Natural occurrence of oviposition and adult emergence of the seed parasitoid wasp *Macrodasyceras hirsutum* Kamijo (Hymenoptera, Torymidae) on *Ilex latifolia* Thunberg in Japan

Etsuro TAKAGI1, Kazunori MATSUO2, Masanori SUZUKI3, Yasumasa ADACHI3, Katsumi TOGASHI4,5

1. Department of Tourism Science, Tokyo Metropolitan University, Minami-Osawa, Hachioji, Tokyo 192-0397, Japan.
2. Biosystemsatics Laboratory, Faculty of Social and Cultural Studies, Kyushu University, Fukuoka 819-0395, Japan.
3. The University of Tokyo Chiba Forest, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Kamogawa City, Chiba 299-5503, Japan.
4. Laboratory of Forest Zoology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.
5. Present address: 20-3-4, Komagome, Toshima-ku, Tokyo 170-0003, Japan.

*Corresponding author’s email: E-mail: e_r@tmu.ac.jp; Tel: +81-42-677-1111*

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**ABSTRACT:** *Macrodasyceras hirsutum* Kamijo (Hymenoptera: Torymidae) has been considered to be a specialized seed parasitoid wasp of *Ilex integra* Thunb. since the description as a new species. A previous study showed that they deposited eggs in seeds of the closely related tree *I. latifolia* when *I. integra* was experimentally absent. However, natural occurrence of oviposition into *I. latifolia* or successful emergence from *I. latifolia* as adults had not been investigated. Thus, to determine whether *M. hirsutum* utilizes *I. latifolia* seeds in the fields, we dissected 536 *I. integra* seeds and 501 *I. latifolia* seeds. *Macrodasyceras hirsutum* larvae were found in 152/307 fertilized seeds of *I. integra*, while found in 16/285 fertilized seeds of *I. latifolia*. Additionally, 12 male and 13 female *M. hirsutum* adults emerged from *I. latifolia* berries collected from field. The present study confirmed natural occurrence of oviposition by *M. hirsutum* females in *I. latifolia* seeds and the emergence of adults from berries.

**KEY WORDS:** *Ilex integra*, *Ilex latifolia*, *Macrodasyceras hirsutum*, seed parasitoid wasp, Torymidae.

**INTRODUCTION**

*Macrodasyceras hirsutum* Kamijo (Hymenoptera: Torymidae) has been considered to be a specialized seed parasitoid wasp of an ornamental tree *Ilex integra* Thunb. (*Aquifoliaceae*) since the description as a new species by Kamijo (1981). Small red berries of *I. integra* surrounded with dark green leaves are attractive to people in winter. However this wasp may reduce the ornamental value because the larvae within seeds keep the berries green (Takagi et al., 2012).

*Macrodasyceras hirsutum* is partially bivoltine; the adults of the overwintered generation emerge between May and June and some insects of the first generation emerge as adults in August (Takagi et al., 2010). Female adults of the overwintered generation selectively lay one to five eggs in each fertilized seed from late May to mid-June (Takagi et al., 2010; Takagi and Togashi, 2013a). The larvae only eat the seeds and never damage the berry flesh (Kamijo, 1981; Takagi et al., 2010, 2012). Only one larva develops in each seed (Takagi et al., 2012). The larvae overwinter in seeds within berries attached to twigs (Takagi et al., 2010, 2012).

Of 25 *Ilex* species distributed in Japan (Yao et al., 2020), *Ilex latifolia* Thunb. (*Aquifoliaceae*) is also planted in gardens and is known as a symbol tree of the post office in Japan. Jian et al. (2017), Park et al. (2019) and Yao et al. (2020) have revealed that *I. latifolia*, *I. integra* and 11 other *Ilex* species constitute a clade termed *Aquifoliun* section in the molecular phylogenetic tree. *Ilex latifolia* and *I. integra* are distributed widely and sympatrically in Japan, although the northern limit is different between them, i.e., Miyagi Prefecture for *I. integra* and Shizuoka Prefecture for *I. latifolia* (Miyawaki et al., 1983; Katsuta et al., 1998). Unlike the two *Ilex* species, the remaining 11 species show restricted ranges; *I. matanoana* Makino, *I. mertensi Maxim., I. percioracea* Tuyama and *I. beecheyi* (Loes.) Makino are endemic to the Bonin Islands and *I. maximowicziana* Loes., *I. dimorphophylla* Koidz., *I. warburgii* Loes and *I. liukiensis* Loes are endemic to the Ryukyu islands (Yao et al., 2020). *Ilex rugosa* F. Schmidt is distributed in high-altitude areas, *I. leucocladula* (Maxim.) Makino in the heavy-snow region, and *I. buergeri* Miq. in the warm areas of western Japan (Satake et al., 1993).

*Ilex latifolia* and *I. integra* are extremely similar in phenology, morphology and reproduction biology. For example, they are dioecious, bird-dispersed, broad-leaved evergreen tree species (Miyawaki et al., 1983). The flower buds are completed by autumn in the leaf axils of the current-year twigs. The flowers open from late March to mid-April in the following year (Katsuta et al., 1998). The pistillate flowers of the two species are characterized by four dysfunctional, small stamens and one large superior ovary with three to five cavities, each
of which contains one ovule enclosed within the endocarp (Katsuta et al., 1998; Takagi et al., 2010). Immediately after flowering, each ovary of pistillate flower starts to develop into a spherical berry irrespective of pollination (Takagi et al., 2010). Both the berries and seeds are smaller in I. latifolia compared to I. integra (Katsuta et al., 1998). Individual I. latifolia and I. integra trees exhibit marked, yearly fluctuations in berry production (Katsuta et al., 1998; Takagi and Togashi, 2012). In addition, female I. integra trees can change their sex and the change of sex is synchronous in individual trees (Takagi and Togashi, 2012), whereas the sex change is unknown in I. latifolia.

Phenological and morphological similarities in berry development and phylogenetic relatedness between I. integra and I. latifolia imply the attack of I. latiflora berries by M. hirsutum. Actually, enclosure experiments with I. latifolia berries alone showed the deposition of the eggs into the seeds and the development into fully grown larvae in the seeds (Takagi and Togashi, 2013b). Thus, to determine whether M. hirsutum utilizes I. latifolia seeds in the fields, this study investigated: (1) natural occurrence of oviposition into I. latifolia seeds by M. hirsutum and (2) the successful emergence of M. hirsutum as adults from I. latifolia berries.

### MATERIALS AND METHODS

Three areas of central Japan were selected to investigate the oviposition and development of M. hirsutum on the two Ilex species. The first was in urban areas of Nishi-Tokyo City, Chofu City and Bunkyo-ku, Tokyo Prefecture, which included the University of Tokyo Tanashi Forest in Nishi-Tokyo City and the campus of the University of Tokyo at Hongo, Bunkyo-ku. The second was in a hilly, rural area covered with forests in Kimitsu and Kamogawa Cities, Chiba Prefecture, which included the University of Tokyo Chiba Forest, where coniferous plantations, natural mixed forests of broad-leaved evergreen and coniferous trees and secondary broad-leaved forests covered the hills. The third was in an urban area of Osaka City, Osaka Prefecture, which included the Nagai Botanical Garden.

To determine the natural occurrence of oviposition by M. hirsutum into I. latifolia, 25 berries were randomly collected from each of four trees in the Tokyo and Chiba study areas in mid-July 2015 (Table 1). In the Osaka study area, 25 berries were randomly collected from a tree in mid-July 2016. As for I. integra, to confirm the oviposition by M. hirsutum in the areas, 15 berries were randomly collected from each of two trees in Nishi-Tokyo City and 30 berries from a tree in Hongo campus of the University of Tokyo in mid-July 2015 (Table 1). In the Chiba study area, 15 berries were randomly collected from each of four trees in mid-July 2015. In the Osaka study area, 15 I. integra berries were randomly collected from a tree in mid-July 2016. The berries on twigs were kept in the dark at 5°C before examination. The berries were dissected under a microscope to record the number of fertilized seeds in each berry and the presence or absence of wasp larvae in each seed.

To compare the proportion of infested seeds with M. hirsutum between I. integra and I. latifolia fertilized seeds, we conducted Fisher’s exact test separately for each study site. The 5 % significance level was adjusted with a Bonferroni correction (α = 0.017). We also used a generalized linear mixed model (GLMM) with binomial distribution linked with logit to compare the proportion of fertilized seeds that were infested with parasitoid wasps between I. integra and I. latifolia. The response variable in the model was the number of wasp-infested seeds out of the fertilized seeds in each berry.
Explanatory variables were tree species as fixed effect and study sites, within which trees were nested, as random effect. *Ilex integra* was used as reference. *P* value was calculated by Wald test. Computation was implemented using R 3.3.2 software (R Core Team, 2016) with package “lme4” (Bates et al., 2015).

To confirm the completion of wasp development in *I. latifolia* seeds in the field, two twigs bearing berries were collected from an *I. latifolia* tree in Hongo campus of the University of Tokyo on 13 May, 2013. The twigs were then placed in plastic bags and kept in the dark at 25°C to capture the wasps.

**RESULTS AND DISCUSSION**

The seed dissection showed that *M. hirsutum* larvae were present in 152/307 fertilized seeds within 135 *I. integra* berries and in 16/285 fertilized seeds within 125 *I. latifolia* berries when the berries were sampled during mid-July in the three study areas (Table 1) (Fig. 1). The proportion of larva-infested seeds was significantly higher in *I. integra* fertilized seeds than in *I. latifolia* ones (Fisher’s exact test, *P* < 0.001 for all sites) (Table 1). GLMM also showed that the proportion of larva-infested seeds was significantly higher in *I. integra* than in *I. latifolia* (coefficient ± SE = −3.28 ± 1.38, *z* = −2.38, *P* = 0.017).

Enclosure experiments with *I. latifolia* berries alone showed the deposition of eggs into the seeds (Takagi and Togashi, 2013b). However, natural occurrence of oviposition into *I. latifolia* seeds by *M. hirsutum* had been unclear. The present study confirmed natural occurrence of oviposition by *M. hirsutum* females in *I. latifolia* seeds. To the best of our knowledge, this is a first record of natural occurrence of oviposition by *M. hirsutum*.

Twelve male and 13 female *M. hirsutum* adults emerged from *I. latifolia* berries that were collected from a tree in Hongo campus of the University of Tokyo in 2013. Takagi and Togashi (2013b) reported their development into mature larvae in the seeds. The present study also confirmed the emergence of adults from *I. latifolia* berries, indicating that *I. hirsutum* completes the life cycle on *I. latifolia*.

*Ilex latifolia* is the closely related species to *I. integra* (Yao et al., 2020), and their phenology and morphology are similar (Miyawaki et al., 1983; Katsuta et al., 1998). It is suggested that these similarity and phylogenetic relatedness between the two *Ilex* species allow the attack of *I. latifolia* berries by *M. hirsutum* and completion of the life cycle on *I. latifolia*.

*Macrodasyceras hirsutum* larvae prevent *I. integra* berries from turning red in autumn – winter (Takagi et al., 2012). Close relatedness between the two *Ilex* species makes us predict that the response of *I. latifolia* to wasp parasitism is similar to that of *I. integra*. If the parasitism by *M. hirsutum* also prevents *I. latifolia* berries from changing their color from green to red, it would reduce the ornamental value. The relationship between the wasp parasitism and berry color in *I. latifolia* remains to be determined.

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**LITERATURE CITED**


