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ORIGINAL ARTICLE

# Stages in the degeneration of flight ability and their interspecific comparisons in the genus *Synuchus* (Coleoptera: Carabidae) in Japan

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**Key words.** Ground beetles, flight muscle, flight traits, hind wing, polymorphism

**Abstract.** The degeneration in flight ability in beetles has rarely been studied in detail with regard to the polymorphism of flight traits within species. However, intraspecific diversity in flight traits means that the flight ability of species is in the process of degenerating, which could provide important insights into how flight ability in beetles degenerates. In this study, the flight muscle and hind wings of the genus *Synuchus* in Japan were studied, which revealed the morphological status of flight traits in 21 species of *Synuchus*. Several species in this genus were found to show intraspecific polymorphisms in the states of the flight muscle and hind wings, and in particular, the very high diversity of different types of hind wings. These results indicate that this genus contains a mixture of species at various stages in the degeneration of the ability to fly.

## INTRODUCTION

The acquisition of the ability to fly was important for insects as it enabled them to disperse and colonize a great variety of environments (Romoser, 1981). On the other hand, the loss of the ability to fly is common in many taxonomic groups, with 10% of all pterygote insects secondarily flightless (Roff, 1990). The order Coleoptera accounts for 40% of all insects in terms of number of species and is a very diverse group (Cai et al., 2022). The degeneration in the ability to fly in beetles is thought to have contributed significantly to their diversification (Ikeda et al., 2012). In particular, many ground beetles have degenerated the ability to fly (Kennedy, 1994; Washimi et al., 2015), which is accompanied by diversification of ecological traits and speciation (e.g., Ikeda et al., 2008; Nishimura et al., 2022).

The degeneration of the ability to fly in insects occurs in the following sequence: reduction in frequency of flight, degeneration of flight muscle, and degeneration of hind wings (Roff, 1986). Over the course of each stage in the degeneration, polymorphism, including dimorphism and trimorphism occur (Den Boer et al., 1980; Harrison, 1980). However, examining morphological traits related to the ability to fly (flight traits), such as the flight muscle and hind wings, requires the removal of hard elytra, and as a consequence there is only information on a few groups, such as the beetles in subfamily Carabinae (Shibuya et al., 2017, 2018). Thus, little is known about the polymorphism of flight traits in beetles. Although only a limited number of beetles show intraspecific polymorphism in flight muscle, *Necrophila japonica*, *Phelotrupes laevistriatus* and some carabid beetles are known to show intraspecific dimorphism (Desender, 2000; Matalin, 2003; Ikeda et al., 2007; Ohta et al., 2009). Three morphological types of hind wings are known in beetles, macropterous, brachypterous and apterous, with brachyptery further divided into subtypes such as brachypterous (short type) and brachypterous (stick type) (e.g., Inaizumi, 1966; Shibuya et al., 2018). These types of hind wings in each species are either monomorphic or polymorphic, with the latter including dimorphism and trimorphism. In monomorphic species, all individuals have the same type of hind wing, whereas dimorphic species have two different types of hind wing and trimorphic species have three (Fujisaki, 1994; Shibuya et al., 2018). In ground beetles, trimorphic hind wings are not reported, and only one combination (macropterous and brachyptous) is known for dimorphism (e.g., Den Boer, 1970; Aukema, 1995; Yamashita et al., 2006). Polymorphic flight traits indicate that flight ability is in the process of degenerating, which is important for clarifying the process of degeneration in ability to fly in beetles.

The genus *Synuchus* Gyllenhal, 1810, a member of the family Carabidae, is a group of ground beetles that have diversified mainly in East Asia, with 37 species on the Japanese archipelago (Mori, 2015). In recent years, it has become increasingly clear that the flight traits of several species in this genus are polymorphic. Hori (2008) sug-

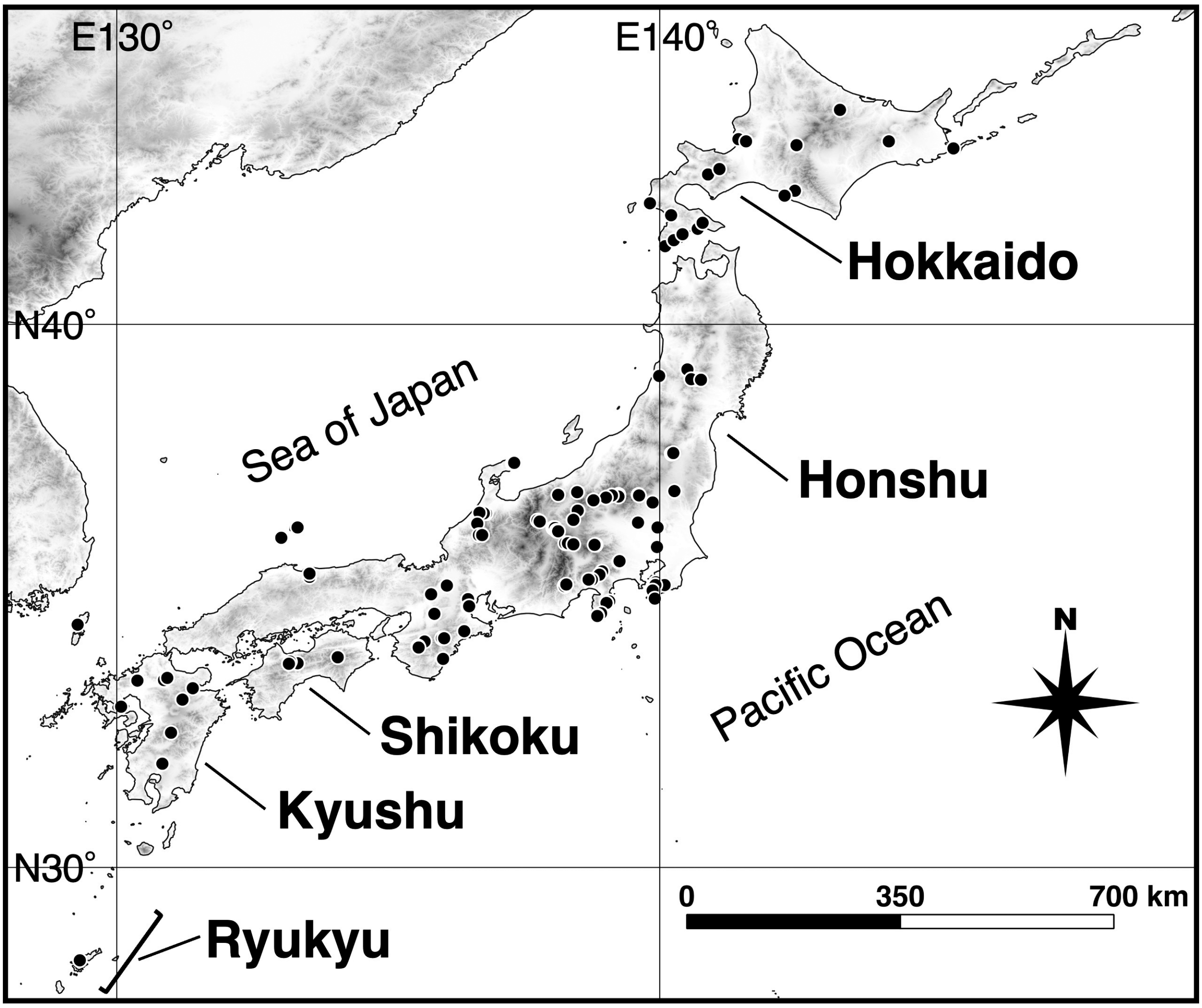


**Fig. 1.** Map of localities sampled in Japan.

gests that flight muscle dimorphism might exist in two species, *Synuchus melantho* and *S. nitidus*, as most individuals are unable to fly, but a few individuals can. Habu (1978) reports that a few individuals of *S. melantho* have hind wings and others do not. Shibuya et al. (2018) report that *S. arcuaticollis* is wing-dimorphic, with macropterous and brachypterous specimens. As these previous studies were only on specific species or populations, a comprehensive collection of samples from across Japan and examination of a larger number of species and individuals is likely to provide a better understanding of the process involved in the degeneration of the ability to fly in the genus *Synuchus*.

In view of the above, the objective of this study is to determine and compare the stages in the degeneration in the ability to fly in each species of *Synuchus*. Specifically, the focus is on the presence or absence of flight muscle and hind wings, which are typical flight traits. The status of these flight traits for each species was assessed by dissection and interspecific differences noted.

## MATERIAL AND METHODS

### Sample collection and Identification

A total of 781 individuals were collected from 110 sites throughout Japan (Hokkaido, Honshu, Shikoku, Kyushu and Ryukyu), as shown in Table S1 and Figs 1 and S1. Pitfall traps and night searching were used to collect samples. Species were identified according to Lindroth (1956), Habu (1978), Tanaka (1985), Morita (2013) and Mori (2015) using a binocular stereomicroscope (SZX-10, Olympus, Tokyo, Japan) and an optical microscope (CX41, Olympus, Tokyo, Japan). The samples included 19 species of *Synuchus* (*Synuchus melantho*, *S. callitheres*, *S. dulcigradus*, *S. nitidus*, *S. cycloderus*, *S. arcuaticollis*, *S. crocatus*, *S. amamioshimae*, *S. angusticeps*, *S. atricolor*, *S. fukuharai*, *S. hikosanus*, *S. montanus*, *S. picicolor*, *S. shibatai*, *S. tanzawanus*, *S. ventricosus*, *S. yamashitai* and *S. yasumatsui*) and two unidentified species. The first is morphologically similar to *S. congruus* (A. Morawitz, 1862), but we also cannot exclude *S. silvester* (Habu, 1955), a species of doubtful status (Habu, 1955, 1978; Morita, 2015). Therefore, it was not identified as either. The morphological characteristics of the second unidentified species differed from those described for all known species. Thus, we assigned *Synuchus* sp. 1 and sp. 2 to the former and latter, respectively, and a total of 21 species were included in this study.

### Morphological analysis

All individuals were dissected and their flight traits recorded. This was done immediately after capture or preservation in 99.5% ethanol. For flight traits, the focus was on flight muscle and hind wings. The conditions of these traits were checked using a microscope. The flight muscle was classified as either present or absent; however, none of the individuals examined in this study had a

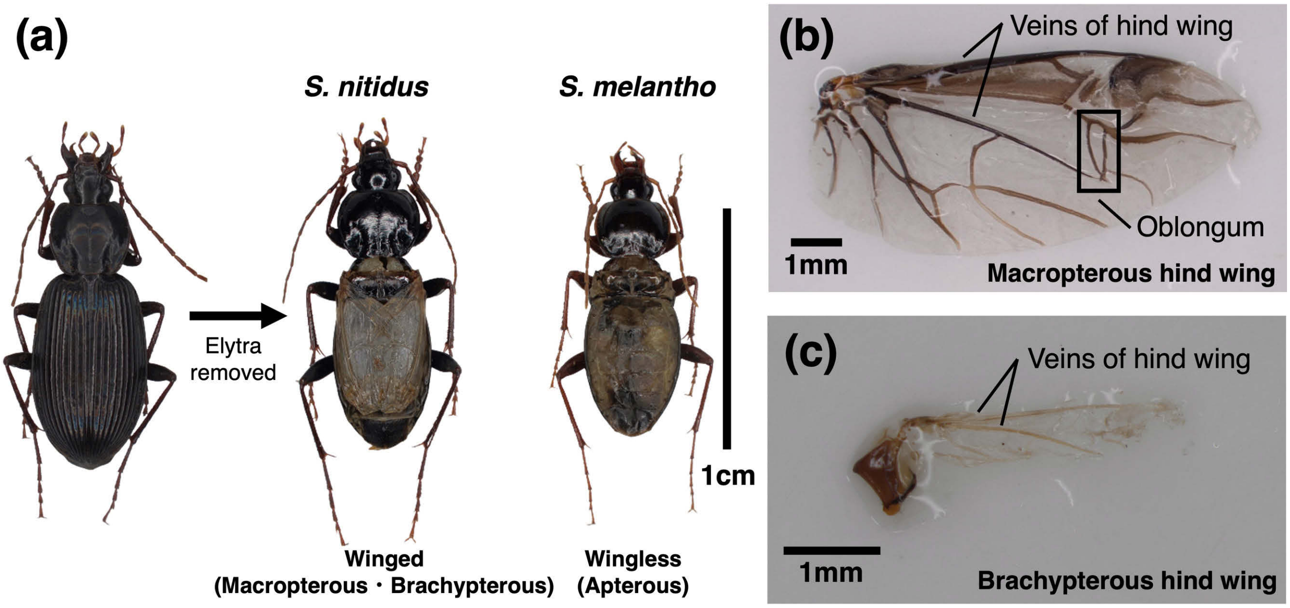


**Fig. 2.** Diversity in the shapes of the hind wings in the genus *Synuchus.* (a) The hind wings after removing the elytra. Scale: 1 cm. (b) Shape of macropterous hind wing. Scale: 1 mm. (c) Shape of brachypterous hind wing. Scale: 1 mm.

flight muscle (see Results). Thus, the species were classified into two groups according to the literature: those with flight muscle and considered to be able to fly and those without flight muscle, which were considered to be flightless. Hori (2008) reports that *Synuchus melantho* and *S. nitidus* were captured by flight interception traps (FIT) in Hokkaido and implied that although most individuals of these two species have no hind wings and are flightless, some individuals have developed hind wings and are able to fly. *S. callitheres* is also captured by malaise traps and light traps on Honshu (Tanaka, 1962; Yahiro, 1997; Nakamura, 2014; Yamauchi & Ishitani, 2020), which similarly indicates that some individuals can fly. Similarly, *S. dulcigradus* is reported captured by light traps on Honshu (Tanaka, 1962) and searchlight traps on Kyushu (So et al., 2022), which also indicates the existence of individuals capable of flying. Based on the above, four species (*S. melantho*, *S. nitidus*, *S. callitheres* and *S. dulcigradus*) were classified as capable of flying and the remaining 17 species as flightless.

According to Shibuya et al. (2018), hind wings can be classified into three types: macropterous (with well-developed veins and an oblongum), brachypterous (with faint veins and without a distinct oblongum) and apterous (without hind wings) (Fig. 2). For those species with hind wings, the appearance frequency of the hind wing (AFHW) for each type of wing and relative macropterous hind wing length (RMHWL) were calculated. After the hind wings were removed, the RMHWLs were determined by measuring body length (BL) and hind wing length (HWL) with GIMP 2.10.22 (The GIMP Development Team) based on images taken with a CCD camera (DP23, Olympus, Tokyo, Japan) and by dividing HWL by BL. Then, the mean value and standard deviation (SD) for each species was calculated. Only macropterous individuals were used to measure HWL except for *S. shibatai*, since brachypterous individuals were rarely collected (see Results). *Synuchus shibatai* is a brachypterous-monomorphic species (see Results), so we calculated the relative brachypterous hind wing length (RBHWL) for this species using the same method. Ten male and 25 female individuals per species collected from six or seven sites were used for two species (*S. nitidus* and *S. cycloderus*) to calculate their RMHWLs (Tables 1, S2) as the sample sizes were large enough. On the other hand, one to five individuals of each sex per species collected from one to five sites for the other six species were used to calculate their RMHWLs or RBHWLs (Tables 1, S2). In carabid beetles, BL and RMHWL are sexually dimorphic, with smaller BLs and thus slightly larger RMHWLs in males (Sota et al., 2000; Shibuya et al., 2018). Therefore, this index was calculated separately for males and females in this study.

In addition to the traits directly related to flight, the shape of metepisternum is also associated with a degeneration in the ability to fly in carabid beetles, with an increased metepisternum width (MW)/metepisternum length (ML) ratio in species with degenerate hind wings (Will, 2004). Thus, MW and ML for one to five individuals were measured for each of 21 species collected from one to five sites and the metepisternum ratio (MR) calculated by dividing MW by ML. Then, the mean value and SD for each species was calculated. To evaluate the relationship between MR and hind wing degeneration, a generalized linear mixed model (GLMM) was constructed using the "lme4" package (Bates et al., 2015) in R v. 4.3.1 (R Core Team, 2013). In this analysis, it was difficult to evaluate intraspecific polymorphisms in hind wings so that simple categories were assumed (macropterous or apterous) and the species broadly classified into two types based on appearance frequency of macropterous hind wings (AFMHW). The species with AFMHW above 85% were regarded as macropterous-dominant and the other species (including all monomorphic apterous species) as apterous-dominant. Seven species with fewer than three individuals and one brachypterous-monomorphic species (*S. shibatai*) were excluded, leaving 13 species in this analysis. Normality of the distribution of the data in the MRs was confirmed using the Shapiro-Wilk test. The MR was determined as a dependent variable and the state of the hind wings (macropterous-dominant or apterous-dominant) as an independent variable. The species were included as a random effect. Then, the likelihood ratio test in R v. 4.3.1 for the GLMM result was carried out. In this genus, there is sexual difference in the shape of the metepisternum in one species (*S. callitheres*; Habu, 1978). Therefore, only female individuals were used in this analysis to rule out the influence of a sexual difference. For all the statistical analyses, the significance level was set at 0.05.

**Table 1.** Number of localities, distributions, sample size, flight ability, types of hind wing, appearance frequency of hind wings (AFHW) for each wing type, and mean (± SD) relative macropterous hind wing length (RMHWL) of 21 *Synuchus* species.

| Species | No. of localities | Distribution* | *N* | Flight ability** | Type of hind wing*** [*N*] | Appearance frequency of hind wings for each wing type*** (%) | Relative macropterous hind wing length**** ± SD [*N*] |
|---|---|---|---|---|---|---|---|
| *Synuchus melantho* (Bates, 1883) | 32 | Hokkaido/Honshu /Shikoku/Kyushu | 152 | + | M[11]/A[141] | M(7.2)/A(92.8) | 0.79 ± 0.00 [2]/0.77 ± 0.03 [4] |
| *S. callitheres* (Bates, 1883) | 2 | Hokkaido/Honshu /Shikoku/Kyushu | 20 | + | M[20] | M(100.0) | 0.81 ± 0.02 [2]/0.79 ± 0.03 [7] |
| *S. dulcigradus* (Bates, 1873) | 17 | Honshu/Shikoku/ Kyushu | 46 | + | M[46] | M(100.0) | 0.84 ± 0.04 [5]/0.78 ± 0.01 [5] |
| *S. nitidus* (Motschulsky, 1861) | 42 | Hokkaido/Honshu /Shikoku/Kyushu | 153 | + | M[153] | M(100.0) | 0.68 ± 0.03 [10]/0.67 ± 0.02 [25] |
| *S. cycloderus* (Bates, 1873) | 48 | Hokkaido/Honshu /Shikoku/Kyushu | 153 | − | M[153] | M(100.0) | 0.77 ± 0.02 [10]/0.76 ± 0.02 [25] |
| *S. arcuaticollis* (Motschulsky, 1860) | 36 | Hokkaido/Honshu /Shikoku/Kyushu | 91 | − | M[82]/B[8]/A[1] | M(90.1)/B(8.8)/A(1.1) | 0.63 ± 0.03 [5]/0.59 ± 0.02 [5] |
| *Synuchus* sp. 1 | 16 | Hokkaido/Honshu /Shikoku | 44 | − | M[4]/B[1]/A[39] | M(9.1)/B(2.3)/A(88.6) | 0.76 [1]/0.74 ± 0.01 [2] |
| *S. crocatus* (Bates, 1883) | 17 | Hokkaido/Honshu /Shikoku/Kyushu | 34 | − | M[4]/A[30] | M(11.8)/A(88.2) | NA/0.74 ± 0.00 [2] |
| *S. shibatai* Habu, 1978 | 1 | Ryukyu | 10 | − | B[10] | B(100.0) | 0.19 [1]/0.19 ± 0.03 [5] |
| *S. amamioshimae* Habu, 1978 | 1 | Ryukyu | 2 | − | A[2] | A(100.0) | − |
| *S. angusticeps* Tanaka, 1962 | 1 | Honshu | 1 | − | A[1] | A(100.0) | − |
| *S. atricolor* (Bates, 1883) | 2 | Honshu | 9 | − | A[9] | A(100.0) | − |
| *S. fukuharai* (Habu, 1955) | 1 | Honshu | 2 | − | A[2] | A(100.0) | − |
| *S. hikosanus* (Habu, 1955) | 1 | Kyushu | 2 | − | A[2] | A(100.0) | − |
| *S. montanus* Lindroth, 1956 | 1 | Honshu/Shikoku | 1 | − | A[1] | A(100.0) | − |
| *S. picicolor* Lindroth, 1956 | 5 | Honshu | 18 | − | A[18] | A(100.0) | − |
| *S. tanzawanus* (Habu, 1955) | 3 | Honshu | 4 | − | A[4] | A(100.0) | − |
| *S. ventricosus* Lindroth, 1956 | 2 | Honshu | 2 | − | A[2] | A(100.0) | − |
| *S. yamashitai* Morita, 2013 | 1 | Honshu | 1 | − | A[1] | A(100.0) | − |
| *S. yasumatsui* (Habu, 1955) | 3 | Shikoku/Kyushu | 5 | − | A[5] | A(100.0) | − |
| *Synuchus* sp. 2 | 11 | Honshu | 31 | − | A[31] | A(100.0) | − |

Sample sizes are shown in [ ]. * Mainly based on Habu (1978), Lindroth (1956) and Morita (2013). *Synuchus* sp. 1 and *Synuchus* sp. 2 were recorded during our field surveys. ** + flight-able; − flightless. Flight ability was assigned on the basis of the literature (see Material and methods). *** M – macropterous; B – brachypterous; A – apterous. **** Calculated as Hind wing length (HW)/Body length (BL). −, all individuals apterous. For only *S. shibatai*, relative brachypterous hind wing length (RBHWL) is shown. Left of / is the male value and right of / is the female value. Since previous studies have shown that this index tends to differ between males and females in this genus, it was calculated separately for each sex (see Shibuya et al., 2018).

## RESULTS

Among the 781 individuals dissected, no tissue was found that was clearly identifiable as flight muscle. Three types of hind wing were recorded, macropterous, brachypterous and apterous (Fig. 2), and most species were monomorphic, with four species macropterous, one species brachypterous and 12 species apterous. However, there were also two dimorphic species (*Synuchus melantho* and *S. crocatus*), in each of which there were both macropterous and apterous individuals, and two trimorphic species (*S. arcuaticollis* and *S.* sp. 1), in each of which there were macropterous, brachypterous and apterous individuals (Table 1). Three apterous-dominant species (*S. melantho*, *S.* sp. 1, and *S. crocatus*) included 7–12% winged individuals, and most of them were macropterous (Table 1); all individuals were wingless in *S. amamioshimae*, *S. angusticeps*, *S. atricolor*, *S. fukuharai*, *S. hikosanus*, *S. montanus*, *S. picicolor*, *S. tanzawanus*, *S. ventricosus*, *S. yamashitai*, *S. yasumatsui* and *S.* sp. 2.

The RMHWLs of the species with macropterous individuals ranged from 0.63 to 0.84 for males and 0.59 to 0.79 for females, with males having greater values than females in all species (Table 1; males of *S. crocatus* could not be measured). The RBHWLs of *S. shibatai* were about 0.19 for males and females. Although macropterous *S. melantho*, *S.* sp. 1 and *S. crocatus* had low values for AFHW (7.2–11.8%), macropterous individuals of these three

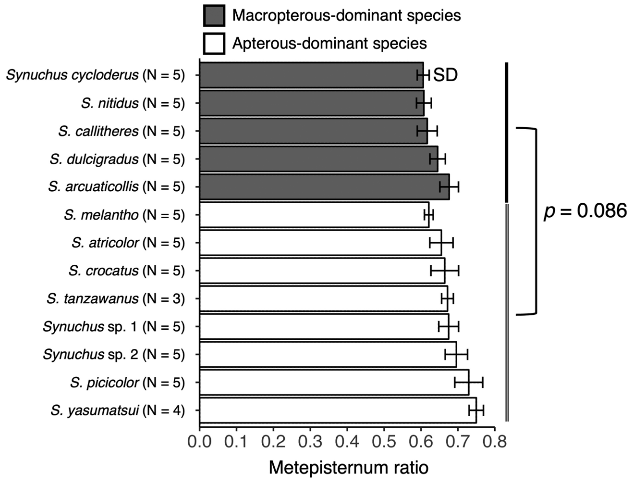


**Fig. 3.** The metepisternum ratios for 13 species of *Synuchus*. Bars show mean (± SD) metepisternum ratio (MR) for each species. *N* indicates number of individuals measured per species. Two types of hind wings are based on the appearance frequency of macropterous hind wings (AFMHW; see Material and methods). *p*-value is the result of the likelihood ratio test based on a generalized linear mixed model (GLMM) between MR and type of hind wings.

species had hind wings whose RMHWLs (ca. 0.75) were equal to those of macropterous-dominant species (Table 1). *S. arcuaticollis* had all three types of hind wings, and most individuals were macropterous (macropterous: 82/91, brachypterous: 8/91, apterous: 1/91). The RMHWL of *S. arcuaticollis* was the smallest for the eight species with macropterous individuals (Table 1).

The MRs also ranged from 0.61 to 0.84 for 21 species, with many species below 0.70 (Table S3). Result of the Shapiro-Wilk test did not confirm the normality of the distribution of the data for the MRs (W = 0.96, $p < 0.05$), so a Gamma distribution and log link function was used in the GLMM. The likelihood ratio test did not support the difference in MR of the two states of the hind wings ($\chi^2 = 2.95$, $df = 1$, $p = 0.086$; Fig. 3, Table S4).

## DISCUSSION

All the 781 individuals of the genus *Synuchus* examined in this study lacked flight muscles, the key trait determining the ability to fly in beetles (Roff, 1986; Ikeda et al., 2008). Furthermore, in this genus, there are currently no known

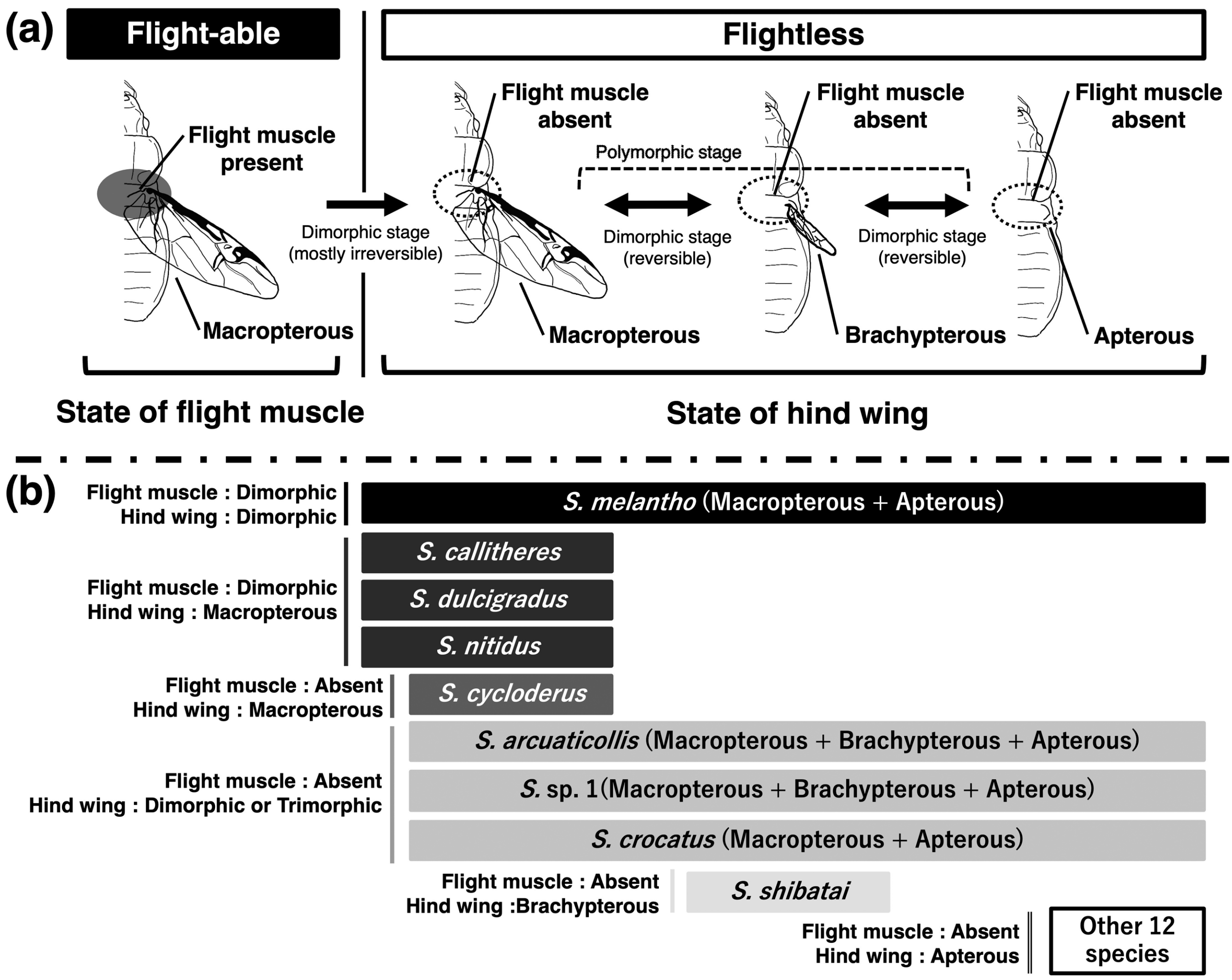


**Fig. 4.** Diagram showing the degeneration in the ability to fly in the genus *Synuchus*. (a) Degeneration of flight ability in insects. (b) States of the ability to fly in each species of *Synuchus*, corresponding to (a). The status of flight muscle in dimorphic species inferred from the literature (see Material and methods).

cases in which flight-capable individuals lose their flight muscles during their lifetime. Based on the above, most individuals of this genus seem to be flightless. However, four species, *Synuchus melantho*, *S. nitidus*, *S. callitheres* and *S. dulcigradus* (see Material and methods), appear to be flight muscle-dimorphic, as literature records indicate that flight-able individuals are present (Tanaka, 1962; Yahiro, 1997; Hori, 2008; Nakamura, 2014; Yamauchi & Ishitani, 2020; So et al., 2022). Although a total of 371 individuals of these four species were dissected in this study, no individual had distinct flight muscles, so it is highly likely that individuals with flight muscles are extremely rare. Even for species of ground beetles with flight muscles, few studies report actual flight behaviour and capturing of the flying individuals (Shibuya et al., 2018), and the flight muscles of some species are reported to dissolve after flight (Desender, 1989; Matalin, 2003; Shibuya et al., 2017). These factors might make it difficult to detect individuals that retain their flight muscles in this genus.

There were three types of hind wings in the genus *Synuchus*: macropterous, brachypterous and apterous. The hind wings of two species (*S. arcuaticollis* and *S.* sp. 1) were trimorphic, those of two other species (*S. melantho* and *S. crocatus*) were dimorphic and those of the remaining 17 species were monomorphic. Polymorphism of the hind wings in ground beetles within the same species is reported in a few cases, but prior to this study, there was only one known type of dimorphism (macropterous and brachypterous; Shibuya et al., 2018). However, two trimorphic species and two dimorphic species with a new combination (macropterous and apterous) were recorded in this study. These findings indicate that this genus is a unique and diverse group of species of ground beetles with different types of hind wings.

Relative hind wing length (RHWL) in ground beetles can be used as an index of the ability to fly (Shibuya et al., 2018), as species with a large RHWL have a higher probability of retaining flight muscles and being able to fly (Thiele, 1977; Shibuya et al., 2017). The RMHWL in this study is identical to the RHWL based on using only macropterous individuals; therefore, it can similarly be used as an index of the ability to fly for each species. The greater RMHWLs of males than females in all cases in this study is in full accord with the results of a previous study by Shibuya et al. (2018). This also fits the general phenomenon in insects that females invest more in reproduction than males and therefore are more likely to degenerate their flight traits (Roff, 1990). This indicates that males are more likely to be able to fly than females in this genus. Shibuya et al. (2018) present a criterion for RHWL in carabid beetles and defined species with a RHWL of 0.75 or less, as those with little or no flight muscle and a low potential for flight based on an analysis of the abilities to fly of many carabid beetles. In this genus, when only male individuals were compared, the RMHWLs were below 0.75 in two species, above 0.75 in five species, whereas when females were compared, four species were below 0.75 and four species above 0.75. Of the four species considered to be able to fly on the basis of reports in the literature, only *S. nitidus* had RMHWLs below 0.75 for both male and female individuals, while the values for the remaining three species were both above 0.75. These results indicate that although RMHWL can generally serve as an index of the ability to fly in this genus, there are some exceptions. In addition, species such as *S. cycloderus* and *S.* sp. 1, which were considered flightless in this study, but had high RMHWLs of 0.75 or more for both males and females or for males only, might include only a few individuals with flight muscles. Of the three species with RMHWLs below 0.75 for both males and females or females only, *S. nitidus*, which is thought to include individuals able to fly, tends to have a shorter HWL and a greater hind wing width than the other species and has a large hind wing area (S. Shibuya, pers. comm.). Therefore, it is possible that the flight ability of this species was not detected by the unitary index of RMHWL. Lindroth (1956) described this species as "Hind wings fully developed and functional", which supports the high probability that there are individuals that can fly in this species. Of the remaining two species, only two female individuals of *S. crocatus* were measured, making the data unreliable, however, the value was only slightly below 0.75. Therefore, by examining more individuals, those that are able to fly might be detected in this species. On the other hand, *S. arcuaticollis* had the smallest RMHWLs (male: 0.63, female: 0.59) of all the species studied, and several brachypterous individuals were also recorded. Therefore, this species is unlikely to be able to fly even if it retains its hind wings. Furthermore, *S. shibatai*, the only brachypterous-monomorphic species, is unlikely to be able to fly as it does not have flight muscles and has a very small RBHWLs (ca. 1/3 of the elytra length; Habu, 1978).

Although all the individuals used in this study were captured using pitfall traps or searching at night (see Material and methods), Den Boer et al. (1980) point out that there are marked differences in HWLs of individuals captured by pitfall traps and those captured by window traps in some pteridimorphic carabid beetles and propose that intraspecific HWLs vary with selection for migration. Given these considerations, it is possible that our results somewhat underestimate the intraspecific AFHW and HWL. It is also likely that individuals captured by FIT, malaise traps, light traps and searchlight traps may have greater HWLs than those recorded in this study.

The GLMM analysis and likelihood ratio test did not reveal a statistically significant relationship between MR and state of the hind wings, but a tendency of an increase in MR accompanied by hind wing degeneration was recorded. Until now, there have been few studies on the metepisternum in carabid beetles and most of them were on its use in taxonomy (e.g. Bousquet, 1996; Baehr & Reid, 2017). The result presented indicate the possibility that the metepisternum might be associated with hind wing degeneration, though the detail of the mechanism is still unknown. In addition, only simple categories of hind wings without intraspecific polymorphisms were considered, and many apterous-monotypic species whose MRs exceeded

0.7 were excluded from this analysis due to the small number of individuals available in this study. The above might have affected the accuracy of the results. Considering these things, a more detailed discussion of the relationship between MR and state of the hind wings in this genus will require further study.

The degeneration in the ability to fly in insects occurs in the following order: reduction in flight frequency, degeneration of flight muscle, and degeneration of the hind wings (Roff, 1986). Furthermore, the degeneration of flight muscle in insects is generally unidirectional. There is only one report of regeneration of flight muscle after previous degeneration in insects (Whiting et al., 2003), for which there is also a rebuttal (Stone & French 2003). The genus *Synuchus* used in this study includes a mixture of species: those with dimorphic flight muscle, without flight muscle, species with macropterous hind wings, species with brachypterous hind wings, species with polymorphic hind wings and species that lack hind wings. The results indicate that there are species at intermediate stages in the degeneration of flight ability in terms of the condition of the flight muscle and hind wings (Fig. 4). In particular, it seems reasonable to assume that the flightless species are derived from the flight muscle-dimorphic species in this genus.

Previous studies on ground beetles have focused on the relationship between the degeneration in the ability to fly and the nature of the environment. Roff (1990) states that it occurs in stable and/or isolated environments, and Den Boer (1970) indicates that macropterous individuals decrease and brachypterous individuals increase with time after a disturbance in species with dimorphic hind wings. Almost all of the species included in this study that have not fully lost their flight muscle or hind wings are widely distributed species, whereas those that have completely lost their ability to fly have narrow distributions, with most of them restricted to mountainous areas above an altitude of 600 m (Tables 1, S1, S2). These results indicate that the ability to fly might be retained in species adapted to a wide range of environments, but lost in species locally distributed in mountainous areas. In carabids, species with polymorphic flight traits have survival advantages over monomorphic species (Kotze & O'Hara, 2003) and the differences in the distributions of the species in this genus might be associated with the polymorphism in flight traits. Shibuya et al. (2017) points out that the hind wings might degenerate in *Synuchus* species in old forests in the less disturbed mountainous areas of the Japanese archipelago. Forests are permanent and spatially extensive habitats that have resulted in the degeneration in the ability to fly in many beetles (Southwood, 1962; Roff, 1990). Some previous studies carried out in the Eastern Alps also reveal that hind wing degeneration in carabid beetles is associated with ecological succession and water balance in the soil and suggest that mountainous forests are one of the environments in which hind wing degeneration is most likely to occur (Brandmayr, 1983, 1991). Therefore, such characteristics of mountainous forests might be associated with the various stages in the degeneration in the ability to fly in the genus *Synuchus* recorded in this study.

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Supplementary Tables S1–S4 and Fig. S1 follow.

**Table S1.** Sampling sites with latitudes, longitudes, and altitudes.

| Site no. | Sampling site | Lat (°N) | Long (°E) | Alt (m) |
|---|---|---|---|---|
| 1 | Atsuta, Ishikari, Hokkaido | 43.41 | 141.45 | 80 |
| 2 | Ao'okuyama, Tobetsu, Hokkaido | 43.37 | 141.59 | 120 |
| 3 | Maruseppu, Engaru, Hokkaido | 43.95 | 143.32 | 360 |
| 4 | Rokugo, Furano, Hokkaido | 43.30 | 142.52 | 280 |
| 5 | Mosetsuri, Tsurui, Hokkaido | 43.37 | 144.22 | 140 |
| 6 | Rusutsu, Rusutsu, Hokkaido | 42.76 | 140.89 | 650 |
| 7 | Jozankei, Sapporo, Hokkaido | 42.86 | 141.10 | 820 |
| 8 | Shidunai, Shinhidaka, Hokkaido | 42.46 | 142.49 | 420 |
| 9 | Takae, Niikappu, Hokkaido | 42.37 | 142.30 | 60 |
| 10 | Bettoga, Nemuro, Hokkaido | 43.24 | 145.41 | 30 |
| 11 | Taiseikumiyako, Setana, Hokkaido | 42.23 | 139.82 | 100 |
| 12 | Tomioka, Otobe, Hokkaido | 42.01 | 140.21 | 175 |
| 13 | Nishidate, Matsumae, Hokkaido | 41.44 | 140.10 | 60 |
| 14 | Sengen, Fukushima, Hokkaido | 41.55 | 140.26 | 155 |
| 15 | Tatekawa, Kikonai, Hokkaido | 41.66 | 140.42 | 30 |
| 16 | Funami, Hakodate, Hokkaido | 41.76 | 140.70 | 80 |
| 17 | Kamedanakano, Hakodate, Hokkaido | 41.87 | 140.79 | 220 |
| 18 | Yoshide, Yuza, Yamagata | 39.05 | 139.99 | 470 |
| 19 | Yunokamiyama, Yuzawa, Akita | 39.17 | 140.51 | 190 |
| 20 | Minase, Yuzawa, Akita | 38.99 | 140.68 | 380 |
| 21 | Takamatsu, Yuzawa, Akita | 38.99 | 140.58 | 1000 |
| 22 | Tsubakikawa, Higashinaruse, Akita | 38.98 | 140.76 | 1030 |
| 23 | Kogainumajiriyamako, Inawashiro, Fukushima | 37.62 | 140.22 | 790 |
| 24 | Kogainumajiriyamako, Inawashiro, Fukushima | 37.63 | 140.25 | 1100 |
| 25 | Tonaka, Tanagura, Fukushima | 36.93 | 140.27 | 960 |
| 26 | Kagesawa, Chikusei, Ibaraki | 36.26 | 139.96 | 40 |
| 27 | Shorenji, Kashiwa, Chiba | 35.90 | 139.95 | 20 |
| 28 | Sakurai, Futtsu, Chiba | 35.25 | 139.93 | 260 |
| 29 | Sasa, Kimitsu, Chiba | 35.20 | 140.09 | 180 |
| 30 | Okuzure, Kyonan, Chiba | 35.20 | 139.92 | 80 |
| 31 | Godo, Minamiboso, Chiba | 35.10 | 139.87 | 80 |
| 32 | Minamiasai, Minamiboso, Chiba | 34.95 | 139.91 | 140 |
| 33 | Kamikuriyama, Nikko, Tochigi | 36.86 | 139.61 | 910 |
| 34 | Kamikuriyama, Nikko, Tochigi | 36.85 | 139.62 | 1040 |
| 35 | Uwadaira, Shioya, Tochigi | 36.72 | 139.87 | 200 |
| 36 | Fuji, Sano, Tochigi | 36.35 | 139.60 | 80 |
| 37 | Tokura, Katashina, Gunma | 36.86 | 139.24 | 1160 |
| 38 | Tokura, Katashina, Gunma | 36.83 | 139.24 | 990 |
| 39 | Fujiwara, Minakami, Gunma | 36.85 | 139.12 | 1000 |
| 40 | Awasawa, Minakami, Gunma | 36.81 | 139.01 | 600 |
| 41 | Hoshimata, Tsumagoi, Gunma | 36.57 | 138.49 | 1230 |
| 42 | Otaki, Chichibu, Saitama | 35.94 | 138.82 | 910 |
| 43 | Otaki, Chichibu, Saitama | 35.94 | 138.80 | 1210 |
| 44 | Matsugaoka, Tokorozawa, Saitama | 35.77 | 139.45 | 80 |
| 45 | Takao, Hachioji, Tokyo | 35.63 | 139.25 | 500 |
| 46 | Uratakao, Hachioji, Tokyo | 35.64 | 139.26 | 210 |
| 47 | Mikuni, Yuzawa, Niigata | 36.76 | 138.78 | 1180 |
| 48 | Suginosawa, Myoko, Niigata | 36.86 | 138.13 | 1230 |
| 49 | Toyosato, Nozawaonsen, Nagano | 36.91 | 138.48 | 1350 |
| 50 | Hotaka-ariake, Azumino, Nagano | 36.39 | 137.75 | 1400 |
| 51 | Hotaka-maki, Azumino, Nagano | 36.37 | 137.79 | 900 |
| 52 | Shinhari, Tomi, Nagano | 36.40 | 138.41 | 1280 |
| 53 | Misayama, Matsumoto, Nagano | 36.25 | 138.04 | 1340 |
| 54 | Misayama, Matsumoto, Nagano | 36.25 | 138.07 | 1720 |
| 55 | Iribeyama, Matsumoto, Nagano | 36.20 | 138.10 | 1480 |
| 56 | Wada, Nagawa, Nagano | 36.19 | 138.13 | 1670 |
| 57 | Harayama, Hara, Nagano | 35.97 | 138.26 | 1240 |
| 58 | Azusayama, Kawakami, Nagano | 35.97 | 138.31 | 1520 |
| 59 | Takane, Hokuto, Yamanashi | 35.95 | 138.41 | 1680 |
| 60 | Shiraidaira, Doshi, Yamanashi | 35.45 | 138.94 | 1070 |
| 61 | Hirano, Yamanakako, Yamanashi | 35.39 | 138.89 | 1360 |
| 62 | Obuchi, Fuji, Shizuoka | 35.30 | 138.75 | 1460 |
| 63 | Awakura, Fujinomiya, Shizuoka | 35.30 | 138.69 | 1080 |
| 64 | Ike, Ito, Shizuoka | 34.90 | 139.07 | 280 |
| 65 | Sugabiki, Izu, Shizuoka | 34.87 | 139.02 | 1050 |
| 66 | Ogamo, Shimoda, Shizuoka | 34.68 | 138.92 | 200 |
| 67 | Kamo, Minami'izu, Shizuoka | 34.64 | 138.85 | 100 |
| 68 | Kano, Minami'izu, Shizuoka | 34.63 | 138.85 | 150 |
| 69 | Kuchisakamoto, Shizuoka, Shizuoka | 35.19 | 138.25 | 1180 |
| 70 | Yokosawa, Shizuoka, Shizuoka | 35.18 | 138.25 | 1300 |
| 71 | Ikawa, Shizuoka, Shizuoka | 35.21 | 138.28 | 1400 |
| 72 | Obishike, Kanazawa, Ishikawa | 36.52 | 136.80 | 900 |
| 73 | Oritani, Kanazawa, Ishikawa | 36.52 | 136.76 | 340 |
| 74 | Okuwa, Kanazawa, Ishikawa | 36.53 | 136.68 | 70 |
| 75 | Nishisara, Hakusan, Ishikawa | 36.33 | 136.64 | 230 |
| 76 | Shiramine, Hakusan, Ishikawa | 36.12 | 136.67 | 820 |
| 77 | Shiramine, Hakusan, Ishikawa | 36.12 | 136.73 | 1200 |
| 78 | Shoin, Suzu, Ishikawa | 37.44 | 137.29 | 20 |
| 79 | Takojima, Suzu, Ishikawa | 37.45 | 137.32 | 0 |
| 80 | Nagasawashinden, Suzuka, Mie | 34.94 | 136.47 | 90 |
| 81 | Takano'o, Tsu, Mie | 34.80 | 136.47 | 80 |
| 82 | Shimojo, Kameyama, Mie | 34.81 | 136.49 | 70 |
| 83 | Aso, Taiki, Mie | 34.35 | 136.40 | 100 |
| 84 | Kamiichiki, Mihama, Mie | 33.84 | 136.01 | 50 |
| 85 | Okishima, Omihachiman, Shiga | 35.19 | 136.08 | 120 |
| 86 | Yoshidakaguraoka, Kyoto, Kyoto | 35.03 | 135.79 | 100 |
| 87 | Byakugoji, Nara, Nara | 34.67 | 135.85 | 190 |
| 88 | Kitamata, Nosegawa, Nara | 34.16 | 135.67 | 1160 |
| 89 | Kamiyukawa, Aridagawa, Wakayama | 34.07 | 135.55 | 1130 |
| 90 | Ryujin, Tanabe, Wakayama | 34.05 | 135.56 | 1280 |
| 91 | Nishihara, Kamikitayama, Nara | 34.22 | 135.99 | 1060 |
| 92 | Nishihara, Kamikitayama, Nara | 34.22 | 136.03 | 1260 |
| 93 | Daisen, Daisen, Tottori | 35.35 | 133.55 | 980 |
| 94 | Daisen, Daisen, Tottori | 35.41 | 133.55 | 660 |
| 95 | Mita, Nishinoshima, Shimane | 36.07 | 133.03 | 320 |
| 96 | Fuse, Okinoshima, Shimane | 36.26 | 133.33 | 420 |
| 97 | Ichiutsuzuro, Tsurugi, Tokushima | 33.87 | 134.07 | 1460 |
| 98 | Kuzuhara, Ino, Kochi | 33.76 | 133.33 | 610 |
| 99 | Nishinokawatei, Saijo, Ehime | 33.79 | 133.19 | 1740 |
| 100 | Terakawa, Ino, Kochi | 33.75 | 133.17 | 1370 |
| 101 | Itaya, Fukuoka, Fukuoka | 33.44 | 130.38 | 700 |
| 102 | Hoshuyama, Toho, Fukuoka | 33.45 | 130.87 | 600 |
| 103 | Hikosan, Soeda, Fukuoka | 33.49 | 130.93 | 800 |
| 104 | Oriyama, Isahaya, Nagasaki | 32.96 | 130.08 | 1000 |
| 105 | Mine, Tsushima, Nagasaki | 34.47 | 129.28 | 30 |
| 106 | Yufuin, Yufu, Oita | 33.30 | 131.40 | 640 |
| 107 | Tano, Kokonoe, Oita | 33.09 | 131.21 | 1320 |
| 108 | Izumi, Yatsushiro, Kumamoto | 32.48 | 131.00 | 1240 |
| 109 | Makizono, Kirishima, Kagoshima | 31.91 | 130.84 | 1060 |
| 110 | Yuwan, Uken, Kagoshima | 28.29 | 129.32 | 480 |

**Table S2.** Numbers of examined samples and individuals of *Synuchus* species with each hind wing type.

| Species | Site number | Number of examined samples | | | |
|---|---|---|---|---|---|
| | | Macroptery | Brachyptery | Aptery | Total |
| *S. melantho* | 2 | 1[0/1/0] | – | 3 | 4 |
| | 3 | – | – | 1 | 1 |
| | 5 | – | – | 1 | 1 |
| | 6 | – | – | 5 | 5 |
| | 8 | – | – | 1 | 1 |
| | 10 | – | – | 23 | 23 |
| | 18 | – | – | 1 | 1 |
| | 19 | – | – | 1[0/0/1] | 1 |
| | 20 | – | – | 1[0/0/1] | 1 |
| | 24 | – | – | 2 | 2 |
| | 25 | 6[1/3/0] | – | 28[0/0/2] | 34 |
| | 33 | 2 | – | 1 | 3 |
| | 34 | – | – | 3 | 3 |
| | 41 | – | – | 1 | 1 |
| | 49 | – | – | 2 | 2 |
| | 48 | – | – | 1 | 1 |
| | 50 | – | – | 3 | 3 |
| | 51 | – | – | 4 | 4 |
| | 52 | – | – | 4[0/0/1] | 4 |
| | 55 | – | – | 7 | 7 |
| | 56 | – | – | 5 | 5 |
| | 57 | – | – | 6 | 6 |
| | 58 | 1 | – | 1 | 2 |
| | 59 | – | – | 5 | 5 |
| | 61 | – | – | 1 | 1 |
| | 62 | – | – | 8 | 8 |
| | 63 | – | – | 4 | 4 |
| | 72 | – | – | 4 | 4 |
| | 77 | – | – | 4 | 4 |
| | 90 | – | – | 1 | 1 |
| | 93 | 1[1/0/0] | – | 1 | 2 |
| | 96 | – | – | 8 | 8 |
| *S. callitheres* | 35 | 9[2/2/2] | – | – | 9 |
| | 74 | 11[0/5/3] | – | – | 11 |
| *S. dulcigradus* | 29 | 1 | – | – | 1 |
| | 32 | 1[1/0/0] | – | – | 1 |
| | 36 | 4[0/1/1] | – | – | 4 |
| | 44 | 1 | – | – | 1 |
| | 64 | 1[0/1/1] | – | – | 1 |
| | 66 | 1 | – | – | 1 |
| | 67 | 2 | – | – | 2 |
| | 68 | 2 | – | – | 2 |
| | 74 | 9[1/2/2] | – | – | 9 |
| | 83 | 6 | – | – | 6 |
| | 84 | 2 | – | – | 2 |
| | 86 | 4 | – | – | 4 |
| | 87 | 7[3/0/0] | – | – | 7 |
| | 102 | 1 | – | – | 1 |
| | 103 | 1[0/1/1] | – | – | 1 |
| | 104 | 2 | – | – | 2 |
| | 106 | 1 | – | – | 1 |
| *S. nitidus* | 1 | 3 | – | – | 3 |
| | 2 | 4[1/0/0] | – | – | 4 |
| | 3 | 6 | – | – | 6 |
| | 4 | 3 | – | – | 3 |
| | 8 | 5[1/3/1] | – | – | 5 |
| | 9 | 2[0/2/0] | – | – | 2 |
| | 19 | 5[0/5/1] | – | – | 5 |
| | 23 | 1 | – | – | 1 |
| | 26 | 26[0/5/1] | – | – | 26 |
| | 27 | 5 | – | – | 5 |
| | 28 | 4[1/0/0] | – | – | 4 |
| | 29 | 2 | – | – | 2 |
| | 30 | 6 | – | – | 6 |
| | 32 | 2[1/0/0] | – | – | 2 |
| *S. nitidus* (contd) | 34 | 2 | – | – | 2 |
| | 36 | 2 | – | – | 2 |
| | 40 | 4 | – | – | 4 |
| | 41 | 2 | – | – | 2 |
| | 42 | 2 | – | – | 2 |
| | 43 | 2 | – | – | 2 |
| | 44 | 2 | – | – | 2 |
| | 45 | 2 | – | – | 2 |
| | 46 | 2 | – | – | 2 |
| | 50 | 2 | – | – | 2 |
| | 51 | 1 | – | – | 1 |
| | 55 | 1 | – | – | 1 |
| | 68 | 2[2/0/0] | – | – | 2 |
| | 70 | 1 | – | – | 1 |
| | 71 | 2 | – | – | 2 |
| | 78 | 2 | – | – | 2 |
| | 79 | 2 | – | – | 2 |
| | 86 | 8[2/5/1] | – | – | 8 |
| | 87 | 4 | – | – | 4 |
| | 90 | 3 | – | – | 3 |
| | 93 | 5 | – | – | 5 |
| | 94 | 2 | – | – | 2 |
| | 95 | 6 | – | – | 6 |
| | 96 | 9[2/5/1] | – | – | 9 |
| | 102 | 4 | – | – | 4 |
| | 103 | 2 | – | – | 2 |
| | 107 | 1 | – | – | 1 |
| | 109 | 2 | – | – | 2 |
| *S. cycloderus* | 3 | 2 | – | – | 2 |
| | 19 | 3[0/3/1] | – | – | 3 |
| | 20 | 4[0/2/0] | – | – | 4 |
| | 21 | 1[1/0/0] | – | – | 1 |
| | 22 | 3[1/0/0] | – | – | 3 |
| | 23 | 2 | – | – | 2 |
| | 24 | 3 | – | – | 3 |
| | 26 | 7[0/5/1] | – | – | 7 |
| | 28 | 2 | – | – | 2 |
| | 30 | 2[2/0/0] | – | – | 2 |
| | 31 | 1 | – | – | 1 |
| | 32 | 4 | – | – | 4 |
| | 34 | 2 | – | – | 2 |
| | 38 | 4 | – | – | 4 |
| | 39 | 4 | – | – | 4 |
| | 40 | 1 | – | – | 1 |
| | 41 | 4 | – | – | 4 |
| | 42 | 2 | – | – | 2 |
| | 43 | 2 | – | – | 2 |
| | 49 | 1 | – | – | 1 |
| | 51 | 2 | – | – | 2 |
| | 52 | 2 | – | – | 2 |
| | 55 | 5 | – | – | 5 |
| | 57 | 1 | – | – | 1 |
| | 64 | 1[1/0/0] | – | – | 1 |
| | 65 | 2 | – | – | 2 |
| | 66 | 1[1/0/0] | – | – | 1 |
| | 68 | 2 | – | – | 2 |
| | 69 | 3 | – | – | 3 |
| | 70 | 2 | – | – | 2 |
| | 71 | 3 | – | – | 3 |
| | 73 | 9[2/5/1] | – | – | 9 |
| | 76 | 1 | – | – | 1 |
| | 79 | 2 | – | – | 2 |
| | 80 | 6 | – | – | 6 |
| | 81 | 4 | – | – | 4 |
| | 86 | 9[2/5/1] | – | – | 9 |
| | 87 | 10 | – | – | 10 |
| | 88 | 2 | – | – | 2 |
| | 89 | 1 | – | – | 1 |
| | 90 | 2 | – | – | 2 |
| | 93 | 6[0/5/1] | – | – | 6 |

| | | | | | |
|---|---|---|---|---|---|
| *S. cycloderus* (contd) | 94 | 6 | – | – | 6 |
| | 96 | 7 | – | – | 7 |
| | 101 | 1 | – | – | 1 |
| | 102 | 4 | – | – | 4 |
| | 103 | 3 | – | – | 3 |
| | 109 | 2 | – | – | 2 |
| *S. arcuaticollis* | 3 | 1 | – | – | 1 |
| | 11 | – | 1 | – | 1 |
| | 12 | 1 | – | – | 1 |
| | 13 | 3[1/0/1] | – | – | 3 |
| | 14 | 1[1/0/0] | – | – | 1 |
| | 15 | 3[1/0/1] | – | – | 3 |
| | 16 | 2 | – | – | 2 |
| | 17 | 2 | – | – | 2 |
| | 18 | 3 | – | – | 3 |
| | 19 | 3[0/1/1] | – | – | 3 |
| | 20 | 2[0/1/1] | – | 1 | 3 |
| | 36 | 3 | 1 | – | 4 |
| | 37 | 3 | 1 | – | 4 |
| | 38 | 1 | – | – | 1 |
| | 39 | 2 | – | – | 2 |
| | 41 | 1 | – | – | 1 |
| | 46 | 1 | – | – | 1 |
| | 47 | 2 | – | – | 2 |
| | 53 | 5[0/1/0] | – | – | 5 |
| | 56 | 1 | – | – | 1 |
| | 57 | 8 | 2 | – | 10 |
| | 58 | 5[1/0/0] | – | – | 5 |
| | 64 | 1 | – | – | 1 |
| | 74 | 1 | – | – | 1 |
| | 75 | 3 | – | – | 3 |
| | 76 | 1 | – | – | 1 |
| | 80 | 2 | – | – | 2 |
| | 81 | 1 | – | – | 1 |
| | 82 | 4 | – | – | 4 |
| | 83 | 3 | – | – | 3 |
| | 85 | 3[1/0/0] | – | – | 3 |
| | 86 | 1 | – | – | 1 |
| | 95 | 3 | 2 | – | 5 |
| | 96 | 2[0/1/1] | 1 | – | 3 |
| | 98 | 1[0/1/0] | – | – | 1 |
| | 105 | 3 | – | – | 3 |
| *Synuchus* sp. 1 | 2 | 1[1/0/0] | – | 3 | 4 |
| | 3 | – | – | 1 | 1 |
| | 6 | – | – | 1 | 1 |
| | 7 | – | – | 8[0/0/1] | 8 |
| | 8 | – | – | 2[0/0/1] | 2 |
| | 18 | – | – | 1 | 1 |
| | 24 | – | – | 1 | 1 |
| | 25 | – | – | 1 | 1 |
| | 37 | – | – | 3 | 3 |
| | 41 | – | – | 2[0/0/1] | 2 |
| | 49 | – | – | 1 | 1 |
| | 52 | – | – | 1 | 1 |
| | 55 | – | – | 1 | 1 |
| | 61 | 1 | – | – | 1 |
| | 71 | 1[0/1/0] | 1 | 4 | 6 |
| | 97 | 1[0/1/0] | – | 9[0/0/2] | 10 |
| *S. crocatus* | 4 | – | – | 1 | 1 |
| | 12 | – | – | 1 | 1 |
| | 16 | 1[0/1/0] | – | 1[0/0/1] | 2 |
| | 17 | – | – | 1[0/0/1] | 1 |
| | 24 | – | – | 1[0/0/1] | 1 |
| | 27 | 1 | – | – | 1 |
| | 33 | – | – | 4[0/0/1] | 4 |
| | 34 | 1[0/1/0] | – | 2 | 3 |
| | 37 | – | – | 3 | 3 |
| | 38 | – | – | 5 | 5 |
| | 39 | – | – | 1 | 1 |
| | 40 | – | – | 5 | 5 |
| *S. crocatus* (contd) | 42 | – | – | 1 | 1 |
| | 47 | 1 | – | – | 1 |
| | 52 | – | – | 2[0/0/1] | 2 |
| | 53 | – | – | 1 | 1 |
| | 93 | – | – | 1 | 1 |
| *S. shibatai* | 110 | – | 10[1/5/5] | – | 10 |
| *S. amamioshimae* | 110 | – | – | 2[0/0/2] | 2 |
| *S. angusticeps* | 92 | – | – | 1[0/0/1] | 1 |
| *S. atricolor* | 45 | – | – | 7[0/0/5] | 7 |
| | 87 | – | – | 2 | 2 |
| *S. fukuharai* | 62 | – | – | 2[0/0/2] | 2 |
| *S. hikosanus* | 108 | – | – | 2[0/0/1] | 2 |
| *S. montanus* | 99 | – | – | 1[0/0/1] | 1 |
| *S. picicolor* | 88 | – | – | 4 | 4 |
| | 89 | – | – | 3 | 3 |
| | 90 | – | – | 2[0/0/1] | 2 |
| | 91 | – | – | 4[0/0/2] | 4 |
| | 92 | – | – | 5[0/0/2] | 5 |
| *S. tanzawanus* | 62 | – | – | 2[0/0/2] | 2 |
| | 65 | – | – | 1 | 1 |
| | 71 | – | – | 1[0/0/1] | 1 |
| *S. ventricosus* | 88 | – | – | 1[0/0/1] | 1 |
| | 91 | – | – | 1 | 1 |
| *S. yamashitai* | 90 | – | – | 1[0/0/1] | 1 |
| *S. yasumatsui* | 97 | – | – | 1[0/0/1] | 1 |
| | 100 | – | – | 3[0/0/1] | 3 |
| | 103 | – | – | 1 | 1 |
| *Synuchus* sp. 2 | 21 | – | – | 1 | 1 |
| | 24 | – | – | 4 | 4 |
| | 43 | – | – | 1 | 1 |
| | 47 | – | – | 1[0/0/1] | 1 |
| | 52 | – | – | 1[0/0/1] | 1 |
| | 54 | – | – | 1 | 1 |
| | 55 | – | – | 5 | 5 |
| | 60 | – | – | 1[0/0/1] | 1 |
| | 71 | – | – | 1[0/0/1] | 1 |
| | 88 | – | – | 7 | 7 |
| | 92 | – | – | 8[0/0/1] | 8 |
| Total | | 473 | 19 | 289 | 781 |

Site numbers correspond to each of the 110 localities in Table S1. Numbers in [ ] indicate the numbers of individuals whose relative macropterous or brachypterous hind wing length (RMHWL or RBHWL) and metepisternum ratio (MR) were measured per each site and species. The left and middle numbers are for relative macropterous or brachypterous hind wing lengths. The left are numbers of the measured male individuals, and the middle are those of female individuals. The right numbers are the numbers of female individuals whose metepisternum ratios were measured.

**Table S3.** Metepisternum ratio (MR) of 21 *Synuchus* species.

| Species | Metepisternum ratio ± SD [*N*] |
|---|---|
| *Synuchus melantho* | 0.62 ± 0.01 [5] |
| *S. callitheres* | 0.62 ± 0.03 [5] |
| *S. dulcigradus* | 0.63 ± 0.02 [5] |
| *S. nitidus* | 0.61 ± 0.02 [5] |
| *S. cycloderus* | 0.61 ± 0.02 [5] |
| *S. arcuaticollis* | 0.68 ± 0.03 [5] |
| *Synuchus* sp. 1 | 0.68 ± 0.02 [5] |
| *S. crocatus* | 0.66 ± 0.04 [5] |
| *S. shibatai* | 0.67 ± 0.05 [5] |
| *S. amamioshimae* | 0.69 ± 0.01 [2] |
| *S. angusticeps* | 0.80 [1] |
| *S. atricolor* | 0.66 ± 0.03 [5] |
| *S. fukuharai* | 0.75 ± 0.06 [2] |
| *S. hikosanus* | 0.74 [1] |
| *S. montanus* | 0.84 [1] |
| *S. picicolor* | 0.73 ± 0.04 [5] |
| *S. tanzawanus* | 0.67 ± 0.02 [3] |
| *S. ventricosus* | 0.66 ± 0.02 [5] |
| *S. yamashitai* | 0.65 [1] |
| *S. yasumatsui* | 0.75 ± 0.02 [5] |
| *Synuchus* sp. 2 | 0.70 ± 0.03 [5] |

Sample sizes are shown in [ ]. This index was calculated using only female individuals for each species.

**Table S4.** Generalized linear mixed model explaining for metepisternum ratio with state of the hind wings as an independent variable.

| Factor | Estimate | SE | *p*-value |
|---|---|---|---|
| State of the hind wings | –0.08 | 0.04 | 0.086 |

*p*-value is based on likelihood ratio test.

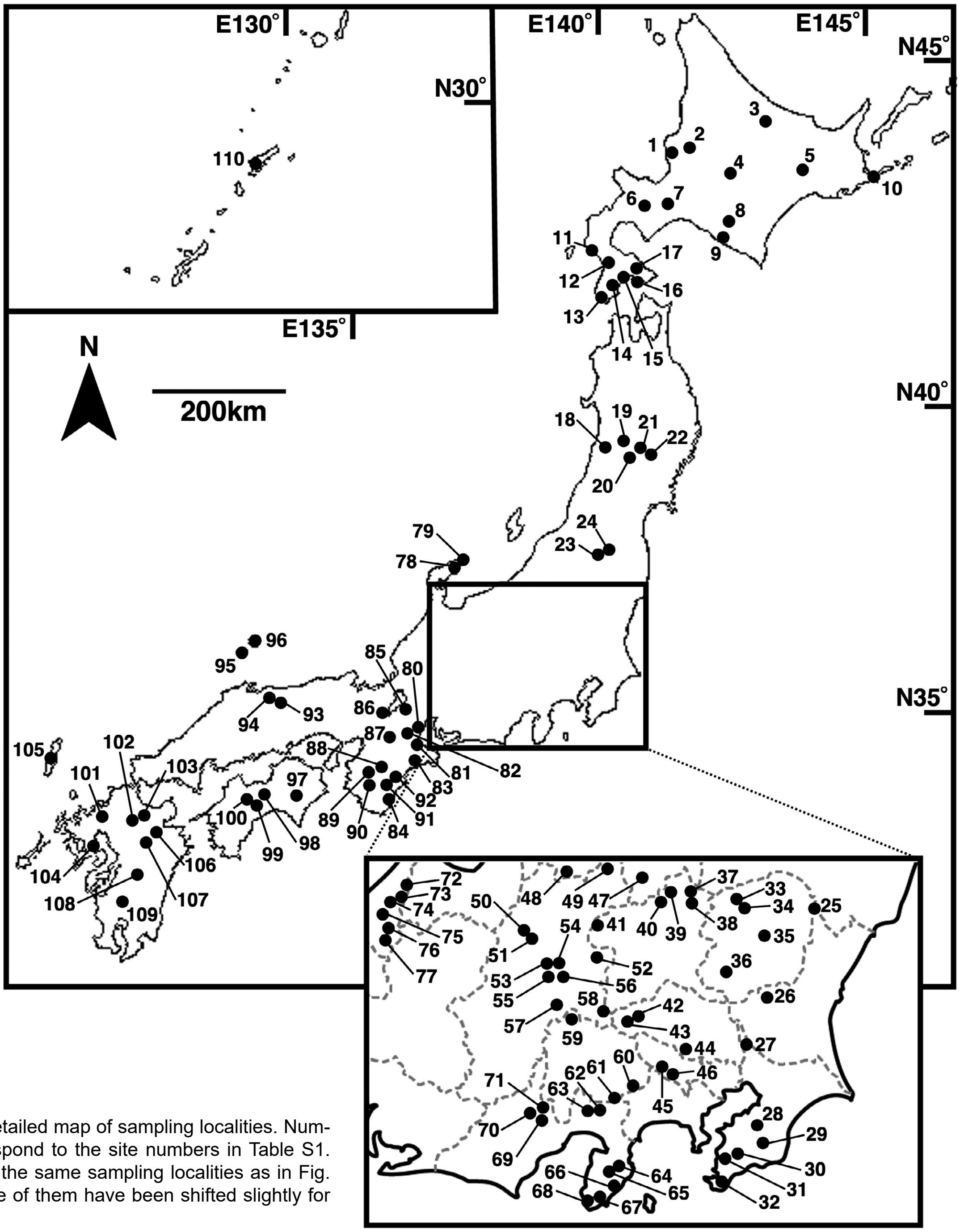


**Fig. S1.** Detailed map of sampling localities. Numbers correspond to the site numbers in Table S1. Dots show the same sampling localities as in Fig. 1, but some of them have been shifted slightly for clarity.