               | 551,40  |
| Purity (Ratio 260/280)              | 2,056   |

cDNA amplification is done using LF and LR primers. Electrophoregram shows a single band with slightly more than 500 bp in length (Figure 1). This is the expected size of PCR products (520 bp).

![Electrophoregram](image1.png)

*Figure 1. Electrophoregram of luciferase cDNA of *Lamprigera* sp (a) and 100 bp DNA ladder (b)*

Figure 2 show luciferase cDNA sequence of *Lamprigera* sp after contig process using Geneious Prime program. This process produce 367 bp luciferase cDNA sequence.
Figure 2. Luciferase cDNA sequences of Lamprigera sp contig by Geneious Prime Program version 2019.2 (Biomatters www.geneious.com). Light blue in nitrogen bases indicates single peak whereas dark blue indicates multiple peaks.

This cDNA sequences are then aligned with luciferase cDNA database of Lamprigera yunnana. BLAST-N analysis show the similarity number between Lamprigera sp and Lamprigera yunnana (Table 4).

| Parameters       | Values       |
|------------------|--------------|
| Total Skor       | 448          |
| Query Cover      | 83%          |
| E Value          | 1e-121       |
| Identity 9       | 2.90%        |
| Accession Number | MK276878.1   |

Table 4 revealed that query cover of luciferase cDNA of Lamprigera sp and Lamprigera yunnana is 83%. It is indicated that these two species do not have the identical luciferase cDNA sequence. Figure 3 show the different nucleotide positions of both species.

Figure 3. Alignment of Luciferase cDNA of Lamprigera sp and Lamprigera yunnana.
As described in Figure 4, luciferase cDNA of Lamprigera sp is most closely related to Lamprigera yunnana.

4. Discussion
In this study we have succeeded in characterizing the cDNA luciferase of Lamprigera sp. This study is began with total RNA isolation and continued with cDNA amplification and sequencing. In this study, total RNA was obtaining with a purity value of 2.059 and a concentration of 551.40 ng/ul. This is a high purity number and a high concentration. The quality of total RNA is qualified to be good if the absorbance ratio (A260/A280) of RNA is in the range 1.8 – 2.1. If the ratio is less than 1.8, it is indicates that RNA is still contaminated with protein [19]. In contrast, if the ratio more than 2., it is indicates that samples is contaminated by DNA [20].

Luciferase gene amplification using LF and LR primers has been successfully carried out. In Figure 1, it can be seen that the amplicon are in the range of 500 bp. This is in accordance with the length of nucleotides that can read by the primer, namely 520 bp. The band show is also a single and thick band, so that this can prove that the primer is designed really sticks and matches to the desired target of the luciferase gene. The amplicon also can be used for the next process, namely sequencing. cDNA sequencing after contiq showed that the length of the luciferase gene obtained was 369 bp. In the Geneious Prime program, the quality of the sequencing can be seen from the color of the nitrogen bases sequence. The bright blue color in the nitrogenous bases means that the confidence value of the sequencing is good. It is can be seen that there is a single peak on the base. In contrast, when the blue color getting darker, the confidence level of the sequencing is getting weaker. In this study, the blue color displayed in the contiq are in the range of light to the dark blue, which means that the nitrogen base sequence can still be tolerated. This is evidenced by the subsequence process, namely BLAST-N and phylogenetic tree.

The luciferase gene sequence was found to have a similarity value of 82% with the Lamprigera yunnana sequence. This indicates that luciferase gene sequence obtained have differences with this
species. This difference can be seen from the alignment of the nitrogenous bases of both genus of Lamprigera. This could imply that the luciferase gene of \textit{Lamprigera} sp is different form \textit{L. yunnana}. This genetic variation might caused by random mating, very large population size, migration, mutation, recombination, and natural selection \cite{21}. To know the relationship of luciferase cDNA of Lamprigera sp to the other of fireflies, a phylogenetic tree was constructed.

The result of the phylogenetic analysis in accordance with Liu et al \cite{16}. The Lamprigera genus is separated from the Luciolinale subfamily and form its own clade. The analysis showed that luciferase cDNA obtained was closely related to \textit{Lamprigera yunnana}. They are in the same genus. The Lamprigera genus is closely related to the Photurinae subfamily (\textit{Phorus pennsylvanica}) compared to the Luciolinale subfamily (genus Luciola, Hotaria and Lampyroidea). Phylgenetic tree construction proves that the luciferase gene sequence found are still closely related to \textit{Lamprigera yunnana}, but it is suspected that this firefly is a new species because the level of similarity to \textit{Lamprigera yunnana} sequence only 82%. Gene Bank sequence are said to be similar if they have a similarity percentage more than 98%. When the similarity percentage is less than 98%, it is considered as unknown. So it is advisable to do further study to make sure the sequence is novel. Lower genetic distance means lower genetic inequality between individuals or the other word it means the relationship between them are close \cite{23}. The closeness of the relationship among populations can be caused by the existence of common ancestor \cite{24}. For this reason, further analysis is needed regarding the identification of species in Lamprigera sp by characterizing the mitochondrial COI gene.

5. Conclusion

The homology percentage of luciferase cDNA sequence of \textit{Lamprigera} sp and \textit{Lamprigera yunnana} is 82%. The differences of luciferase sequence DNA might contribute to the different color of light emitted. However, \textit{Lamprigera} sp and \textit{Lamprigera yunnana} have closest relationship.

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