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We describe here the complete nucleotide sequence and the exon-intron structure of the luciferase gene of the Hotaria-group fireflies, H. unmunsana, H. papariensis, H. tsushimana and H. parvula. The luciferase gene of H. unmunsana, H. papariensis and H. tsushimana spans 1,950 bp and is six introns and seven exons coding for 548 amino acid residues. Two different luciferase genes, F7 and Fc, in H. unmunsana were cloned and their sequences were determined. The deduced protein sequence of H. unmunsana luciferase gene F7 showed amino acid differences at 1 position with Fc, but identical to that of H. papariensis luciferase gene P1 and P2. Two different luciferase genes, S1 and S2, in H. tsushimana were determined. The deduced protein sequences of S1 and S2 were different from 1 position and 2 positions in H. unmunsana luciferase F7, respectively. Furthermore, novel luciferase gene in H. parvula F174 was cloned and compared with the known H. parvula luciferase gene. The major differences between the two H. parvula luciferase genes were two amino acids deletion in the N-terminal region of F174 and PV3 as compared with the known H. parvula luciferase with 548 amino acid residues, and amino acid differences were identified at 4 positions. Phylogenetic analysis confirmed the deduced amino acid sequences of the Hotaria luciferase genes to belonged to the same subfamily, Luciolinae, demonstrating that H. unmunsana luciferase gene is more closely related to H.

papariensis and H. tsushimana than to H. parvula. We also analyzed COI gene of Hotaria-group fireflies. The deduced protein sequence of COI gene of H. unmunsana was identical to that of H. papariensis and H. tsushimana, but different from 3 positions in H. parvula. In conclusion, our results suggest that H. unmunsana, H. papariensis and H. tsushimana are very closed or, at least in luciferase and COI genes, same species.