Electronic Supplementary Material (ESI) for Photochemical & Photobiological Science

Biochemical characteristics and gene expression profiles of two paralogous luciferases from the Japanese firefly *Pyrocoelia atripennis* (Coleoptera, Lampyridae, Lampyrinae): Insight into the evolution of firefly luciferase genes

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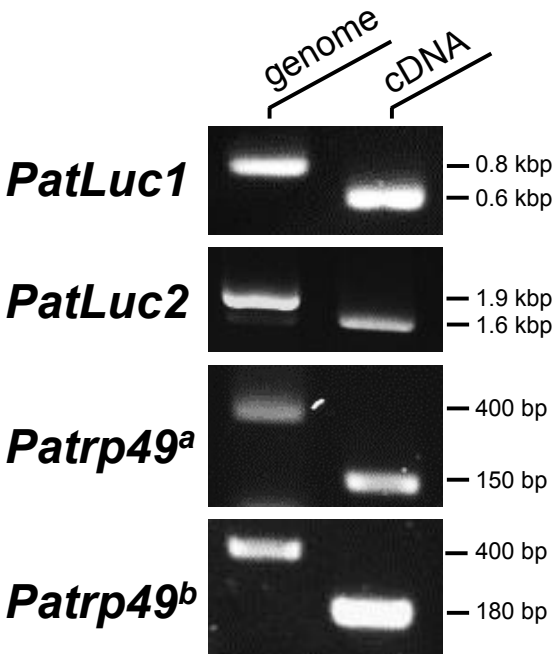

Fig. S1. RT-PCR and genomic PCR of *PatLuc1* (A), *PatLuc2* (B) and *Patrp49* (C, D). The RT-PCR products (cDNA) are distinguishable from the genomic PCR (genome) products by the length of the amplicon.

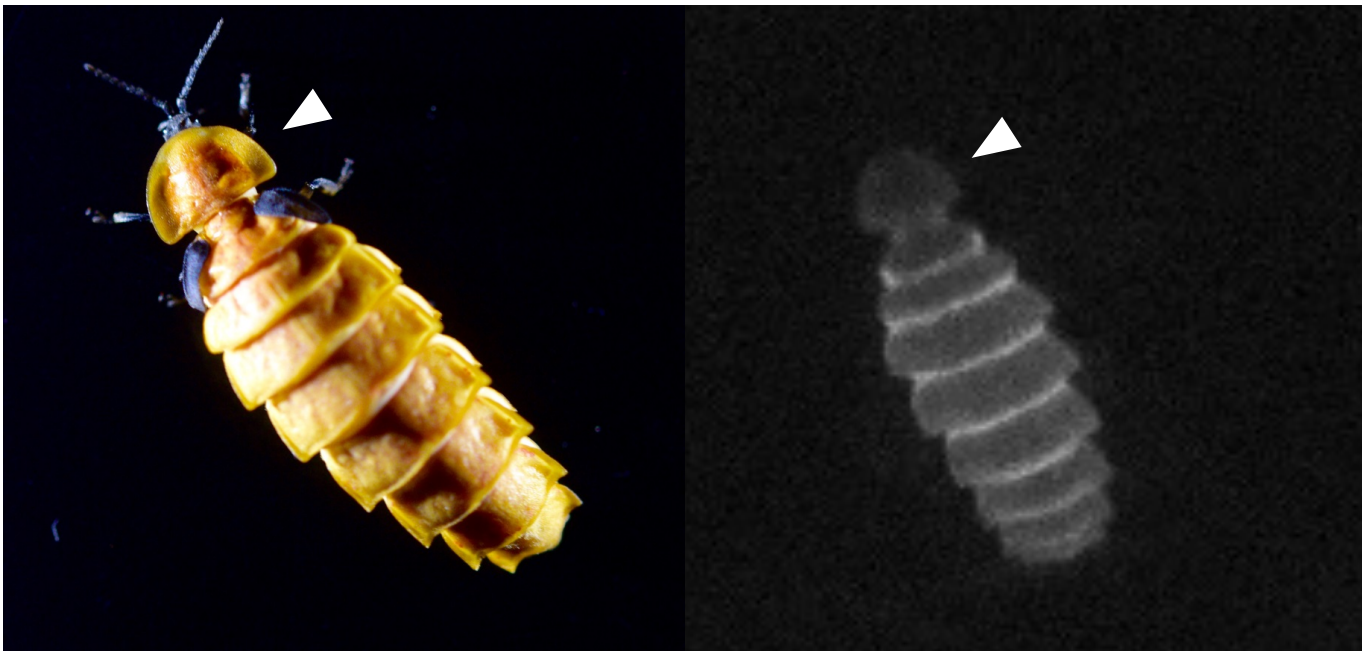

Fig. S2. Non-lantern bioluminescence of female adults. The luminescence is observed throughout the body including the prothorax (arrowhead). The luminescence is stronger between segments.

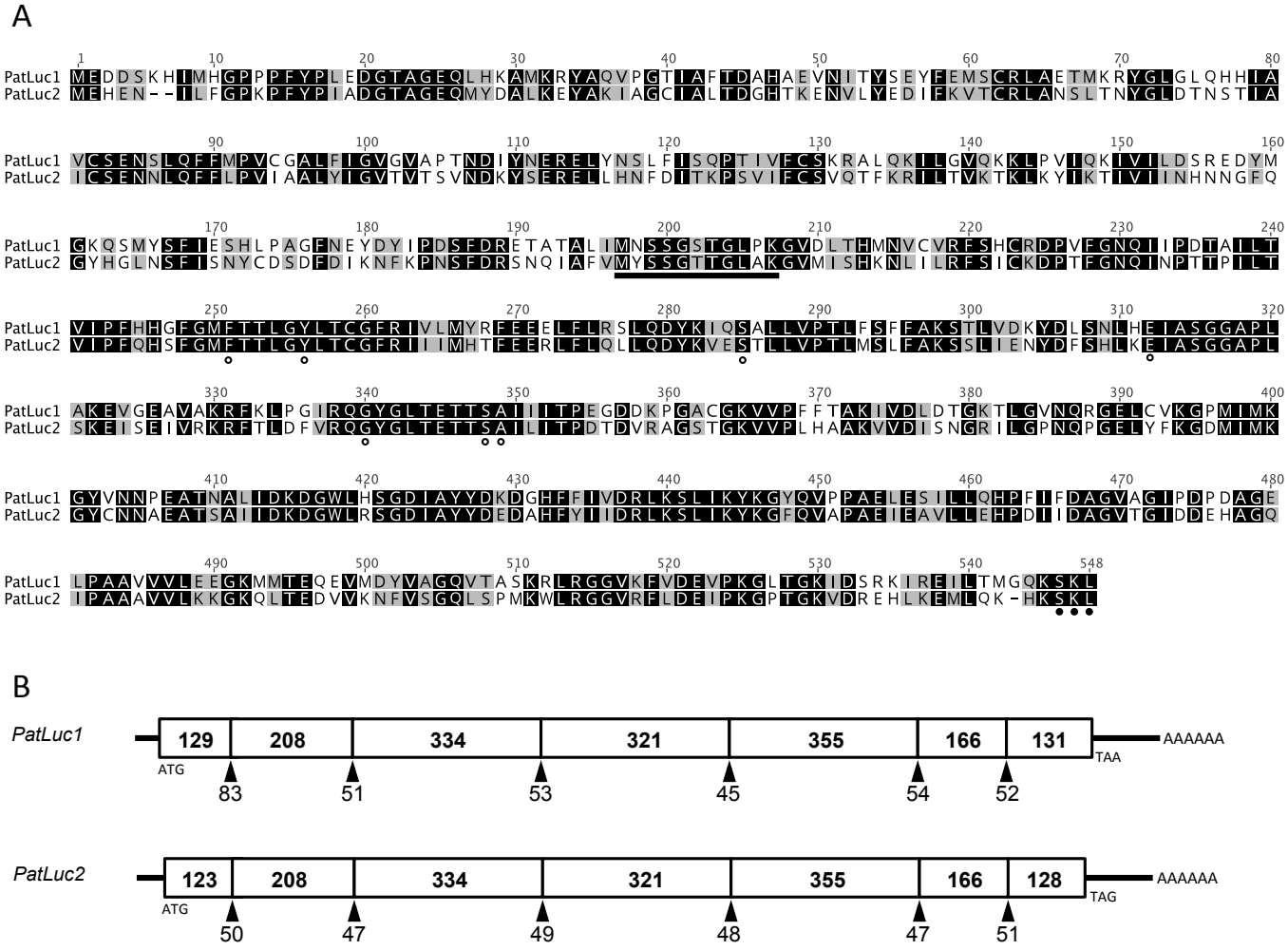

Fig. S3. (A) Pairwise alignments of PatLuc1 and PatLuc2. Underline indicates the AMP-biding domain, open circles indicate putative firefly luciferin-binding sites (Nakatsu et al., 2006), and closed circles indicate the PST sequence. Identical amino acid residues and similarities according to the Blosum62 matrix are boxed in black and grey, respectively. (B) Gene structures of *PatLuc1* and *PatLuc2*. Both CDSs are divided by six introns. Numbers in each box indicate nucleotide length. Numbers under arrowheads indicate intron size.

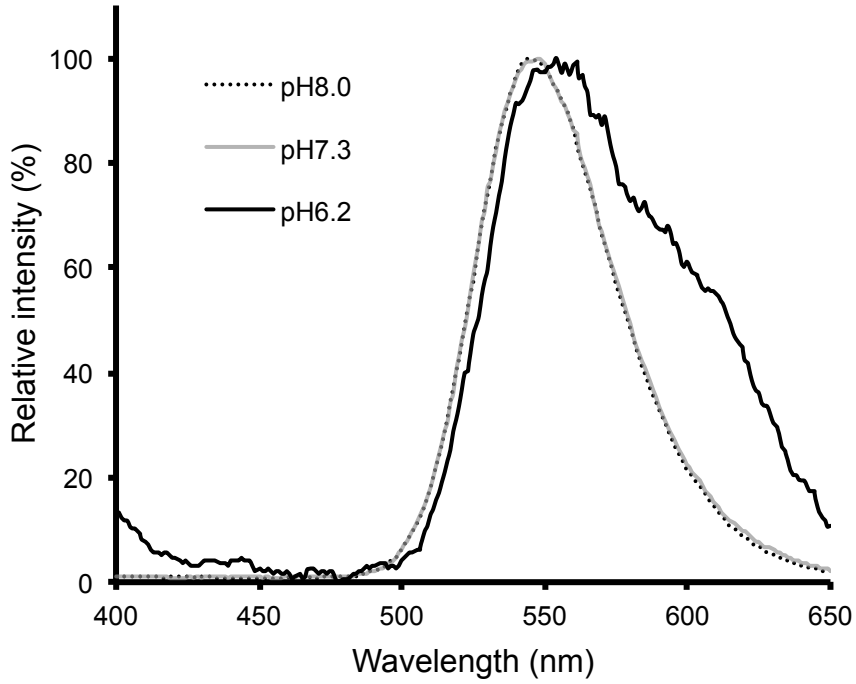

Fig. S4. *In vitro* luminescence spectra of native luciferase extracted from larval lanterns at pH 6.2 (black line), pH 7.3 (gray line), and pH 8.0 (dots).

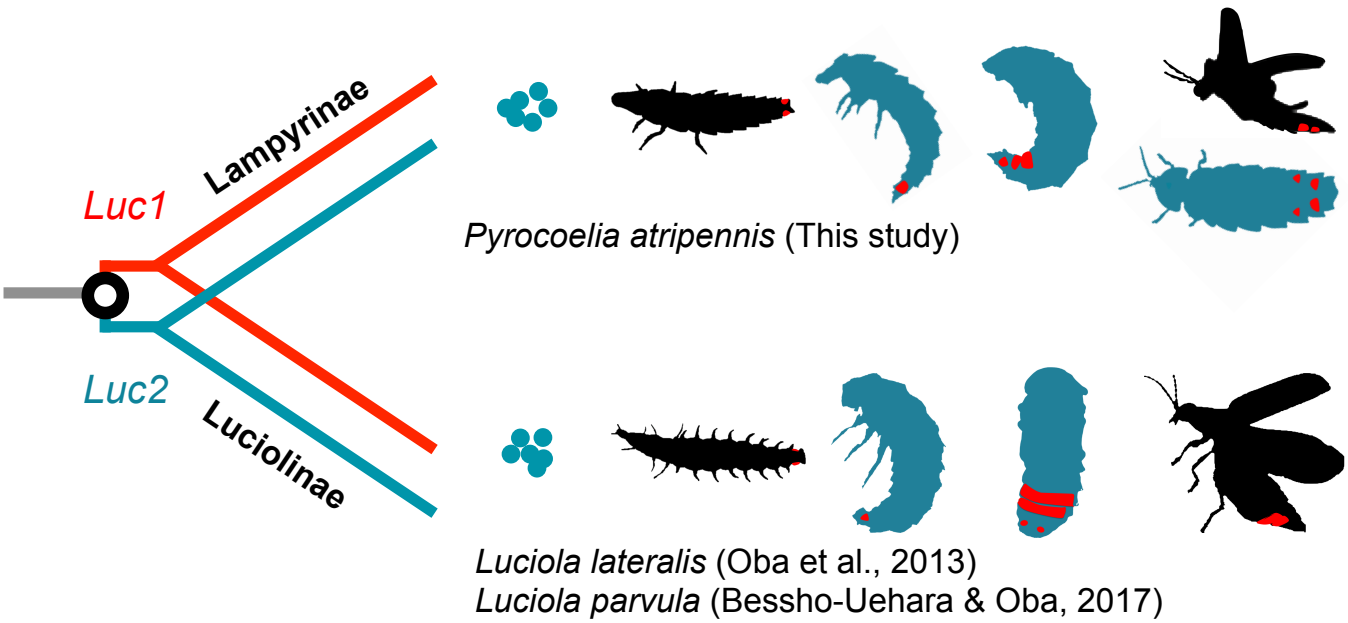

Fig. S5. Schematic diagram of the evolutionary process of firefly luciferases. The ancestral firefly luciferase gene split into *Luc1* (red line) and *Luc2* (blue line) by gene duplication (black circle). In each developing stage (silhouettes), the areas filled in red and blue indicate where *Luc1* and *Luc2* are expressed, respectively.

Table S1. Primer list and PCR conditions.

Name of primer	Sequence（5' - 3'）	Description
Molecular cloning of *PatLuc1* [a,b,c]		
PyrocoeliaLuc1-f1	GGTTCCAGGGACAATTGC	Forward primer
PyrocoeliaLuc1-r3	TGCAGGAAGTTCACCGGC	Reverse primer
PyrocoeliaLuc1-r4	TTCTAATTCGGCAGGCGG	Reverse primer
PatLuc1-5RACE1	GTAAACCAAGTCCGTACCTC	Reverse primer for 5' RACE-PCR
PatLuc1-5RACE2	ATAGTGCACCGCATACAGGC	Reverse primer for 5' RACE-PCR
PatLuc1-3RACE3	CCCAGAAGCAACAAATGC	Forward primer for 3' RACE-PCR
PatLuc1-3RACE4	GATGGATGGTTACACTCTGG	Forward primer for 3' RACE-PCR
PatLuc1-5f	CTGGTTCCTGAGACACTAACGC	Forward primer for cloning complete CDSs
PatLuc1-3r	GTGCACATCCCATTGTTAGC	Reverse primer for cloning complete CDSs
Molecular cloning of *PatLuc2* [b,c,d,e]		
LH1	GGWWCHACYGGNYTNCCNAA	Degenerated forward primer for luciferase homologs
LH2	ACYGGNYTNCCNAARGGNGT	Degenerated forward primer for luciferase homologs
LH3	ACYTGRTANCCYTTRTAYTT	Degenerated reverse primer for luciferase homologs
LH4	TGRTANCCYTTRTAYTTDAT	Degenerated reverse primer for luciferase homologs
LucUdig-r3	TCWGTHARWCCRTANCCYTG	Degenerated reverse primer for firefly luciferase
PatLuc2-5RACE1	GACACCTTTAGGCAGACC	Reverse primer for 5' RACE-PCR
PatLuc2-5RACE2	GGTGTGGTGGGATTGATTTG	Reverse primer for 5' RACE-PCR
PatLuc2-3RACE3	GCTAGTTCCGACCCTAATG	Forward primer for 3' RACE-PCR
PatLuc2-3RACE4	GAGATAGCATCAGGTGGAGC	Forward primer for 3' RACE-PCR
PatLuc2-5f	CGTGTGCAATTTCGAGTGAAC	Forward primer for cloning complete CDS and genomic sequence
PatLuc2-3r	CTGAAATATTCAAATGTCACTAATA	Reverse primer for cloning complete CDSs and genomic sequence
Construction of heterologous expression plasmid [c,e]		
pCZP-PatLuc1-f	CAGGGGCCCGAATTCCGAATGGAAGATGATAGTAA	Forward primer for constructing pCold-ZZ-P-PatLuc1 and cloning

		genomic sequence
pCZP-PatLuc1-r	ACCTATCTAGACTGCAATTACAATTTGGATTTTTG	Reverse primer for constructing pCold-ZZ-P-PatLuc1 and cloning genomic sequence
PatLuc2-XhoI	GGACTCGAGATGGAACACGAA	Forward primer for constructing pCold-ZZ-P-PatLuc2
PatLuc2-XbaI	GGGTCTAGAGTACACATTAAAAG	Reverse primer for constructing pCold-ZZ-P-PatLuc2
Molecular cloning of *Lprp49* [f]		
fireflyrp49-f1	RGGWCAATACYTRATGCC	Degenerated forward primer for *ribosomal protein 49*
fireflyrp49-r2	CGAATSGAAAGTTGSGCAGC	Degenerated reverse primer for *ribosomal protein 49*
fireflyrp49-r3	RCGMAGACGTGCATGTCC	Degenerated reverse primer for *ribosomal protein 49*
Semi-quantitative RT-PCR [g,h,i]		
PatLuc1-3RACE3	CCCAGAAGCAACAAATGC	Forward primer
PatLuc1-3r	GTGCACATCCCATTGTTAGC	Reverse primer
PatLuc2-5f	CGTGTGCAATTTCGAGTGAAC	Forward primer
PatLuc2-3r	CTGAAATATTCAAATGTCACTAATA	Reverse primer
Patrp49-f1	ATGCCCAACATTGGTTACGG	Forward primer
Patrp49-r1	CGAATGGAAAGTTGGGCAGC	Reverse primer
Patrp49-f2	ACGGTTCCAATGCAAGAACAC	Forward primer
Patrp49-r2	GAAAGTTGGGCAGCACGTTC	Reverse primer

W = A + T, R = A + G, M = A + C, K = T + G, Y = T + C, S = G + C, H = A + C + T, B = G + C + T, V = A + G + C, D = A + G + T, N = A + G + C + T. PCR conditions were as follows. [a]PCR conditions for isolating a partial gene of *PatLuc1* using degenerated primer sets: 5.0 min at 95℃; 25-40 cycles of 30 s at 95℃, 1.0 min at 55℃, and 1.5-2.5 min at 72℃; followed by a final extension of 7.0 min at 72℃. [b]RACE-PCR conditions with the primer sets were: 5.0 min at 95℃; 25 cycles of 30 s at 95℃, 1.0 min at 53-56℃, and 2.0 min at 72℃; followed by a final extension of 7.0 min at 72℃. [c]PCR conditions for cloning complete CDSs and construction of expression plasmids with primer sets were: 2.0 min at 96℃; 30 cycles of 10 s at 98℃, 15 s at 55-58℃, and 30 s at 72℃; followed by a final extension of 5.0 min at 72℃. [d]Degenerated PCR conditions for isolating luciferase homologs with degenerated primer sets were: 5.0 min at 95℃; 25-35 cycles of 15-30 s at 95℃, 1.0 min at 44℃, and 1.1-2.5 min at 72℃; followed by a final extension of 7.0 min at 72℃. [e]PCR conditions for cloning genomicsequences with

primer sets were: 2.0 min at 96℃; 40 cycles of 10 s at 98℃, 5-15 s at 55℃, and 0.5-1.0 min at 72℃; followed by a final extension of 5.0 min at 72℃. [f]Degenerated PCR conditions for *Patrp49* with degenerated primer sets were: 5.0 min at 95℃; 5 cycles of 30 s at 95℃, 30 s at 45℃, and 1.0 min at 72℃; 10 cycles of 30 s at 95℃, 30 s at 50℃, and 1.0 min at 72℃; 30 cycles of 30 s at 95℃, 30 s at 55℃, and 1.0 min at 72℃; followed by a final extension of 7.0 min at 72℃. [g]Semi-quantitative RT-PCR conditions for *PatLuc1* with primer sets were: 5.0 min at 94℃; 25-30 cycles of 30 s at 94℃, 20 s at 52℃, and 50 s at 72℃; followed by a final extension of 7.0 min at 72℃. [h]Semi-quantitative RT-PCR conditions for *PatLuc2* with primer sets were: 5.0 min at 94℃; 35 cycles of 30 s at 94℃, 20 s at 52℃, and 2.0 min at 72℃; followed by a final extension of 7.0 min at 72℃ or 5.0 min at 94℃; 5 cycles of 30 s at 94℃, 20 s at 52℃, and 2.0 min at 72℃; 27-35 cycles of 30 s at 94℃, 20 s at 55℃, and 2.0 min at 72℃; followed by a final extension of 7.0 min at 72℃. [i]Semi-quantitative RT-PCR conditions for *Patrp49*; with primer sets were: 5.0 min at 95℃; 25 cycles of 20 s at 95℃, 20 s at 55℃, and 40 s at 72℃; followed by a final extension of 5.0 min at 72℃.