



Coexistence of Two Species of *Harmonia* Ladybird Beetles (Coleoptera: Coccinellidae) on the Taiwan Red Pine *Pinus taiwanensis* on Alishan in Taiwan: *Harmonia yedoensis* Takizawa and a New Record of *Harmonia quadripunctata* (Pontoppidan)

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ABSTRACT

The existence and identity of the ladybird beetle *Harmonia yedoensis* on the Taiwan red pine *Pinus taiwanensis* at the middle elevation of Alishan in Central Taiwan were confirmed, based on the male genitalia and larvae as well as the morphology of the larvae reared from gravid females newly collected in the field. Life history characteristics, sexual size dimorphism, and prey species of the Alishan *H. yedoensis* population were evaluated. On the same host plant, we observed adults of coexisting *H. quadripunctata*, which is a new record in Taiwan.

Key words: *Harmonia*, new record, larval morphology, sexual size dimorphism, Alishan, Taiwan

The ladybird beetle *Harmonia yedoensis* Takizawa (Coleoptera: Coccinellidae) is a specialized predator of aphids geographically distributed in Japan (the islands of Kyushu, Shikoku, Honshu, Yakushima and Ryukyu, but with no reports from Hokkaido), Korea, China (not confirmed) and Taiwan (Kurosawa *et al.*, 1982; Reznik *et al.*, 2015). In its natural habitat of central Japan, *H. yedoensis* feeds on the giant pine aphid *Cinara pini* (Linnaeus), and Thunberg's pine aphid *Eulachnus thunbergii* (Wilson) both found on pine trees (Osawa and

Ohashi, 2008). Although *H. yedoensis* is commonly found in urban areas during late April and May in Japan, it has been rarely collected in subtropical Taiwan, with the exception of a few sporadic records in central and southern Taiwan (Sasaji, 1982). One study collected adult *Harmonia* specimens, identified as *H. yedoensis*, on Alishan (Fenchihu and Alishan of Chiayi Co.) on 1965 April 9 and 12 (Sasaji, 1982). Confirming the species identification of these records is difficult because the adult of *H. yedoensis* is

indistinguishable from its sibling species *H. axyridis* Pallas. This is due to the fact that each of these two species has four types of multicolored elytra markings (e.g., Fig. 1a). Two morphs were identified from the specimens in Taiwan: morph *succinea* with and without color spots and morph *axyridis* (Sasaji, 1982). These two *Harmonia* species can only be distinguished by minute morphological differences in the male genitalia (Sasaji, 1981; Nakagawa and Sasaji, 1988). However, the morphological characteristics of the larvae are more distinct and thus more useful for identifying *H. yedoensis*. The third and fourth instar larvae of *H. yedoensis* possess a pair of orange dorsolateral tergal spines on the abdomen of I-VI and I-VII, respectively (Sasaji, 1977), whereas both the third and fourth instar larvae of *H. axyridis* have these orange spines only on the abdomen of I-V. The existence of *H. yedoensis* on Alishan is currently unclear and requires additional evidence and confirmation. In this study, we collected and confirmed the existence and identity of *H. yedoensis* on Alishan in Central Taiwan based on the male genitalia and larvae reared from gravid females newly collected in the field. During the process, the congeneric species *H. quadripunctata* was found.

One larva and eight adult *Harmonia* beetles (male, $n = 6$; female, $n = 2$, all being morph *succinea* without a black spot; Fig. 1a & b and Fig. 2, upper two specimens) were obtained by beating the branches of several Taiwan red pines (*Pinus taiwanensis*, Pinaceae) and by using an insect net at a rest stop located near the 132 km mark on Rd. 18 of the Tataka area on Alishan between 2015 June 9 and 12 (N 23°29.176', E 120°53.214', elevation 2816 m). From these same pine trees, the probable aphid prey of this *Harmonia* beetle was collected and preserved in 95% ethyl alcohol for later identification. The beetles were kept alive in plastic centrifuge tubes and transported to the laboratory. Upon arrival in the laboratory they were individually maintained in plastic Petri dishes (9 cm in diameter and 1.5 cm high) at 22°C with 8-day/16-night cycles and 70% relative humidity. The beetles were provided each day with a surplus of frozen *Ephestia kuehniella* Zeller eggs (Beneficial Insectary®, Redding, CA, USA). The size of the beetles was

measured using a caliper. The body size of the field-collected adults revealed substantial sexual dimorphism, with the females being significantly ($t = -2.67$, $p < 0.05$) larger than the males (female: 7.36 ± 0.24 mm, mean \pm S.E., $n = 2$; male: 6.63 ± 0.14 , $n = 6$). The two females mated frequently with the six males in the plastic Petri dishes. It took six days for the eggs to hatch. The duration of the immature stages was as follows: larvae, 13 days (first instar larvae, 3 days; second instar, 3 days; third instar, 2 days; and fourth instar, 5 days); prepupa, one day; and pupa, seven days. The third instar larvae collected on Alishan had orange dorsolateral tergal spines only on the abdomen of I to V (fifth was light orange and sixth was black; Fig. 1b). For the eight males and females reared from a gravid *Harmonia* female collected at the site, all fourth instar larval stages of these individuals ($n = 16$) exhibited orange dorsolateral tergal spines on the abdomen of I-VII (Fig. 1c), a color pattern consistent with the larval description of *H. yedoensis* by Sasaji (1977). The body size of the reared adults (all were morph *succinea* without a black spot) revealed no sexual dimorphism ($t = -0.50$, $p = 0.63$; female: 7.13 ± 0.19 mm, $n = 8$; male: 7.00 ± 0.19 , $n = 8$). After the beetles died, a male specimen from the insect collection was dissected and identified as *H. yedoensis* based on its genital morphology (Fig. 2 of Sasaji 1981).

We compared the body size of *H. yedoensis* adults (all were morph *succinea*, with black spots; female: 6.63 ± 0.28 mm, $n = 4$; male: 5.87 ± 0.23 , $n = 6$) that were field-collected from the Ryukyu Islands (Karimata, Miyako Island, Okinawa) on 2014 March 6 (N 24°88.640', E 125°27.993', elevation 17.5 m) with those collected from Alishan. The male body size in the Alishan population was significantly larger ($t = -2.66$, $p < 0.05$) than that in the Ryukyu population. The same trend was observed for females in both populations, although the difference was not significant ($t = -1.99$, $p = 0.12$). The only aphids collected from the same host plants of *H. yedoensis* in Alishan were identified as *E. thunbergii* (Wilson) (Tao, 1990). We did not collect any other aphid species on the same plants, suggesting that *E. thunbergii* is likely to be the prey of *H. yedoensis*. Our results provide the first new record of *H. yedoensis* in Alishan of Taiwan since 1965.

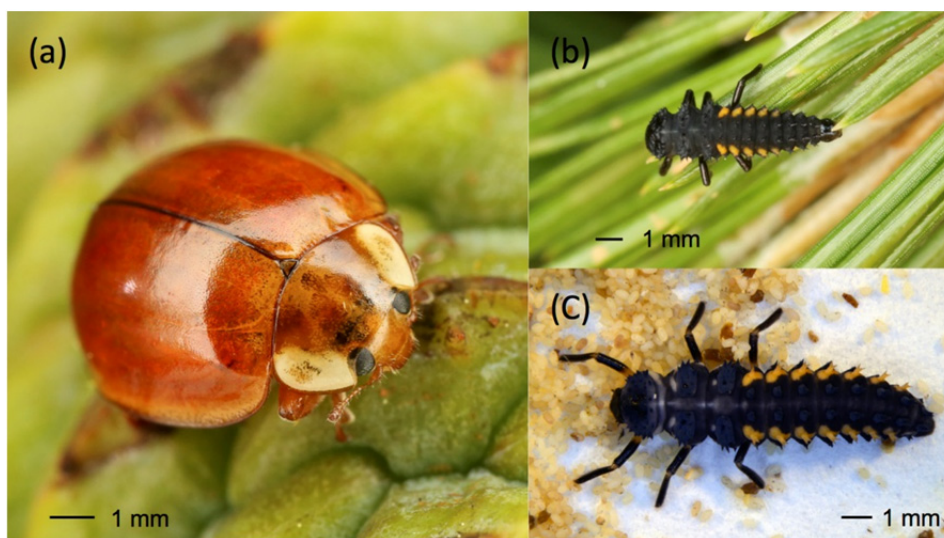


Fig. 1. The adult (a), 3rd instar (b) and 4th instar larva (c) of Alishan *Harmonia yedoensis* [photo by Shih-Chieh Huang (a, b) and Naoya Osawa (c)].



Fig. 2. The adult of Alishan *Harmonia yedoensis* [upper: male (left) and female (right)] and *H. quadripunctata* [bottom: male (left) and female (right)] (photo by Naoya Osawa).

We collected nine adults of *H. quadripunctata* (Pontoppidan) from the same pine tree in Alishan (Fig. 2, lower two specimens identified on the basis of male genitalia). The adult body size of *H. quadripunctata* from Alishan indicated substantial sexual dimorphism: females significantly larger ($t = -5.48, p < 0.001$) than males (female: 6.87 ± 0.18 mm, $n = 2$; male: 5.77 ± 0.09 , $n = 7$). *Harmonia quadripunctata* is considered an Eurasian species found mainly on conifers. Its distribution is recorded in Europe (e.g., Hodek and Honek, 1996), Turkey (Aslan and Uygun,

2005), India (Kapur, 1963), China (Yu *et al.*, 2000) and Russia (Kuznetsov, 1997). Furthermore, *H. quadripunctata* has recently been recorded in North and South America (Vandenberg, 1990; Majka and McCorquodale, 2006; Gonzales, 2012), where it may have been accidentally introduced. We could not determine whether the population of *H. quadripunctata* in Taiwan was distributed naturally, although forests still exist on Alishan, particularly at higher elevations. Additional studies evaluating the natural distribution of *H. quadripunctata* are warranted.

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References

- Aslan MM, Uygun N.** 2005. The aphidophagous coccinellid (Coleoptera: Coccinellidae) species in Kahramanmaras, Turkey. *Turk J Zool* 29: 1-8.
- Gonzales G.** 2012. Lista actualizada de especies de Coccinellidae (Insecta: Coleoptera) presentes en Chile. Available at <http://www.coccinellidae.cl>.
- Hodek I, Honek A.** 1996. Ecology of Coccinellidae. Kluwer Academic Publ., Dordrecht, The Netherlands. 464 pp.
- Kapur AP.** 1963. The taxonomic status and further description of *Harmonia expallida* Weise, (Col., Coccinellidae), feeding on *Adelges* species (Hem., Adelgidae) in North-West India. *Entomophaga* 8: 199-203.
- Kurosawa Y, Hisamitsu S, Sasaji H.** 1982. The Coleoptera of Japan in Color Vol. III. Hoikusha Publ., Osaka Japan. 500 pp. (in Japanese)
- Kuznetsov VN.** 1997. Lady Beetles of the Russian Far East. Memoir No. 1. Center for Systematic Entomology and The Sandhill Crane Press, Florida USA. 248 pp.
- Majka CG, McCorquodale DB.** 2006. The Coccinellidae (Coleoptera) of Maritime Provinces of Canada: new records, biogeographic notes, and conservation concerns. *Zootaxa* 1154: 49-68.
- Nakagawa K, Sasaji H.** 1988. Elytral colour pattern polymorphisms and their inheritance in *Harmonia yedoensis* Takizawa (Coleoptera: Coccinellidae). *Ent J Fukui* 2: 37-48. (in Japanese)
- Osawa N, Ohashi K.** 2008. Sympatric coexistence of sibling species *Harmonia yedoensis* and *H. axyridis* (Coleoptera: Coccinellidae) and the roles of maternal investment through egg and sibling cannibalism. *Eur J Entomol* 105: 445-454.
- Reznik SY, Dolgovskaya MY, Ovchinnikov AN, Belyakova NA.** 2015. Weak photoperiodic response facilitates the biological invasion of the harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). *J Appl Ent* 139: 241-249.
- Sasaji H.** 1977. Larval characters of Asian species of the genus *Harmonia* Mulsant (Coleoptera: Coccinellidae). *Mem Fac Educ Fukui Univ (Ser. II, Nat. Sci.)* 27: 1-18.
- Sasaji H.** 1981. Biosystematics on *Harmonia axyridis*-complex (Coleoptera: Coccinellidae). *Mem Fac Educ Fukui Univ (Ser. II, Nat. Sci.)* 30: 59-79.
- Sasaji H.** 1982. A revision of the Formosan Coccinellidae (III): subfamily Coccinellinae (Coleoptera). *Mem Fac Educ Fukui Univ (Ser. II, Nat. Sci.)* 31: 1-49.
- Tao C-C.** 1990. Aphid-Fauna of Taiwan Province, China. Taiwan Provincial Museum, Taipei, Taiwan. 327 pp. (in Traditional Chinese)
- Vandenberg NJ.** 1990. First North American records for *Harmonia quadripunctata* (Pontopiddian) (Coleoptera: Coccinellidae). A lady beetle native to the Palaearctic. *Proc Entomol Soc Wash* 92: 407-410.
- Yu G, Montgomery ME, Yao D.** 2000. Lady beetles (Coleoptera: Coccinellidae) from Chinese hemlocks infested with the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). *Coleopterists Bull* 54: 145-199.

共棲於臺灣阿里山區二葉松之兩種 *Harmonia* 瓢蟲 (鞘翅目：瓢蟲科)：隱斑瓢蟲 (*Harmonia yedoensis*) 與新記錄之四斑瓢蟲 (*Harmonia quadripunctata*)

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摘 要

本研究使用雄蟲生殖器與野生雌蟲所產幼蟲之形態特徵鑑定隱斑瓢蟲 (*Harmonia yedoensis*)，並重新確認此物種棲息於臺灣阿里山中海拔地區的二葉松 (*Pinus taiwanensis*)。文中詳述此瓢蟲族群的生活史、性別體型雙態性和獵物種類。我們在相同的二葉松植株上發現臺灣地區之新記錄種—四斑瓢蟲 (*H. quadripunctata*)。

關鍵詞：*Harmonia*、新記錄、幼蟲形態、性別體型雙態性、阿里山、臺灣