Short Note

Male Morphological Dimorphism in the Genji Firefly *Nipponoluciola cruciata* in Central Japan

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Male Morphological Dimorphism in the Genji Firefly *Nipponoluciola cruciata* in Central Japan

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Abstract: The Japanese Genji firefly *Nipponoluciola cruciata* is famous for geographic variation in flash pattern. Such geographic variation has also been well studied in terms of ecology and molecular phylogeny. However, there are very few studies on geographic variation in morphology of this species. This study focused on morphological characteristics of males of this species in Yamanashi and Gunma Prefectures separated by the Kanto Mountains in central Japan. Previous studies have shown that this species in the two prefectures exhibits different genetic features as well as different flash patterns. This study aimed to explore morphological characteristics of males of this species and performed multivariate analysis using measurements previously published in the literature. As a result, allometric analysis between body length and elytra width suggested that this species exhibits different allometric patterns between the two prefectures. Moreover, cluster analysis of measurements of genitalia suggested that this species is completely separated into two different groups between the two prefectures. These results suggest the importance of further morphological studies to clarify the divergence of this species.

Keywords: Male Dimorphism; Allometriy; Cluster; Gunma; Yamanashi

1. Introduction

The aquatic firefly *Nipponoluciola cruciata* (formerly named *Luciola cruciata*, Coleoptera, Lampyridae) is commonly called Genji-botaru and one of the most popular insects in Japan [1]. Interestingly, this species shows geographic variation in interflash intervals, which vary from about 2 s in western Japan to about 4 s in eastern Japan [2–4]. Molecular phylogenetic studies have also found genetic differentiation between the 4-s and 2-s flash types [5–8]. However, there are very few studies on geographic differences in morphological characteristics of this species. Ohba [9] examined several morphological measurements of this firefly such as body length, body width, pronotum length, and pronotum width throughout Japan. However, he found no significant geographic differences in mean values of measurements. He also showed geographic differences in regression of pronotum length on pronotum width but did not further multivariate analysis to compare regression lines. Imasaka [10,11] also examined several morphological measurements of this species throughout Japan but did not perform detailed statistical analysis.

Fortunately, the present author found that Imasaka [10,11] documented morphological measurements of this species in detail. Therefore, in this article, the author aims to reanalyze his data and statistically clarify characteristics of this species varying geographically.

This article focuses on morphological characteristics of this species in Gunma and Yamanashi Prefectures, central Japan (Figure 1). According to previous studies [4,12,13], this species in Gunma belongs to the 4-s flash type and in Yamanashi belongs to the 3-s flash type. In terms of geographic characteristics, the Kanto Mountains exist between the two prefectures. Previous studies [4,12–14] inferred that the divergence of *N. cruciata* in central Japan occurred between the 4-s flash type and the common ancestor of the 2-s and 3-s flash types about 11 Ma in the Miocene in relation to the uplifting of the Kanto Mountains. Therefore, the present author hypothesized that statistical differences would be also observed in the measurements of Imasaka [10,11] between the two prefectures.

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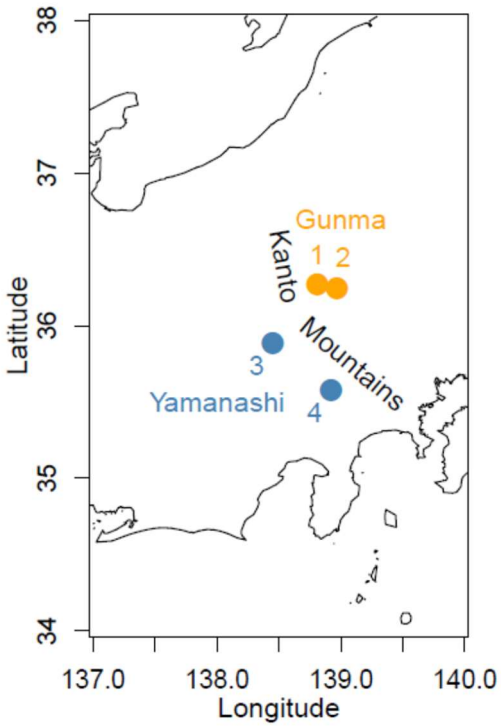

Figure 1. Map of the study area in Gunma and Yamanashi Prefectures, central Japan. Numbers denote the following localities for specimens used in this study: 1. Tomioka, Gunma; 2. Yoshii, Gunma; 3. Takane, Yamanashi; 4. Tsuru, Yamanashi.

2. Materials and Methods

The data used in this study were morphological measurements of male *N. cruciata* collected at two sites in Gunma Prefecture and two sites in Yamanashi Prefecture, central Japan as shown in Figure 1. They were obtained from Table 2 of Imasaka [10] and Table 2 of Imasaka [11].

The measurements used this study are as follows: body length, elytra width (width of the basal part), and lengths of six parts of genitalia. The statistical analysis was performed using two multivariate analysis methods: allometric analysis between body length and elytra width and cluster analysis of lengths of six parts of genitalia. The data of genitalia contain missing values. Specimens with missing values were excluded from cluster analysis. The specimen B82 shows an extremely small value of the part j of genitalia (Table 2 of Imasaka [11]). This was assumed to be a misprint and therefore this specimen was excluded from cluster analysis.

According to previous studies on the firefly *Luciola parvula* [15,16], allometric equations were applied to the data of body length and elytra width using standardized major axis regression with the smatr package in the R software [17]. The data were $\log_{10}$-transformed into the log-log relationship between body length (x) and elytra width (y). Consequently, the $\log_{10}$-transformed allometric equation was expressed as:

$$\log_{10} y = \log_{10} a + b \log_{10} x$$

where a and b are constants. The constant b is the slope of the line, also known as the allometric coefficient.

The hierarchical cluster analysis of the lengths of six parts of genitalia was performed with the function hclust of the R software [17] using Ward's method with Euclidean distance.

3. Results

As shown in Figure 2, the two allometric lines did not differ significantly in slope ($\chi 2 = 2.07$, df = 1, $p = 0.150$) but did differ significantly in elevation (y-intercept) ($\chi 2 = 8.31$, df = 1, $p = 0.004$). The

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common slope ($b = 1.40$) was significantly different from 1 ($\chi 2 = 9.00$, df = 2, $p = 0.011$), which indicated a positive allometry between body length and elytra width.

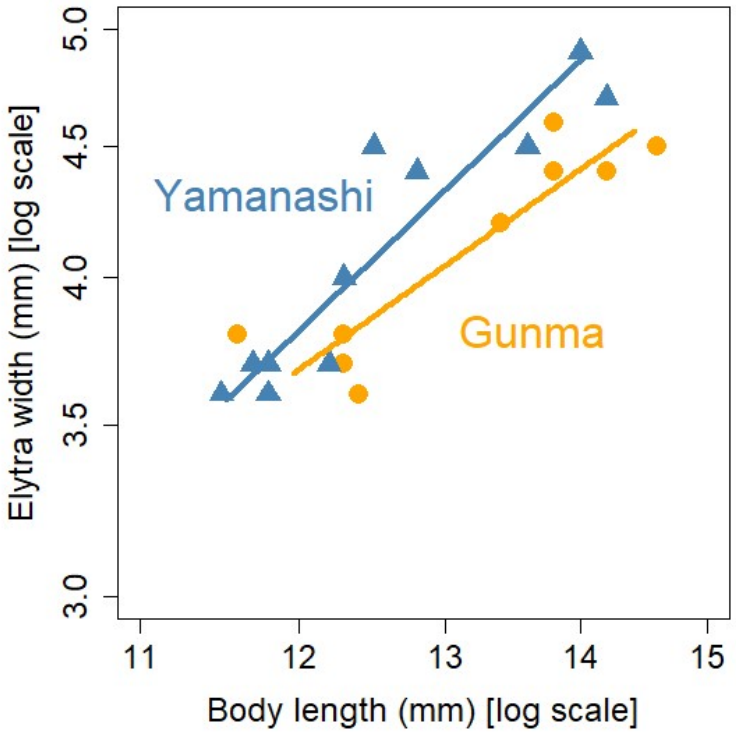

Figure 2. Allometric relationship between body length and elytra width in *N. cruciata* in Gunma and Yamanashi Prefectures, central Japan. The standardized major axis regression lines were separately fitted to each prefecture.

As shown in Figure 3, the results of cluster analysis showed that Gunma and Yamanashi specimens were completely classified into two major clusters.

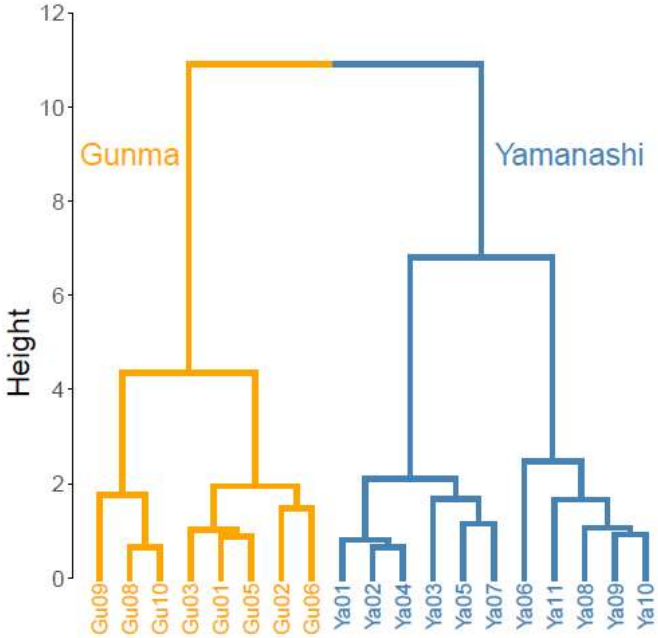

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Figure 3. Dendrogram of cluster analysis of measurements of genitalia in male *N. cruciata* in Gunma and Yamanashi Prefectures, central Japan. The specimens collected in Gunma and Yamanashi Prefectures are represented by Gu and Ya, respectively, in the *x*-axis label.

4. Discussion

The results of allometric analysis showed that the allometry of *N. cruciata* in Gunma and Yamanashi Prefectures is represented by two parallel lines. These findings are similar to the allometric patterns of the Japanese terrestrial firefly *Luciola parvula* observed in Nagano Prefecture, central Japan [15]. However, as shown in Figure 2, there remains a possibility that *N. cruciata* in Gunma and Yamanashi Prefectures may show different allomeric slopes. Further studies are required with larger sample size to determine the allometric patterns of *N. cruciata* in central Japan.

The results of cluster analysis revealed that *N. cruciata* shows different shapes of male genitalia between Gunma and Yamanashi Prefectures. As mentioned above, this species in Gunma belongs to the 4-s flash type and in Yamanashi belongs to the 3-s flash type [4,12,13]. Therefore, this species of different flash types may exhibit different shapes of male genitalia in other areas. However, few studies have explored the relationship between morphology and flash types in this species. Ohba [18] mentioned that the 4-s type occasionally lacks a black marking in the pronotum but did not refer to differences in internal organs such as genitalia.

Ohba [9] suggested that variations in size of external structures in *N. cruciata* are larger within populations than among populations. However, the results of this study showed that multivariate analysis can be useful to detect size variations in both external and internal structures. The male morphological dimorphism of this species observed in this study may be associated with topography in this area. Further morphological studies are required to clarify the divergence of this species especially in mountainous areas of central Japan.

4. Conclusion

This article explored differences in morphological characteristics of the Japanese Genji firefly *Nipponoluciola cruciata* between Yamanashi and Gunma Prefectures separated by the Kanto Mountains in central Japan. Two multivariate analysis methods were performed using measurements previously published in the literature. First, allometric analysis between body length and elytra width suggested that this species exhibits different allometric patterns between the two prefectures. Second, cluster analysis of measurements of genitalia suggested that this species in the two prefectures is completely classified into different groups. These results suggest the importance of further morphological studies to clarify the divergence of this species.

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Conflicts of Interest: The author declares no conflicts of interest regarding the publication of this paper.

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