

Phylogenetic Relationships of the Fireflies Co-occurring in Korean and Japanese Territories Analyzed by Luciferase and Mitochondrial DNA Sequences

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In Korean Peninsula including neighboring islands and Japanese Islands identical firefly species or the species belonging to same genera occur together in both territories. These geographic firefly species, nonetheless, have never been subject to taxonomic consideration together until recently, lacking clear species status and phylogenetic relationships. A recent serial study of these fireflies using luciferase gene and/or portions of mitochondrial DNA sequences provided some insight into these populations in terms of validity of species name, phylogenetic relationships, and speciation event. In this article, thus, we have reviewed the recent progress on phylogenetic and/or population genetic aspects of these species, i.e., *Hotaria*-group fireflies, *Luciola lateralis*, and *Pyrocoelia rufa* to better understand the firefly species in these regions.

Key words: Fireflies, Coleoptera, *Hotaria*, *Luciola*, and *Pyrocoelia*, Luciferase gene, mtDNA, COI

Introduction

Fireflies are thought to be a heart-touching organism by their beautiful lights, and, thus, often appear in poems, songs, and stories. Further, they are often regarded as a biological barometer for healthy environment, because the occurrence of fireflies in a given area suggests that a light

interference, which is one of the indicatives of urbanization is not severe and the luminous signal for sexual purpose is effective.

Fireflies started to dwell on earth from the Cenozoic era of about 70,000 years ago and now more than 2,000 species are found in everywhere in the world, except for the South and the North Poles (Minami, 1983).

In Asian region, fireflies occurring in Japan have extensively been studied. In the early 19th, a checklist of the fireflies in Japanese Islands and adjacent regions presented only nine species (Okada, 1930). However, thereafter, Nakane (1968), Satô (1978), and Nakane and Ohba (1981) enumerated 24, 34, and 39 species, respectively, by the discovery of new species mainly from the Ryukyu Islands, located in the southwest of Japan. Recently, 46 lampyrid species in eight genera belonging to four sub-families and one rhagophthalmid are listed in the Japanese firefly fauna (Suzuki, 2001).

Unlikely the situation in Japan, only a few species have been documented in Korean mainland, neighboring offshore islets, and on a remote Island, Jeju, Korea. After the first report on the presence of *Lucidina biplagiata* belonging to Lampyrinae (Heyden, 1887) Okamoto (1924) reported the presence of *Luciola cruciata* on a remote island, Jeju, located about 100 km southwestward away from Korean Peninsula. Later, Doi (1931, 1932) reported two new species (*Hotaria unmunsana* and *H. papariensis*) and three unlisted species (*L. lateralis*, *Pyrocoelia rufa*, and *Lampyrus noctiluca*) from Korean mainland. Later, Cho (1957) listed a total of seven species in a checklist of Korean Coleoptera and this continued until 1970th. Later, Kim and Nam (1981) removed *L. cruciata* in the checklist, because they failed to find any evidence of occurrence in Korea. Currently, one representative checklist of the Korean insects added one doubtful species, *L. accensa*,

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Table 1. List of fireflies found historically and currently in Korea (Jeong, 1994; Kim *et al.*, 2003)

Species	Current status
Family Lampyridae	
Subfamily Drilastinae	
<i>Drilaster unicolor</i> Lewis, 1895	not found recently
Subfamily Luciolinae	
<i>Luciola lateralis</i> Motschulsky, 1860	widely found
<i>Hotaria papariensis</i> Doi, 1932	synonym to <i>H. unmunšana</i>
<i>Hotaria unmunšana</i> Doi, 1931	widely found
Subfamily Lampyrinae	
<i>Lampyris notiluca</i> Linn, 1767	rarely found
<i>Lucidina accensa</i> Gorham, 1883	not found recently
<i>Lucidina biplagiata</i> Motschulsky, 1866	not found recently
<i>Pyrocoelia rufa</i> Olivier, 1886	widely found

and now lists seven species belonging to five genera in two subfamilies (Table 1; Jeong, 1994; Kim *et al.*, 2003).

Kim *et al.* (2004) recently reported three year's research result on firefly habitats and found 86 spots for *L. lateralis*, 132 spots for *P. rufa*, and 116 spots for *H. papariensis* in Korean mainland and neighboring islets and on a remote Jeju Island. He also found *L. noctiluca* in a few localities later (Kim, personal communication), but did not find the remaining *Drilaster unicolor*, *L. accensa*, and *L. biplagiata* at all (Kim *et al.*, 2003).

Among those *L. lateralis* occurs widely in Korean Peninsula, Jeju Island, and four major Japanese Islands (Hokkaido, Honshu, Shikoku, and Kyushu) (Fig. 1). In the case of *P. rufa*, it occurs widely in Korean Peninsula, but, in Japan, it occurs only on one island, Tsushima, located

about 50 km away from the nearest Korean mainland (Fig. 1). In the case of *Hotaria*, *H. papariensis* and *H. unmunšana* are widely spread in Korea, but not found in Japan, whereas *H. tsushimana* occurs only on Tsushima Island in Japan and *H. parvular* occurs widely in three Japanese Islands (Honshu, Shikoku, and Kyushu), but these fireflies are not found in Korean mainland and neighboring islets at all (Fig. 1).

Although an identical species or close species belonging to same genus commonly occur in Korean mainland, neighboring islands, and Japanese territory it was only recent that the populations of these fireflies together were subjected to investigation on the species status and genetic relationships. Thus, in this study, we have reviewed available studies on taxonomic, phylogenetic, and population

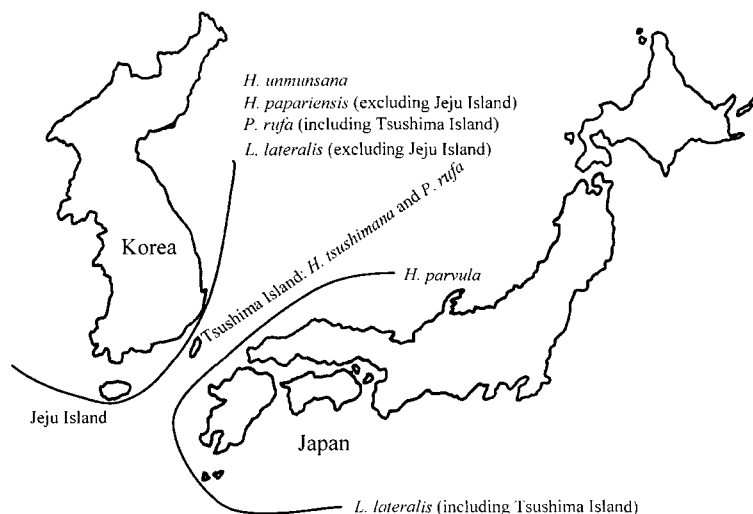


Fig. 1. Schematic illustration of the distribution range of the firefly species occurring both in Korean and Japanese territories. The distributional information was obtained from Matsumura (1928), Ohba (1983, 2000), Suzuki *et al.* (1993), and Kim *et al.* (2003, 2004).

Table 2. List of firefly luciferase (family Lampyridae) genes known so far

Species	GenBank no.	# amino acids	References
Lampyridae			
Lampyrinae			
<i>Photinus pyralis</i>	M15077	550	de Wet <i>et al.</i> (1987)
<i>Photinus pyralis</i>	X84846	550	Croizier (unpublished)
<i>Photinus pyralis</i>	X84847	550	Croizier (unpublished)
<i>Photinus pyralis</i>	X84848	550	Croizier (unpublished)
<i>Photinus pyralis</i>	U03687	550	Bonin <i>et al.</i> (unpublished)
<i>Cratomorphus distinctus</i>	AY633557	547	Viviani <i>et al.</i> (2004)
<i>Pyrocoelia rufa</i>	AF328553	548	Lee <i>et al.</i> (2001)
<i>Pyrocoelia miyako</i>	L39928	548	Ohmiya <i>et al.</i> (1995)
<i>Lampyris noctiluca</i>	X89479	547	Sala-Newby <i>et al.</i> (1996)
<i>Lampyris noctiluca</i>	AY447204	547	Li <i>et al.</i> (2003)
Luciolinae			
<i>Luciola mingrelica</i>	S61961	548	Devine <i>et al.</i> (1993)
<i>Luciola cruciata</i>	M26194	548	Masuda <i>et al.</i> (1989)
<i>Luciola lateralis</i>	X66919	548	Tatsumi <i>et al.</i> (1992)
<i>Luciola lateralis</i>	AY181996	548	Kim <i>et al.</i> (2004)
<i>Luciola lateralis</i>	AY192560	548	Kim <i>et al.</i> (2004)
<i>Luciola lateralis</i>	AY192561	548	Kim <i>et al.</i> (2004)
<i>Luciola lateralis</i>	U49181	548	Cho <i>et al.</i> (1999)
<i>Luciola lateralis</i>	U51019	548	Cho <i>et al.</i> (1999)
<i>Luciola lateralis</i>	Z69619	548	Cho <i>et al.</i> (1999)
<i>Luciola lateralis</i>	Z49891	548	Cho <i>et al.</i> (1999)
<i>Hotaria tsushimana</i>	AF486804	548	Choi <i>et al.</i> (2003)
<i>Hotaria tsushimana</i>	AF486801	548	Choi <i>et al.</i> (2003)
<i>Hotaria papariensis</i>	AF486802	548	Choi <i>et al.</i> (2003)
<i>Hotaria papariensis</i>	AF486803	548	Choi <i>et al.</i> (2003)
<i>Hotaria unmunšana</i>	AF486800	548	Choi <i>et al.</i> (2002)
<i>Hotaria unmunšana</i>	AF420006	548	Choi <i>et al.</i> (2002)
<i>Hotaria parvula</i>	L39929	548	Ohmiya <i>et al.</i> (1995)
Photurinae			
<i>Photuris pennsylvanica</i>	U31240	545	Ye <i>et al.</i> (1997)
<i>Photuris pennsylvanica</i>	D25415	552	Zenno <i>et al.</i> (unpublished)
<i>Photuris pennsylvanica</i>	D25416	552	Zenno <i>et al.</i> (unpublished)

genetic aspects of the four firefly species, where luciferase gene and portions of mtDNA sequences were used as genetic markers.

Some aspects of luciferase gene and insect mitochondrial DNA

Luciferase catalyzes the oxidation of luciferin to oxylu-

ciferin in the presence of ATP, magnesium, and oxygen, resulting in bioluminescence (DeLuca, 1976). Along with the commercial interest, firefly luciferase genes are favored as a reporter gene in many organisms such as bacteria (Jacobs *et al.*, 1993), cellular slime moulds (Howard *et al.*, 1988), plants (Millar *et al.*, 1992), silkworm (Vikas *et al.*, 1995), and mice (DiLella *et al.*, 1988; Kondo *et al.*, 1992). The enzymatic assay of the luciferase is mainly preferred due to several merits, *i.e.*, sensitivity, rapidity,

and non-invasive method of quantification. Up to date, more than 30 luciferase genes have been isolated from about 12 firefly species belonging to family Lampyridae (Table 2). These were cloned and sequenced with a diverse interest such as an over-expression of the gene for the monitoring of the promoter activity (e.g., de Wet *et al.*, 1987), connection between bioluminescence spectra and primary structure (e.g., Viviani *et al.*, 1999), illustration of genomic structure (e.g., Cho *et al.*, 1999), and domain structure (e.g., Ye *et al.*, 1997). Along with these purposes, some firefly luciferase genes were cloned and sequenced with a phylogenetic interest. For example, Ye *et al.* (1997) cloned and sequenced a firefly luciferase from *Photuris pennsylvanica* in part to illustrate the domain structure of the luciferase. Further, the cDNA sequence was utilized for the comparison with the luciferases of other members of Luciolinae, and found a close relationship among *Luciola* species belonging to subfamily Luciolinae. In fact, fireflies use light signals in sexual communication and produce species-specific flashing pattern. Thus, the primary structure of the enzyme interacting with the substrate luciferin to produce bioluminescence may provide some insight into the genetic relatedness among close species.

Mitochondria play a central role in metabolism (Brand, 1997), apoptosis (Kroemer *et al.*, 1998), disease (Fliss *et al.*, 2000), and aging (Van Remmen and Richardson, 2001). They are the site of oxidative phosphorylation, essential for the production of ATP, as well as a variety of other biochemical functions (Boore, 1999). The metazoan mitochondrial DNA (mtDNA) has a remarkably conserved gene content, containing 37 genes: 13 protein-coding genes, 2 ribosomal RNA genes, and 22 transfer RNA genes (Wolstenholme, 1992). Additionally, it contains a control region known in insect mtDNA as the adenine (A) + thymine (T)-rich region, which contains the replication origin for both mtDNA strands in *Drosophila* species (Fauron and Wolstenholme, 1980; Clary and Wolstenholme, 1985).

MtDNA is characterized to have a relatively simple genetic structure, a straightforward form of transmission without recombination, and rapid rate of change that is up to ten times faster than nuclear DNA counterparts (reviewed by Avise, 1994). Due in part to these properties various mitochondrial regions have been used to gain information on the population genetic structure and/or evolutionary relationships of insect species (Xiong and Kocher, 1991; Gasparich *et al.*, 1995; Zhang *et al.*, 1995; Besansky *et al.*, 1997; Bae *et al.*, 2001; Kim *et al.*, 2003; Lee *et al.*, 2003). In particular, the mitochondrial cytochrome *c* oxidase subunit I (COI) sequence is preferentially employed as taxon "barcodes" for animal identifi-

cation (Hebert *et al.*, 2003).

***Hotaria*-group fireflies**

Hotaria unnumsana and *H. papariensis* occur widely in Korea during late May ~ middle July (Kim *et al.*, 2004) and were first recorded on the basis of the specimens collected on Mt. Unmun located in southern part of Korean Peninsula (Doi, 1931) and *H. papariensis* from far northern locality Paba-li (Doi, 1932), respectively. These two species are seemingly very alike, and the major morphological difference is a color pattern of the pronotum: orange-red in *H. unnumsana* and yellowish brown with semicircular speckles at the first thorax in *H. papariensis* (Doi, 1931, 1932). This distinction was further supported in a morphological study by Sim (2001): the presence of two dots in the light organ located at the fifth abdominal node, smaller male size, and larger female size in *H. papariensis*. However, Sim's finding (2001) is seemingly faulty in that the two species were pre-divided on the basis of Doi's criteria (1931, 1932), in which the yellowish brown semicircular speckles on the first thorax was employed as a prime criterion to distinguish one from another species. Also, Sim and Kwon (2001) investigated the regional distribution of the two species and found that roughly *H. papariensis* was dominant in the mid part of Korean Peninsula, whereas *H. unnumsana* was abundant in the southern part. However, they basically found both species in every place surveyed, except for a remote Island, Jeju, Korea, where only *H. unnumsana* was found. Based on these results the two species of *Hotaria* are sympatric. This finding casts a doubt why these two species occur together in an ecologically identical place.

Hotaria parvula, which occurs widely in Japan was first recorded by Kiesenwetter (1874) and Nakane (1970) later reported another species, *H. tsushimana*, endemic only to Tsushima Island, Japan. Before the genus *Hotaria* was erected *Hotaria* species were placed into *Luciola*. Earlier Yuasa (1937) erected a new genus *Hotaria* separately from *Luciola* based on several features such as possession of degenerative back wings in females, larger body size in males, and terrestrial larval stages of *Hotaria*-group fireflies, although some of these features are not universal (e.g., terrestrial life during larval stage). Later, McDermott (1966) and Ballantyne (1968) put the genus *Hotaria* into subgenus of *Luciola*, but currently *Hotaria* is treated as an independent genus in the Japanese firefly checklist (Ohba, 1998). Acting in concert with the revision by Yuasa (1937), the two previous Korean *Luciola* species (*H. unnumsana* and *H. papariensis*) also were treated as *Hotaria* fireflies (Sim and Kwon, 2000).

Although islands are a source of adaptive radiation and Tsushima Island may be a good place to sustain an endemic firefly species like *H. tsushimana* this taxonomic hypothesis seems to be premature until the neighboring source populations are carefully surveyed. This may be true particularly because Tsushima Island is only 50 km away from Korean Peninsula, and the southern Korean coastline is characterized by numerous small islands that lie within 1 ~ 3 km of the mainland. Furthermore, Tsushima Island, Korean offshore islets and also the Korean mainland were joined by the lowered sea level about at 20,000 years ago when the last glacial advanced (Ohshima, 1990). Thus, it would be reasonable to speculate that *H. tsushimana* occurring on Tsushima Island may have some relatedness with the species occurring in Korean Peninsula.

In such situation, Suzuki (1997) reported the phylogenetic relationships of 27 Japanese fireflies including two *Hotaria* species (*H. tsushimana* and *H. parvula*) using a portion of mitochondrial 16S ribosomal RNA (16S rRNA). Later, Kim *et al.* (2000) sequenced an identical portion of mtDNA from two geographic samples of *H. papariensis*, along with other firefly species occurring in Korea and incorporated the sequence data to Suzuki's data (1997). The phylogenetic analysis did not show species-based clustering among *H. tsushimana* and two *H. papariensis* samples (Figs. 6, 7 in Kim *et al.*, 2000). Based on this result Kim *et al.* (2000) suggested that the *H. papariensis* and *H. tsushimana* may not be independent species and cast a strong doubt on the validity of the species status of *H. tsushimana*.

In this circumstance, Ohmiya *et al.* (1995) and later Choi *et al.* (2002) respectively reported a cDNA sequence of the luciferases composed of 1,644 nucleotides with an open reading frame of 548 amino acid residues each from *H. parvula* and *H. unmunšana*. Choi *et al.* (2003) subsequently reported the complete genomic sequence of the luciferase from *Hotaria*-group fireflies (excluding *H. parvula*) and found a strong relatedness among *H. papariensis*, *H. unmunšana*, and *H. tsushimana* in that the three species share an identical length (1,950 bp) and exon/intron structure (Fig. 2). Further, amino acid sequence

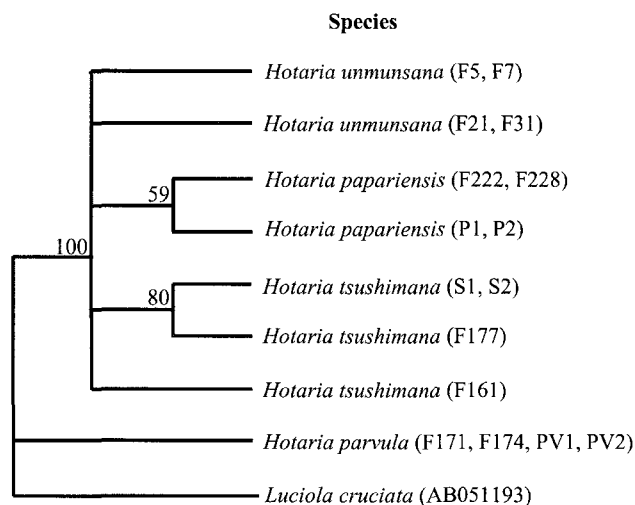


Fig. 3. A phylogenetic tree obtained by the analysis of mitochondrial COI nucleotide sequences of the *Hotaria*-group firefly using homologous sequences of *L. cruciata* as an outgroup (Choi *et al.*, 2003).

variation was low among three *Hotaria*-group fireflies including several isoforms (0 ~ 3 amino acid residues), and found no discernible difference on the basis of the amino acid difference. However, they found an equidistant amino acid difference of the *H. parvula* from the other three *Hotaria* species (10 ~ 11 amino acids) (Choi *et al.*, 2003). In the same report, Choi *et al.* (2003) performed phylogenetic analysis among *Hotaria*-group fireflies with amino acid sequences of luciferases, and obtained an unresolved branches among *H. papariensis*, *H. unmunšana*, and *H. tsushimana*, but *H. parvula* was well separated from others (Fig. 4 in Choi *et al.*, 2003). Furthermore, they sequenced a 438-bp region of the mitochondrial COI gene from several samples of the four *Hotaria* species and found an identical amino acid sequences in all samples of three *Hotaria* species, except for *H. parvula*, where amino acid sequences of *H. parvula* samples differed by three common positions from other *Hotaria* species (Choi *et al.*, 2003). Nucleotide sequences, on the other hand, resulted in two or three haplotypes in each *H. papariensis*, *H. unmunšana*, and *H. tsushimana*, except

A

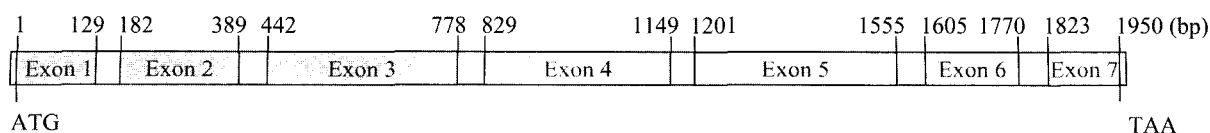


Fig. 2. Genomic organization of the luciferase gene of *H. papariensis*, *H. unmunšana*, *H. tsushimana* (Choi *et al.*, 2003).

for *H. parvula*, in which one identical haplotype was resulted from four samples. The nucleotide sequence divergence among these species ranged from 1.87% (eight nucleotides) to 5.7% (25 nucleotides), but no species-specific distinction was available. On the other hand, *H. parvula* was well distinguished from the other three species, with the sequence divergence ranging from 9.6% (42 nucleotides) to 11.9% (52 nucleotides). Further, phylogenetic analysis based on nucleotide sequences of mitochondrial COI gene also showed unresolved branches among haplotypes of three *Hotaria* species, but *H. parvula* was well separated from the other three *Hotaria* species (Fig. 3). Based on these information Choi *et al.* (2003) concluded that *H. papariensis*, *H. unmunšana*, and *H. tsushimana* are not diverged to each other enough to be considered as independent species.

Although *H. papariensis*, *H. unmunšana*, and *H. tsushimana* together did not subject to a thorough morphological consideration they have been regarded to be very similar species (Suzuki, 2001). Recently, Kim *et al.* (2004) examined morphological aspects of *H. unmunšana* and *H. papariensis* and found that the dispersed semicircular speckles on the dorsal thorax, which is the most distinguishable morphological feature, were not absolute in *H. papariensis* and found a polymorphism within species and sometimes between species, suggesting that the two species may not be different species.

Although several authors suggested *H. papariensis*, *H. unmunšana*, and *H. tsushimana* to be identical species (Suzuki, 2001; Choi *et al.*, 2003; Kim *et al.*, 2004) further decisive conclusion should be made after more aspects on these species are considered (*i.e.*, lightning pattern). Also, data on geographic samples of *Hotaria* species are not yet available, except for *H. parvula*, where allozyme data were employed (Suzuki, 1993). Therefore, data on geographic samples of *Hotaria*-group fireflies would be greatly appreciated, particularly because samples from biased locality may lead to the false taxonomic conclusion if intra-specific variation is substantial.

Luciola lateralis

Luciola lateralis occurs in Korea, northeast China, Sakhalin, the Kuril Islands, and four major islands of Japan (Hokkaido, Honshu, Shikoku, and Kyushu). In Korea, it occurs widely, but in extremely limited habitats such as terraced rice fields and wet fields during June to July (Kim *et al.*, 2004). The species was first recorded from the samples collected from several localities in Korean Peninsula (Doi, 1931). A developmental characteristic study by indoor-rearing suggested that some individuals undergo

more than one year for emergence as adult fireflies, whereas others take only one year (Kim *et al.*, 2001).

This species is unique in that it occurs both in Korean and Japanese main territory widely. The populations of the species in each territory have been studied independently, and it is only recent that populations of both territories were considered together for genetic aspects, although limited. Kim *et al.* (2001) investigated the genetic structure of the Korean populations of *L. lateralis* using mitochondrial COI gene sequences and found a genetic subdivision corresponding to the major mountain range in Korea. For the Japanese populations, Suzuki *et al.* (2001) presented the randomly amplified polymorphic DNA pattern of only Japanese populations of *L. lateralis*. The study was aimed to illustrate the possible genetic differentiation between the Honshu and Hokkaido populations, which were recognized as two flashing pattern-based ecological types, but it was inconclusive (Ohba *et al.*, 1993). In the course to find out genetic relationships of the Korean fireflies to Japanese fireflies, Kim *et al.* (2000) analyzed a portion of mitochondrial 16S rRNA from one sample of Korean *L. lateralis* and combined the data with a previous report by Suzuki (1997). In this analysis, two territorial samples of *L. lateralis* clustered together, excluding other species of *Luciola*, but the pairwise nucleotide divergence between them suggested the presence of a substantial nucleotide difference between them when the divergence estimate of other pairs of *Luciola* species is considered.

In the other course of study, Tatsumi *et al.* (1992) reported a cDNA sequence of the luciferase from *L. lateralis* collected from Japan. Later, Cho *et al.* (1999) also reported a total of two amino acid sequence types of the cDNAs from samples collected from one of the most firefly-abundant localities, Muju, located in southern part of Korean Peninsula. Recently, Kim *et al.* (2004) additionally reported three types of luciferase genomic DNAs from the samples collected from two Korean mainland localities, Muju and Boun and found two types from Muju samples (names as MJ1 and MJ 2) and one type from Boun samples (names as BU). The two types from Muju were found to be identical to the luciferase types found in Cho *et al.* (1999), resulting in a total of three Korean types of *L. lateralis* luciferases. The cDNA length of the *L. lateralis* luciferase was identical among all geographic samples and also species of *Luciolinae* subfamily (Table 2). Also, the total length and exon/intron composition of the genomic DNA of the *L. lateralis* luciferase gene were identical among Korean *L. lateralis* samples as 1,197 bp from start codon to before-stop codon (not provided for Japanese sample) (Kim *et al.*, 2004).

Using the sequence information of the luciferase types

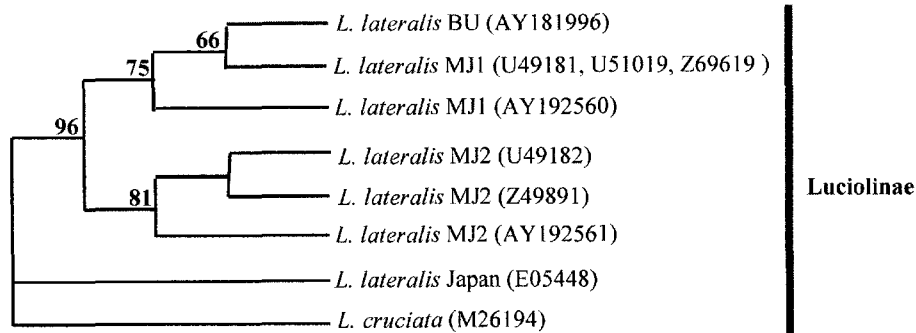


Fig. 4. A phylogenetic tree for aligned nucleotide sequences of the *L. lateralis* firefly luciferase. The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1,000 replicates. The outgroup chosen was *Luciola cruciata* (Masuda *et al.*, 1989). The GenBank numbers within parenthesis indicate identical sequences. Because the stop codon of *L. lateralis* Japan (E05448; Tatsumi *et al.*, 1992) is not available in the GenBank, all the stop codons of the remaining luciferase genes were removed from this analysis (Kim *et al.*, 2004).

Kim *et al.* (2004) compared amino acid sequence divergence among territorial samples of *L. lateralis*, and found an equidistant divergence of the Japanese type from the three Korean types (0.9% ~ 1.1%), although among-Korean types ranged only from 0.2% to 0.4%. In the phylogenetic analysis a distant relationship of the Japanese type from three Korean types was observed, forming relatively strong group among three Korean types (Kim *et al.*, 2004). Nucleotide-based comparison among the three Korean luciferase types turned into six types, and the divergence estimate also supported a distant relationship of the Japanese type from the six Korean types (2.0% ~ 2.3%), whereas among-Korean types ranged only from 0.2% to 0.7%. Phylogenetic analysis using nucleotide sequence was more robust than amino acid sequence-based tree in that the Korean types were clustered with a higher bootstrap support (Fig. 4). Based on these results, Kim *et al.* (2004) concluded that there is a genetic boundary between the territorial *L. lateralis* and suggested the Nmahe Sea that lies between Korean Peninsula and Japan as the most likely source of genetic isolation of the two territorial populations.

More population-oriented study was performed by Suzuki *et al.* (2004). They amplified mitochondrial COII gene from the samples collected from 46 Japanese localities and two Korean localities, and digested the PCR products with several restriction enzymes. They expected genetic subdivision between the Honshu and Hokkaido populations, which were recognized as two separate groups based on inter-flash interval of mate-seeking males (Ohba *et al.*, 1993), but they found no discernible difference between them. Instead, what they found was two distinct haplotypes from Korean samples, and found higher genetic distance, presented as *p* value (0.081), between Korean and Japanese haplotypes than those within Japan

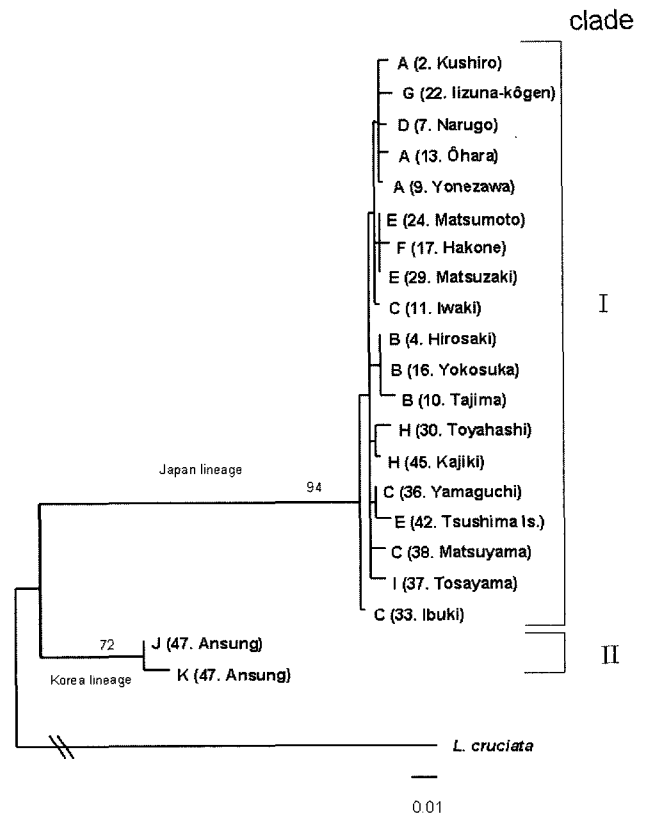


Fig. 5. Maximum likelihood tree of COII gene haplotypes in *Luciola lateralis* under the TrN + G model. A to K are haplotypes of the populations in parentheses. Bootstrap scores greater than 60 % are shown (Suzuki *et al.*, 2004).

(Suzuki *et al.*, 2004). In their phylogenetic analysis they also found the presence of independent territory-based lineages (Fig. 5).

Considering the current available results by several reports (Suzuki *et al.*, 2001; Kim *et al.*, 2004; Suzuki *et*

al., 2004) genetic isolation, caused by geographic isolation seemed to have occurred between territorial *L. lateralis* populations in Korea and Japan. However, still more population-oriented studies are needed to have a firm conclusion on this issue. For example, future study may need samples collected from rather wide Korean area, and these data can be put into Suzukis data (2004), which covered extensive geographic areas in Japan.

Pyrocoelia rufa

Pyrocoelia rufa has been recorded to occur in China, in Japan, on Tsushima Island, and in Korean Peninsula (Kim and Nam, 1981). In Korea, it occurs widely during August to September (Kim, 1999) and, thus, the adult period of the *P. rufa* is later than *Hotaria* and *Luciola* occurring in Korea. The species is known to occur in various habitats such as stream areas, wet fields, and mountain areas (Kim *et al.*, 2003). Developmental characteristic study by indoor-rearing suggested that most individuals undergo only one year for emergence as adult with sufficient feed and proper environment, whereas some take more than one year (Kim *et al.*, 2003). Adult female of *P. rufa* possesses degenerative back wings, incapable of flying. In Japan, adult males are known to be active both during day and night, but, in Korea, such behavior was only observed on Jeju Island (Kim, personal communication).

Matsumura (1928) long ago classified the Jeju Island population as *P. saishutonis*, the Tsushima Island population as *P. tsushimana*, and the Korean Peninsula one as *P. coreana* on the basis of morphological data. However, until recently this hypothesis was not tested at all either by morphological or molecular approach. In such situation, Kim *et al.* (2000) sequenced a portion of mitochondrial 16S rRNA gene of the two geographic samples of *P. rufa*: one from Korean Peninsula and the other from Jeju Island and incorporated the sequence data to the pre-existing Suzuki's data (1997), in which the sequence data of one *P. rufa* collected from Tsushima Island is available. In their phylogenetic analysis, the individual from Korean Peninsula clustered together with the Tsushima Island individual, excluding the Jeju Island *P. rufa*, although a clustering of the Korean samples was expected.

In a subsequent population-oriented investigation, Lee *et al.* (2000, 2003) collected the *P. rufa* from Korean Peninsula, Jeju Island, and Tsushima Island and sequenced a portion of mitochondrial COI gene. Sequence analysis resulted in 20 haplotypes, being subdivided into two reciprocally monophyletic groups with the minimum nucleotide divergence of 3.7% (15 nucleotides) (Fig. 6). In the perspective of geographic distribution, one haplotype

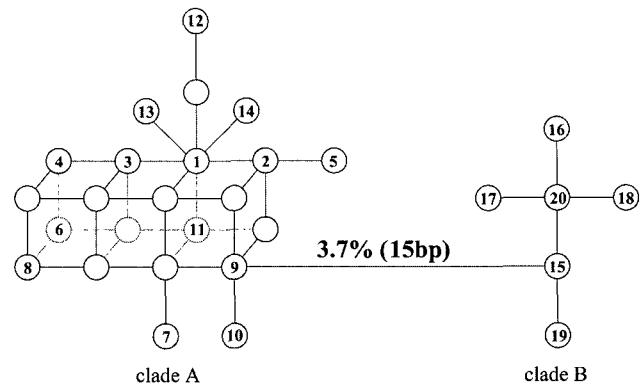


Fig. 6. Parsimonious one-step median networks analysis among 20 *P. rufa* haplotypes. Each bar indicates one nucleotide difference from the neighboring haplotype and the empty circles indicates the hypothetical haplotypes, which were not found in this study. Numbers on each circle denote haplotype name, omitting the antecedent alphabets, PR. Note that PR9 in clade A and PR15 in clade B require 15 mutational steps (3.7%) to connect between clade A and clade B (Lee *et al.*, 2003).

group was found throughout Korean Peninsula and coastal islets and also Japanese Tsushima Island, whereas the other haplotype group was found exclusively on Jeju Island, suggesting genetic differentiation of the Jeju Island population (Lee *et al.*, 2003). Analysis of population genetic structure of *P. rufa* further supported the genetic differentiation of Jeju Island population from remaining regional populations, supporting a long-ago hypothesis of Matsumura (1928). Genetic differentiation of Jeju *P. rufa* was interpreted as an accumulation of independent genetic divergence from Korean Peninsula populations after the retreat of the last glacial advance at the time of 12,000 years ago, by referring the geological data of Oshima (1990). On the other hand, the Tsushima *P. rufa* did not gain any support as an independent species due to one haplotype shared with that of one coastal islet, Namhe, and to phylogenetic outcome, in which haplotypes of Tsushima *P. rufa* clustered together with those found in Korean mainland and coastal islets (Lee *et al.*, 2003). Taken these together, Lee *et al.* (2003) suggested that the Jeju *P. rufa* seems to have gained independent genetic divergence from remaining populations, but this taxonomic suggestion is solely based on mtDNA.

Currently, luciferase gene information of the *P. rufa* is available for the sample collected from Korean Peninsula (Lee *et al.*, 2001), but not for those from Tsushima Island and Jeju Island. Thus, luciferase gene sequence of the *P. rufa* collected from those regions would be greatly appreciated in order to infer species status among local populations of *P. rufa*. Also, genetic information of the *P. rufa*

from a wide distributional region of China and North Korea, known to be *P. rufa* habitats, is needed to draw further complete taxonomic conclusion on *P. rufa* populations. Li *et al.* (2003) recently reported the genomic DNA sequences of luciferase gene of each one *P. rufa* collected from Korean Peninsula and Huwan, China, which is located ~1,000 km away from Korean mainland, and found some length variation among introns and amino acid substitution in the luciferase gene, whereas the length of amino acid (548) and exon/intron structure (seven exons and six introns) were identical. In the same study, the analysis of a portion of mitochondrial COI sequences of the Chinese *P. rufa* revealed a substantial sequence divergence from pre-existing Korean and Japanese haplotypes (6.9% ~ 8.4%) (Lee *et al.*, 2003). These results suggest the necessity of collective study covering samples collected from whole distributional range of *P. rufa* including China with the molecular markers derived both from nuclear and mtDNA to have a firm conclusion. In recent, Bae *et al.* (2004) reported the complete mtDNA sequence of *P. rufa* (17,739 bp) collected from Muju, Korea. The complete mitochondrial genome contains 37 genes and one hypervariable A+T-rich region, which are variable in evolutionary rate. Thus, this sequence information may be applied to design primers covering various regions of mtDNA, which reveal different evolutionary rate to address questions on geographic variations of *P. rufa* and species status of the populations.

In summary, we have reviewed recent reports on geographic variation and phylogenetic relationships of the firefly species, occurring in both Korean and Japanese territories. Mitochondrial DNA and luciferase gene sequence data provided some insight into these species in terms of validity of species name, phylogenetic relationships, and speciation event. Phylogenetic analysis using luciferase gene and mitochondrial COI sequences suggested that only *H. unmunisana* and *H. parvula* seem to be valid species among *Hotaria*-group fireflies. In the case of *Luciola*, geographic samples of the *L. lateralis* collected in Korea are well differentiated from those collected in several Japanese localities, suggesting that speciation may have occurred between two territorial populations. Phylogenetic analysis and population structure of the *P. rufa* on the basis of mitochondrial COI sequences suggested the validity of species status for Jeju Island population, but not for Tsushima Island population, although other molecular markers including luciferase gene are urgent to resolve this issue. To have further conclusive result, still more data on the distributional range and diverse molecular markers are required in the future.

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