

A taxonomic issue of two species of *Trissolcus* (Hymenoptera: Platygasteridae) parasitic on eggs of the brown-winged green bug, *Plautia stali* (Hemiptera: Pentatomidae): resurrection of *T. plautiae*, a cryptic species of *T. japonicus* revealed by morphology, reproductive isolation and molecular evidence

Kazunori Matsuo · Yoshimi Hirose ·
Norman F. Johnson

Received: 2 October 2013 / Accepted: 12 March 2014 / Published online: 25 March 2014
© The Japanese Society of Applied Entomology and Zoology 2014

Abstract *Trissolcus plautiae* (Watanabe) is known as a major egg parasitoid of the brown-winged green bug *Plautia stali* Scott, which is a serious pest of various fruit trees in Japan. Although *T. plautiae* was synonymized with *T. japonicus* (Ashmead) in 1981, both scientific names have been used for the same egg parasitoid of *P. stali* for about the past 30 years because of their taxonomic confusion. To promote an effective IPM program for *P. stali* using its egg parasitoids, we attempted to resolve the confusion by the use of a variety of methods. On detailed observation of adult morphology, we found that sublateral setae on the T1 are present in *T. plautiae* and absent in *T. japonicus*, and that this morphological difference is corroborated by more subtle differences between the two species. This finding supports the view that they are different species. The view was also supported by the results of mating experiments to determine the reproductive isolation of *T. plautiae* from *T. japonicus* and DNA analysis of these two species. We conclude that *T. plautiae* is a

cryptic species of *T. japonicus* and resurrect *T. plautiae* from *T. japonicus* stat. rev.

Keywords Host range · mtDNA · Egg parasitoid · Telenominae

Introduction

The brown-winged green bug, *Plautia stali* Scott (Hemiptera: Pentatomidae), is a serious pest of various fruit trees, such as apple, pears, citrus, persimmon, and peach, in Japan because feeding by *P. stali* leads to deterioration of fruit quality (e.g., Ide et al. 1997; Murai and Ogasawara 2003; Sugiura et al. 2007; The Japanese Society of Applied Entomology and Zoology 2006). In spring, overwintering adults of *P. stali* lay their eggs on conifers, such as Japanese cedar and Japanese cypress, and then adults of the first generation move to orchards to feed on fruits. Because the growing area of conifers covers at least 21 % of Japan (Forestry Agency, Japan 2011), it is extremely difficult to suppress *P. stali* populations on conifers using chemical insecticides. At present, chemical insecticides are often applied to orchards for their control. Thus, the pest management system largely relies on chemical insecticides, but it has a risk of causing the resurgence of other fruit-tree pests, such as coccoids and spider mites (Morishita 2005; Tsutsumi 2004). Under such a situation, integrated pest management by different control measures including chemical insecticides are needed for this stink bug pest. Natural enemies of the pest are among the different control measures, and egg parasitoids are the most promising control agents as natural enemies of *P. stali* (Goto and Adachi 2004; Ohno 1987a, b).

As an egg parasitoid of *P. stali*, Watanabe (1954) first described *Asolcus plautiae* Watanabe (Hymenoptera:

K. Matsuo
Entomological Laboratory, Graduate School of Bioresource and
Bioenvironmental Sciences, Kyushu University,
Fukuoka 812-8581, Japan

Present Address:
K. Matsuo (✉)
1185 Haibara, Waki, Mima, Tokushima 779–3601, Japan
e-mail: matsuosudachi@yahoo.co.jp

Y. Hirose
Kyushu University, Fukuoka 812-8581, Japan

N. F. Johnson
Department of Evolution, Ecology and Organismal Biology,
The Ohio State University, Columbus, OH 43212, USA

Platygastridae, but Scelionidae at that time of his description) from Zentsuji, Kagawa, Japan. His description was based on nine females and one male bred from field-collected eggs of *P. stali* and ten females and one male probably reared from field-collected eggs of *P. splendens* Distant. Tachikawa et al. (1977) used the scientific name *Trissolcus plautiae* (Watanabe) instead of *A. plautiae* because Masner and Muesebeck (1968) transferred *A. plautiae* to the genus *Trissolcus*. Recording four species as egg parasitoids of *P. stali* reared in Fukuoka, Japan, Tachikawa et al. (1977) stated that *T. plautiae* seems to be the most effective in eliminating *Plautia* eggs among the four species. Yamada and Miyahara (1979) demonstrated that *T. plautiae* is the most dominant egg parasitoid of *P. stali*, thus corroborating the statement of Tachikawa et al. (1977). Since then, the view that *T. plautiae* has considerable ability to be an effective control agent against *P. stali* has been supported by several workers, such as Goto and Adachi (2004), Ohno (1987a, b), and Toyama and Mishiro (2010).

Four years after Hirashima and Yamagishi (1981), Tachikawa et al. (1977) synonymized *T. plautiae* with *T. japonicus* (Ashmead), described as *Dissolcus japonicus* by Ashmead (1904) on the basis of a single female collected from Hakone, Kanagawa, Japan, without host record. One of the authors, YH, considered further confirmation was required for the taxonomic treatment proposed by Hirashima and Yamagishi (1981) because they did not examine the type specimens of *T. plautiae* that had been preserved in Hokkaido University in Sapporo, Japan. Preceding the comparison of morphological characteristics between types of *T. plautiae* and *T. japonicus*, YH sent some specimens of *Trissolcus* that were reared from eggs of *P. stali* to one of the authors, NJ, in 1983 and requested him to compare the specimens with the type of *T. japonicus* that had been deposited in the US National Museum. In 1984, NJ found one substantial difference among them: the specimens from YH have a pair of sublateral setae on the T1, whereas the type of *T. japonicus* does not have the setae. Because the type of *T. plautiae* was reared from an egg of *P. stali*, specimens from YH might be *T. plautiae*. Therefore, this finding strongly suggested that *T. plautiae* and *T. japonicus* are different from each other.

As a result of unpublished information that it is possible that there are two species involved, two scientific names, i.e., *T. plautiae* (Watanabe) and *T. japonicus* (Ashmead), have been used for an egg parasitoid of *P. stali* in Japan since Hirashima and Yamagishi (1981): *T. plautiae* by Arakawa and Namura (2002), Goto and Adachi (2004), Ohno (1987a, b, 1999), and Toyama and Mishiro (2010) and *T. japonicus* by Buhl (1996), Johnson (1992), Ryu and Hirashima (1984), and Talamas et al. (2013).

As exemplified by Masner (1958), the genus *Trissolcus*, which was referred to as *Asolcus* by him, contains many

species characterized by a high degree of variability in adult morphology. Thus, we see the possibility that cryptic species could be frequently found in this genus. The first case of finding cryptic species in the genus is that of *T. crypticus* Clarke reported by Clarke (1993) as a cryptic species of *T. basalis* (Wollaston). Cryptic species of parasitoids may differ in their biological traits that affect their efficiency as control agents, such as *Torymus* species (Hymenoptera: Torymidae) attacking *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), a serious pest of chestnut production (Moriya et al. 2003; Yara et al. 2010). We therefore consider precise identification is essential to promote IPM programs. In this context, we attempted to resolve the above-mentioned confusion about the taxonomy of the two species, *T. plautiae* and *T. japonicus*. In this article, we present the results of morphological, biological and genetic studies of these two species to confirm their taxonomic status.

Materials and methods

Insect collecting and culture

A host culture was established not only for rearing *Trissolcus* wasps, but also for obtaining hosts for host deployment to collect these wasps in the field and for laboratory mating experiments to determine reproductive isolation of *T. plautiae* from *T. japonicus*. The culture was initiated using *P. stali* adults, which were collected from Fukuoka, Japan, in 2011–2012. The adults were kept in plastic cages containing filter paper, water and peanuts under 23 °C and 14L–10D conditions.

In the host deployment in the field, egg clusters of *P. stali*, which were laid on filter paper, were attached to leaves of *Morus alba* Linnaeus (Moraceae) by paper clips during the period from May to September 2012 in Hakozaki, Fukuoka. Each egg cluster was deployed for 2–4 days. On the occasion of the host deployment, we collected egg masses of *P. stali* and *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) that were laid under natural conditions to clarify natural host ranges of *Trissolcus* wasps. After being brought back into the laboratory, the deployed and field-collected eggs were kept under 23 °C and 14L–10D conditions until adult wasp emergence. Adults of parasitoid wasps were preserved in 99 % ethanol for morphological observation and DNA analysis.

Morphological examination and terminology

For microscopic study, the ethanol-stored specimens were dried by the method described in Matsuo and Yukawa (2009). Specimens were observed under a binocular

microscope (LEICA S8APO). Several specimens were gold-coated for microphotography with a JEOL JSM-5600LV scanning electronic microscope. Male genitalia were mounted on slides in Canada balsam using ethanol and clove oil. Drawings were made with the aid of a drawing tube. For morphological comparison, we paid special attention to characteristics mentioned by Clarke (1993) and those of the pleural and genital morphologies that had not been described in the earlier studies (Ashmead 1904; Hirashima and Yamagishi 1981; Ryu and Hirashima 1984; Watanabe 1954). Morphological terminology of adults follows usage in Mikó et al. (2007) for the head and mesosoma, Johnson (1984) for the metasoma, and Polaszek and Kimani (1990) for the male genitalia.

Specimens examined and depositories

The holotypes of *T. plautiae* and *T. japonicus* are deposited in the Hokkaido University Museum, Hokkaido, Japan (HUM), and the US National Museum of Natural History, Washington, DC, USA (USNM), respectively. For morphological comparison, we examined these holotypes. We also examined *Trissolcus* specimens reared from eggs of *P. stali* deposited in the collection of the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Japan (ELKU). We further examined specimens used in Arakawa and Namura (2002), Ohno (1981, 1987a, b,

1999), and Toyama and Mishiro (2010) that have been kept in the ELKU.

Mating experiment and statistical analysis

For mating experiments, adults of *T. plautiae* and *T. japonicus* were kept in glass tubes (8 mm in width and 50 mm in height) with honey under the same conditions as those of *P. stali*. Laboratory experiments to determine reproductive isolation of *T. plautiae* from *T. japonicus* were conducted using 0-day-old females and 0–4-day-old males under 23 °C and 14L–10D conditions. An unmated female and male were introduced to the same glass tube (8 mm in width and 50 mm in height) for mating. Four mating combinations were tested: *T. japonicus* (♂) × *T. plautiae* (♀), *T. plautiae* (♂) × *T. japonicus* (♀), *T. japonicus* (♂) × *T. japonicus* (♀) and *T. plautiae* (♂) × *T. plautiae* (♀). The number of replicates conducted for each mating test was varied from 75 to 84. A pair of wasps was isolated 24–36 h after copulation. To each isolated female, 6–28 eggs of *P. stali* (0–2 days old) were presented for 24–36 h to assess mating success. The egg masses were then removed and kept in the tube until offspring emerged. Females that produced female offspring were regarded as successfully mated. We compared the percentage of mating success among the four combinations. The significance of results in the mating test was assessed with a chi-square test.

Table 1 Specimens of two species of *Trissolcus* and an outgroup taxon which were used for DNA analysis

Species	Host insect	Associated plant ^a	Collecting site (collector ^c)	Date of emergence	No. of individuals	Accession no.
<i>T. japonicus</i>	<i>Plautia stali</i>	<i>Morus alba</i> ^b	Fukuoka, Fukuoka (KaM)	ix 2012	3 females	AB847129-30, 43
<i>T. japonicus</i>	<i>P. stali</i>	<i>M. alba</i> ^b	Fukuoka, Fukuoka (KaM)	x 2012	4 females	AB847137, 44-46
<i>T. japonicus</i>	<i>P. stali</i>	<i>M. alba</i> ^b	Ninomiya, Kanagawa (TM)	24–26 vii 2012	3 females	AB847131-32, 36
<i>T. japonicus</i>	<i>Halyomorpha halys</i>	<i>Distylium racemosum</i>	Fukuoka, Fukuoka (KaM)	v 2012	4 females	AB908179-82
<i>T. plautiae</i>	<i>P. stali</i>	<i>M. alba</i> ^b	Fukuoka, Fukuoka (KaM)	ix 2012	3 females	AB847133-35
<i>T. plautiae</i>	<i>P. stali</i>	<i>M. alba</i> ^b	Ninomiya, Kanagawa (TM)	28–30 viii 2012	3 females	AB847138-39, 47
<i>T. plautiae</i>	<i>P. stali</i>	<i>M. alba</i>	Tsukuba, Ibaraki (MT)	11–16 viii 2011	3 females	AB847140-42
<i>T. plautiae</i>	<i>H. halys</i>	<i>Elaeocarpus zollingeri</i>	Fukuoka, Fukuoka (WK)	8–10 vi 2012	4 females	AB908183-86
Out group						
<i>Telenomus chrysopae</i>						
	Unidentified chrysopterid	<i>Quercus glauca</i>	Chikushino, Fukuoka (KiM)	17 x 2011	1 female	AB847148

^a Plant on which eggs of *P. stali* or *H. halys* were laid

^b Eggs of *P. stali* that were laid on filter paper were attached to leaves of the associated plant by paper clips

^c Name of collectors: KaM, Kazunori Matsuo; KiM, Kiyoko Matsunaga; MT, Masatoshi Toyama; TM, Toshiharu Mita; WK, Wanggyu Kim

DNA analysis

Specimens stored in 99 % ethanol were used for DNA analysis (Table 1). For every individual specimen, total DNA was extracted from the whole body with the DNeasy Blood and Tissue Kit following the manufacturer's instructions. The primers used in the analysis were: forward, CI-J-1751 5'-GGA TCA CCT GAT ATA GCA TTC CC-3' and reverse, CI-N-2191 5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3' (Simon et al. 1994). A region of the cytochrome oxidase subunit I (COI) gene of mitochondrial DNA was amplified by following procedures: denaturation step at 94 °C for 60 s, annealing for 90 s at 48 °C and extension at 72 °C for 90 s, with 35 cycles being performed. The sequencing reaction was performed using the BigDye Terminator Cycle Sequencing Kit and an ABI 3100 sequencer. The sequence data were analyzed with the neighbor-joining (NJ) method using MEGA 4 (Tamura et al. 2007). In constructing an NJ tree, *Telenomus chrysopae* Ashmead (Hymenoptera: Platygasteridae) that had been reared from an egg of an unidentified chrysopid (Neuroptera) in Fukuoka, Japan, was employed as an outgroup taxon. Evolutionary distances were computed by Kimura's two-parameter distances (Kimura 1980). The resulting trees were subjected to bootstrap analysis (Efron 1982; Felsenstein 1985) with 1,000 replications using MEGA 4.

Results

Morphological examination

We reconfirmed NJ's observation that *T. plautiae* and *T. japonicus* were distinguished by the presence or absence of the sublateral setae on the T1 based on morphological comparison between their type and other specimens. *Trissolcus plautiae* has a pair of sublateral setae on the T1, whereas *T. japonicus* does not (Figs. 1–4). This characteristic was available in both sexes in discriminating these two species. Additional morphological characters are given in the descriptions below. Specimens used in Arakawa and Namura (2002), Ohno (1981, 1987a, b, 1999), and Toyama and Mishiro (2010) had a pair of sublateral setae on the T1. This finding showed that they were identical to *T. plautiae*. Thus, the ecological traits revealed by the aforementioned papers should be regarded as those of *T. plautiae*. The result of morphological examination of specimens deposited in the ELKU (Table 2) demonstrated that both *T. plautiae* and *T. japonicus* parasitize *P. stali* eggs and have sympatric distributions.

Mating experiments

Most females used in intraspecific mating combinations successfully reproduced female offspring, whereas females

of interspecific mating combinations completely failed to reproduce female offspring (Fig. 19). Mating success was significantly different between *T. japonicus* (♂) × *T. plautiae* (♀) and *T. japonicus* (♂) × *T. japonicus* (♀) ($p < 0.01$); *T. japonicus* (♂) × *T. plautiae* (♀) and *T. plautiae* (♂) × *T. plautiae* (♀) ($p < 0.01$); *T. plautiae* (♂) × *T. japonicus* (♀) and *T. japonicus* (♂) × *T. japonicus* (♀) ($p < 0.01$); *T. plautiae* (♂) × *T. japonicus* (♀) and *T. plautiae* (♂) × *T. plautiae* (♀) ($p < 0.01$).

DNA analysis

The length of the amplified COI gene fragment was 424 bp. DNA sequencing data supported the results of morphological examination and mating experiments with high bootstrap values (Fig. 20). The maximum and minimum sequence divergences between *T. plautiae* and *T. japonicus* were 13.9 and 13.4 %, respectively.

Taxonomy

We resurrect *T. plautiae* from *T. japonicus* based on the results of morphological observation, mating experiments, and DNA analysis. Host records are restricted to field-collected hosts.

Trissolcus plautiae (Watanabe, 1954) stat. rev.

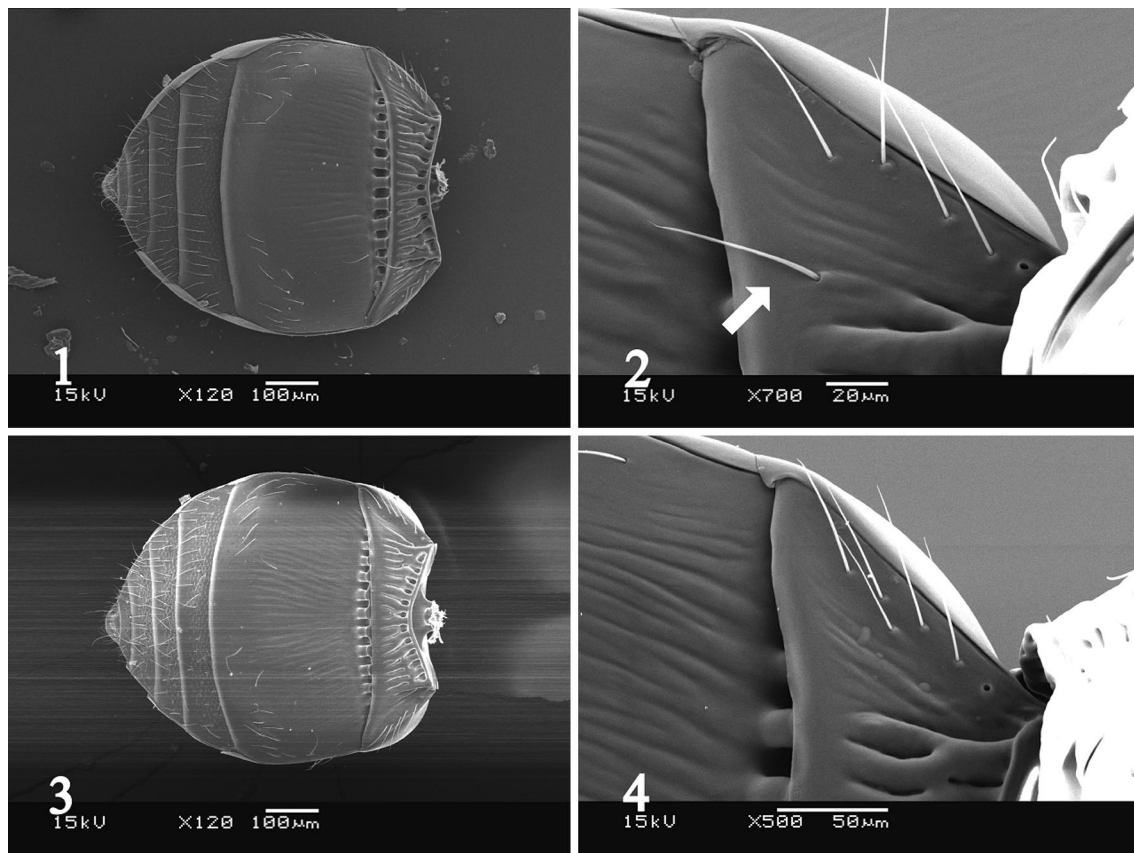
Figs. 1, 2, 5–10, and 17

Asolcus plautiae Watanabe, 1954, Trans. Shikoku Entomol. Soc. 2: 18 (original description).

Trissolcus plautiae (Watanabe): Masner and Muesebeck, 1968, Bull. U.S. Nat. Mus. 270: 72 (generic transfer).

Trissolcus plautiae (Watanabe): Hirashima and Yamagishi, 1981, J. Fac. Agr. Kyushu Univ. 25: 153 (junior synonym of *Trissolcus japonicus* (Ashmead)).

Specimens examined. Holotype: female, emerged on 4 vii 1952 from an egg of *P. stali* collected by T. Kobayashi from Zentsuji, Kagawa, Japan (HUM). Paratypes: five females and one male, same data as holotype; nine females and one male, emerged on 20 vi 1951 from eggs of a *Plautia* species (probably *P. splendens* Distant) on *Catalpa ovata* G. Don (Bignoniaceae) collected by T. Kobayashi from Zentsuji, Kagawa, Japan (HUM). Other specimens: one female and one male, emerged on 11–16 viii 2011 from eggs of *P. stali* on *Pinus thunbergii* Parlatores (Pinaceae) collected by K. Matsuo on 4 viii 2011 from Hakozaki, Fukuoka, Fukuoka, Japan (ELKU); four females and three males, emerged in x 2012 from eggs of *P. stali* deployed on *M. alba* and collected by K. Matsuo in ix 2012 from Hakozaki, Fukuoka, Fukuoka, Japan (ELKU); four females, emerged on 25 v 2012 from eggs of *H. halyis* on *Distylium racemosum* Siebold et Zuccarini



Figs. 1–4 *Trissolcus plautiae* and *T. japonicus*. 1: Female of *T. plautiae*, metasoma dorsal view. 2: Female of *T. plautiae*, side of T1, an arrow points toward the sublateral setae. 3: Female of *T. japonicus*, metasoma dorsal view. 4: Female of *T. japonicus*, side of T1

Table 2 Number of individuals of two *Trissolcus* species reared from field-collected eggs of *P. stali* in Japan. These specimens have been deposited in the collection of the ELKU

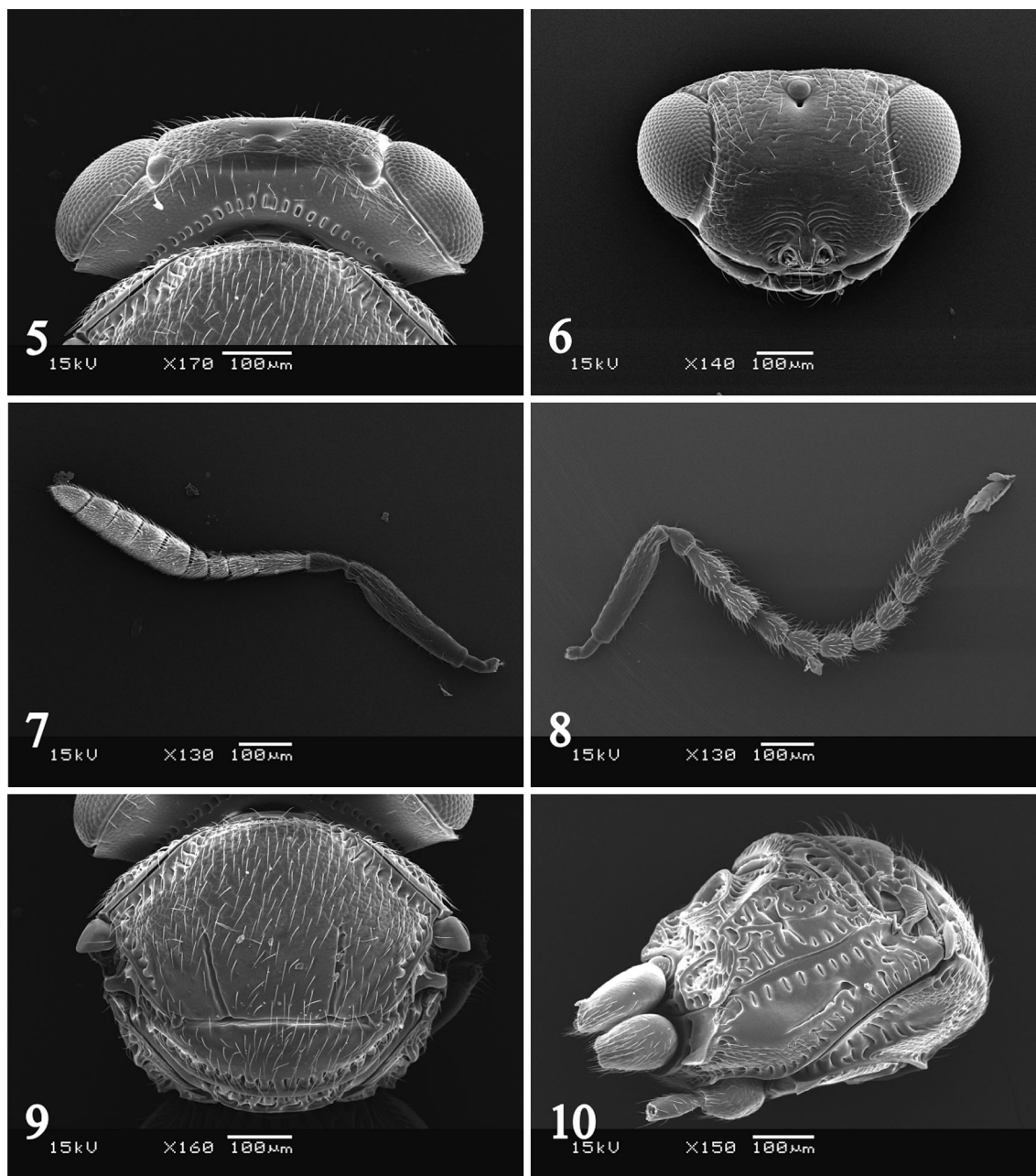
Collecting site (collector ^a)	Date of egg collecting	Associated plant ^b	No. of <i>T. japonicus</i>		No. of <i>T. plautiae</i>	
			Male	Female	Male	Female
Kashihara, Nara (MO)	14 ix 1978	<i>Cryptomeria japonica</i>	0	1	0	1
Takatori, Nara (MO)	20 ix 1978	<i>Cr. japonica</i>	0	0	1	1
Gojo, Nara (MO)	21 viii 1980	<i>Viburnum odoratissimum</i>	0	0	0	11
Sanmaiden, Tenri, Nara (MO)	30 v 1983	<i>Morus alba</i>	3	2	19	11
Asuka, Nara (MO)	1 viii 1985	<i>Diospyros kaki</i>	1	3	0	9
Hamakita, Hamamatsu, Shizuoka (AT)	11 vi 1988	<i>M. alba</i>	2	1	0	7
Hamakita, Hamamatsu, Shizuoka (AT)	23 vi 1988	<i>M. alba</i>	1	1	1	18
Hamakita, Hamamatsu, Shizuoka (AT)	29 vi 1988	<i>Chamaecyparis obtusa</i>	0	1	0	0
Chiba, Chiba (HF)	1 vi 1987	<i>M. alba</i>	1	5	0	0
Chiba, Chiba (HF)	15 vi 1987	<i>Ch. obtusa</i>	0	3	0	6

^a Name of collectors: AT, Asao Takahashi; HF, Hiroshi Fukuda; MO, Michihiro Oda

^b Plant on which eggs of *P. stali* were laid

(Hamamelidaceae) collected by K. Matsuo on 17 v 2012 from Hakozaki, Fukuoka, Fukuoka, Japan (ELKU). For other specimens examined, see Table 2.

Redescription. Female. Body length 1.2–1.7 mm. Head, mesosoma, and metasoma black. Antenna black except for A1, which is brownish at base, sometimes A1 and A2



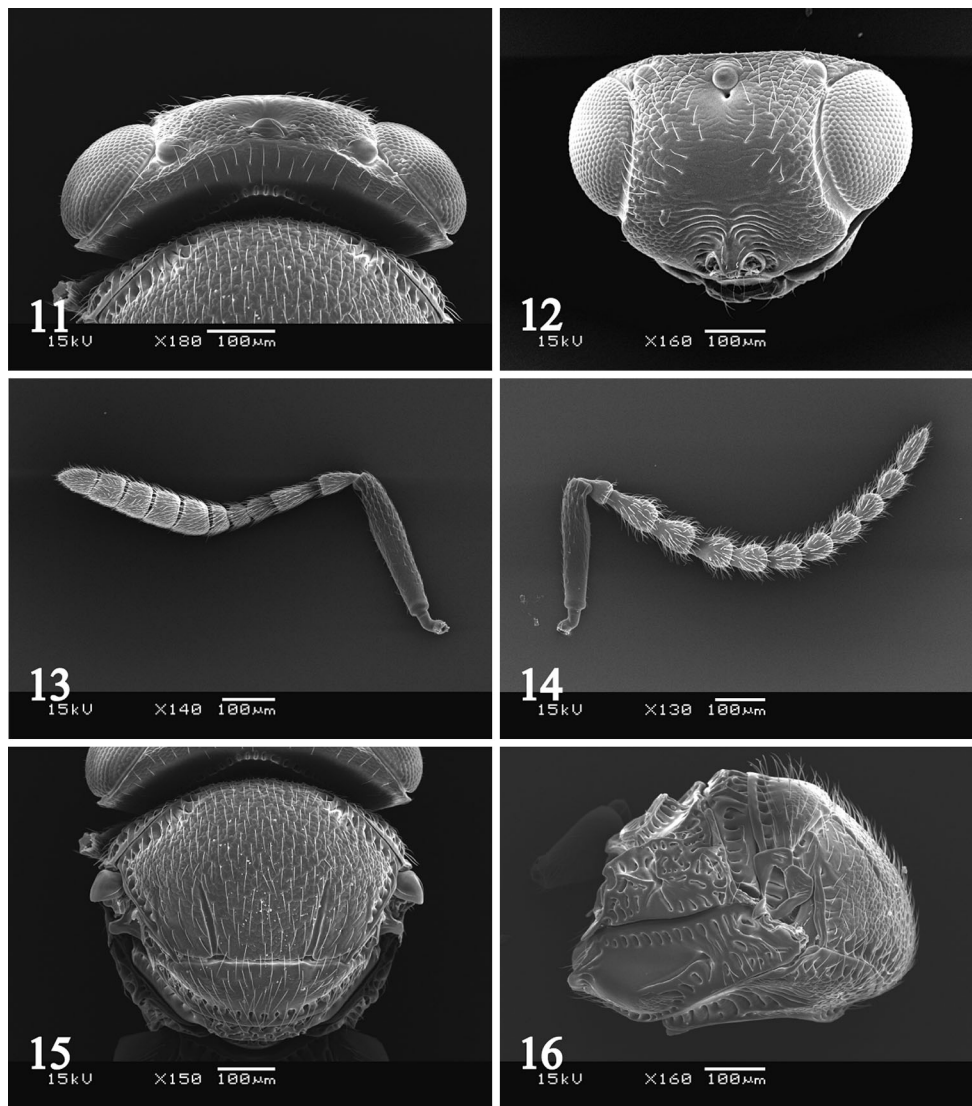
Figs. 5–10 *T. plautiae*. 5: Female head, dorsal view. 6: Female head, frontal view. 7: Female antenna. 8: Male antenna. 9: Female mesosoma, dorsal view. 10: Female mesosoma, lateral view

brown. Coxae of all legs black; all femora infuscate; all tibiae brownish yellow.

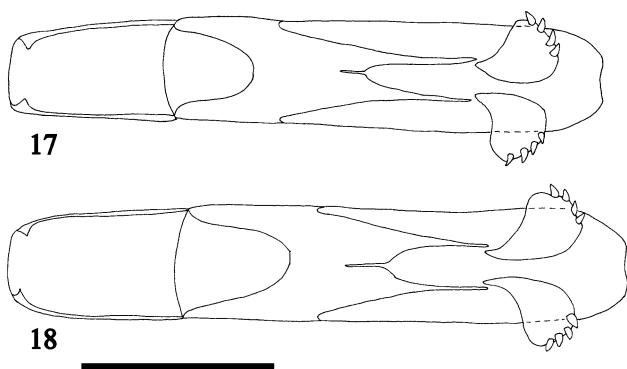
Head 1.1–1.2 times as wide as mesosoma; hyperoccipital carina complete but not very sharp (Fig. 5); lateral ocellus connected to orbital furrow by a short sulcus; orbital furrow smooth, expanded ventrally; eye with very short setae; frons shiny with coriaceous microsculpture, below precellar pit with extensive smooth area; a longitudinal groove present below precellar pit in larger individuals; frontal depression transversely striated (Fig. 6).

Antenna 11 segmented; A3 distinctly longer than A2 (Fig. 7).

Mesosoma with distinct notauli (Fig. 9); mesoscutum coarsely sculptured, smoother posteriorly; scutellum shiny and smooth, sometimes with superficial reticulation; setal base on scutellum smooth; netrion well developed (Fig. 10); mesopleural carina connects to pleural pit, not reaching ventral margin of mesopleuron; postacetabular patch coriaceous, with several setae; mesepisternum below postacetabular patch sometimes with numerous (15–20)



Figs. 11–16 *T. japonicus*. 11: Female head, dorsal view. 12: Female head, frontal view. 13: Female antenna. 14: Male antenna. 15: Female mesosoma, dorsal view. 16: Female mesosoma, lateral view



Figs. 17–18 *T. plautiae* and *T. japonicus*. 1: Male genitalia of *T. plautiae*. 2: Male genitalia of *T. japonicus*. Scale bar: 0.1 mm

short setae; posterior mesepimeral area smooth; anterior extension of metapleuron toward mid coxa long and acute.

T1 with a pair of sublateral setae (Figs. 1, 2); T2 smooth, sparsely setose laterally; T3 and beyond punctulate anteriorly.

Male. Differs from female as follows: Body length 1.2–1.4 mm. Antenna with 12 segments (Fig. 8). Genitalia (Fig. 17); basal ring 0.3–0.4 times as long as aedeago-volsellar shaft; aedeago-volsellar parallel sided; end of laminae volsellars strongly incised; digitus with four digital teeth; aedeagal lobe weakly emarginated or sometimes entirely truncate.

Distribution. Japan.

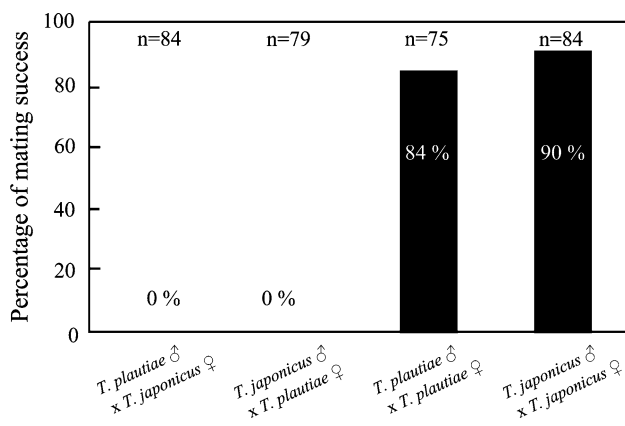


Fig. 19 Percentage of mating success of inter- and intraspecific mating in *T. plautiae* and *T. japonicus*. The number of replicates is shown above each bar

Host information. This species parasitizes eggs of *P. stali* and *H. halys*.

Trissolcus japonicus (Ashmead, 1904)

Figs. 3, 4, 11–16, and 18

Dissolcus japonicus Ashmead, 1904, J. New York Entomol. Soc. 12: 73 (original description).

Trissolcus japonicus (Ashmead): Masner and Muesebeck, 1968, Bull. U.S. Nat. Mus. 270: 72 (generic transfer).

Trissolcus japonicus (Ashmead): Hirashima and Yamagishi, 1981, J. Fac. Agr. Kyushu Univ. 25: 153 (senior synonym of *Trissolcus plautiae* (Watanabe)).

Trissolcus halyomorphae Yang: Talamas et al., 2013, J. Hym. Res. 33: 116 (junior synonym of *Trissolcus japonicus* (Ashmead)).

Specimens examined. Holotype: female, collected by A. Koebele from Hakone, Kanagawa, Japan (USNM, type no. 7127). Other specimens: four females and three males, emerged in x 2012 from eggs of *P. stali* deployed on *M. alba* and collected by K. Matsuo in ix 2012 from Hakozaki, Fukuoka, Fukuoka, Japan (ELKU); four females, emerged on 25 v 2012 from eggs of *H. halys* on *D. racemosum* collected by K. Matsuo on 17 v 2012 from Hakozaki, Fukuoka, Fukuoka, Japan (ELKU). For other specimens examined, see Table 2.

Morphology. Female and male. Morphological characteristics are largely identical to those of *T. plautiae*, differing in the following: upper frons almost entirely sculptured, smooth field limited to area just below the preocellar pit; microsculpture of mesoscutum more extensive, reaching transscutal articulation between notauli; mesoscutellum with coriaceous microsculpture anteriorly; T1 without sublateral setae.

Distribution. Japan and P.R. China (Yang et al. 2009).

Host information. This species parasitizes eggs of *P. stali* and *H. halys*.

Discussion

Taxonomic remarks

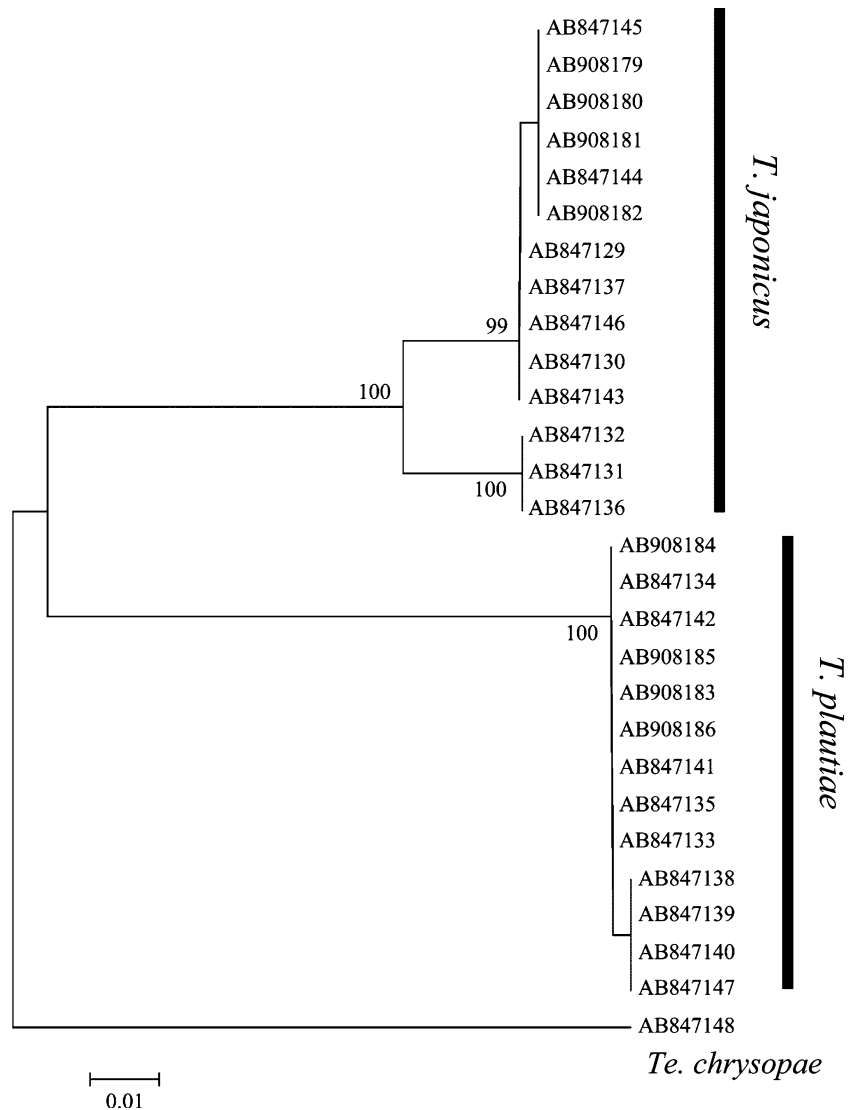
Talamas et al. (2013) have recently synonymized *T. halyomorphae* Yang with *T. japonicus*. The characters noted in their paper are consistent with our observations. Some of the features noted by Yang et al. (2009), such as the presence of a longitudinal groove below the median ocellus, may be influenced by host size in that the host from which those specimens were reared, *H. halys*, is larger than *P. stali*. The effect of host size on character expression in these parasitoids remains to be carefully examined. Similarly, in the absence of controlled rearing conditions, the morphological differences in sculpture that we report here would likely be treated as insignificant individual variation. The fact that the differences are observed under identical temperature and photoperiod and from specimens reared from the same host species suggests that they accurately reflect that *T. plautiae* and *T. japonicus* are different species.

Host range

Arakawa and Namura (2002) showed that *T. plautiae* can utilize eggs of *H. halys* under laboratory conditions. We newly confirmed that *H. halys* is one of the natural hosts of *T. plautiae* in Japan. Yang et al. (2009) recorded *H. halys* as a natural host of *T. japonicus* (= *T. halyomorphae*) from P.R. China. We have also reared *T. japonicus* from field-collected eggs of *H. halys* in Fukuoka and showed that the Japanese population of *T. japonicus* parasitizes *P. stali* and *H. halys* as natural hosts.

Ryu and Hirashima (1984) recorded *Elasmucha putoni* Scott (Hemiptera: Acanthosomatidae) as one of the hosts of *T. plautiae* or *T. japonicus*. However, they never provided information on voucher specimens with such collecting data and their depositories. Therefore, we were unable to identify which species, *T. plautiae* or *T. japonicus*, parasitizes eggs of *E. putoni*. *Elasmucha putoni* females guard their eggs (Honbo and Nakamura 1985; Kudo 1990), but *P. stali* females do not. Thus, egg parasitoids of *E. putoni* may be required to develop parasitic strategies to overcome host parental care. In this context, a series of oviposition behaviors of *T. plautiae* and *T. japonicus* (antennation, ovipositing, and marking) takes much time and would make it impossible to parasitize *E. putoni* eggs. We believe

Fig. 20 Neighbor-joining tree for *T. plautiae* and *T. japonicus* based on 424 bp of the mtDNA COI gene. Bootstrap values are indicated for nodes gaining more than 90 % support in 1,000 replications. *Telenomus chrysopae* was used as an outgroup species. DDBJ accession numbers are shown in Table 1



that the host *E. putoni* recorded by Ryu and Hirashima (1984) needs to be reconfirmed.

Acknowledgments We thank Dr. K. Ohno, Prof. R. Arakawa, Dr. I. Adachi, Dr. H. Goto, Dr. M. Toyama, Mr. M. Oda, Mr. A. Takahashi, Mr. H. Fukuda, Dr. T. Mita, Mr. Wanggyu Kim, Mr. Y. Tazuhara, Mr. N. Baba, Mr. N. Kitano, Ms. K. Akiyama, and Ms. Matsunaga for providing us valuable specimens. We also thank Dr. M. Buffington, Mr. T. Nuhn, and Dr. M. Ohara for allowing us to examine the type specimens of *T. japonicus* and *T. plautiae*; Prof. Y. Abe and Dr. T. Ide for their support in taking SEM images; Emeritus Prof. J. Yukawa, Dr. N. Mizutani, Mr. Y. Higashiura, and Mr. R. Ito for providing information on the rearing system to keep *P. stali*; Dr. M. Maruyama for his support in DNA analysis; and two anonymous reviewers for their critical comments on the manuscript. KM thanks Emeritus Prof. O. Tadauchi, Dr. L. J. Westover, Dr. S. Kamitani, and Mr. D. Yamaguchi for their support in various ways. This study was supported partly by the Global COE Program (Center of excellence for Asian conservation ecology as a basis of human-nature mutualism), Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan.

References

- Arakawa R, Namura Y (2002) Effects of temperature on development of three *Trissolcus* spp. (Hymenoptera: Scelionidae), egg parasitoids of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). *Entomol Sci* 5:215–218
- Ashmead WH (1904) Descriptions of new Hymenoptera from Japan. I. *J N Y Entomol Soc* 12:65–84
- Buhl PN (1996) Two new species of Scelionoidea from East Asia (Hymenoptera: Platygasteridae et Scelionidae). *Phegea* 24: 127–130
- Clarke AR (1993) A new *Trissolcus* Ashmead species (Hymenoptera: Scelionidae) from Pakistan: species description and its role as a biological control agent. *Bull Entomol Res* 83:523–527
- Efron B (1982) The jackknife, the bootstrap, and other resampling plans. Society for Industrial and Applied Mathematics, Philadelphia 85 pp
- Felsenstein J (1985) Confidence limits on phylogenies—an approach using the bootstrap. *Evolution* 39:783–791
- Forestry Agency, Ministry of Agriculture, Forestry and Fisheries, Japan (2011) Annual Report on Forest and Forestry in Japan

- Goto H, Adachi I (2004) Development of immature stages of *Trissolcus plautiae* (Hymenoptera: Scelionidae), an egg parasitoid of *Plautia crossota stali* (Heteroptera: Pentatomidae). Jpn J Appl Entomol Zool 48:213–218 (in Japanese with English abstract)
- Hirashima Y, Yamagishi K (1981) Redescriptions of the types of some Japanese Scelionidae (Hymenoptera: Proctotrupoidea) preserved in the United States National Museum. J Fac Agric Kyushu Univ 25:153–159
- Honbo Y, Nakamura K (1985) Effectiveness of parental care in the bug *Elasmucha putoni* Scott (Hemiptera: Acanthosomatidae). Jpn J Appl Entomol Zool 29:223–229 (in Japanese with English summary)
- Ide Y, Iwanaga H, Yasunishi T, Suetsugu N, Tashiro N, Matsuzaki M (1997) Damage of Satsuma mandarin heavily attacked by stink bugs at the young fruit stage. Proc Assoc Pl Prot Kyushu 43:110–113 (in Japanese)
- Johnson NF (1984) Systematics of Nearctic *Telenomus*: classification and revisions of the *Podisi* and *Phymatae* species groups (Hymenoptera: Scelionidae). Bull Ohio Biol Sur 6:1–113
- Johnson NF (1992) Catalog of world species of Proctotrupoidea, exclusive of Platygasteridae (Hymenoptera). Mem Am Entomol Inst 51:1–825
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120
- Kudo S (1990) Brooding behavior in *Elasmucha putoni* (Heteroptera: Acanthosomatidae), and a possible nymphal alarm substance triggering guarding responses. Appl Entomol Zool 25:431–437
- Masner L (1958) Some problems of the taxonomy of the subfamily Telenominae. Transaction of the 1st international conference on insect pathology and biological control. Prague, pp 375–385
- Masner L, Muesebeck CFW (1968) The types of Proctotrupoidea (Hymenoptera) in the United States National Museum. Bull US Natl Mus 270:1–143
- Matsuo K, Yukawa J (2009) Two new species of *Torymus* Dalman, 1820 (Hymenoptera: Torymidae) parasitizing *Celticecis japonica* Yukawa and Tsuda, 1987 (Diptera: Cecidomyiidae) that induce leaf galls on *Celtis* species (Ulmaceae) in Japan. Entomol Sci 12:261–269
- Mikó I, Vilhelmsen L, Johnson NF, Masner L, Péntzes Z (2007) Skeletomusculature of Scelionidae (Hymenoptera: Platygastroidea): head and mesosoma. Zootaxa 1571:1–78
- Morishita M (2005) Resurgence of Japanese mealybug, *Planococcus kraunhiae* (Kuwana), induced by a synthetic pyrethroid cypermethrin in persimmon. Ann Rep Kansai Pl Prot 47:125–126 (in Japanese with English summary)
- Moriya S, Shiga M, Adachi I (2003) Classical biological control of the chestnut gall wasp in Japan. In: Proceedings of the 1st international symposium on biological control of arthropods, Honolulu, pp 407–415
- Murai T, Ogasawara H (2003) Damage caused by fruit-piercing stink bugs in European pear in Aomori. Ann Rep Soc Pl Prot North Japan 54:177–181 (in Japanese)
- Ohno K (1981) Intraspecific competition between larvae of *Trissolcus plautiae* (Watanabe) (Hymenoptera: Scelionidae), an egg parasitoid of the brown-winged green bug *Plautia stali* Scott (Heteroptera: Pentatomidae). Proc Assoc Pl Prot Kyushu 27:158–160 (in Japanese with English summary)
- Ohno K (1987a) Effect of host age on parasitism by *Trissolcus plautiae* (Watanabe) (Hymenoptera: Scelionidae), an egg parasitoid of *Plautia stali* Scott (Heteroptera: Pentatomidae). Appl Entomol Zool 22:646–648
- Ohno K (1987b) Ovarian development, fecundity and sex ratio of *Trissolcus plautiae* (Watanabe) (Hymenoptera: Scelionidae), an egg parasitoid of the brown-winged green bug, *Plautia stali* Scott (Hemiptera: Pentatomidae). Jpn J Appl Entomol Zool 31:385–390 (in Japanese with English summary)
- Ohno K (1999) Brood guarding in *Trissolcus plautiae* (Watanabe) (Hymenoptera: Scelionidae), an egg parasitoid of the brown-winged green bug, *Plautia crossota stali* Scott (Heteroptera: Pentatomidae). Entomol Sci 2:41–47
- Polaszek A, Kimani SW (1990) *Telenomus* species (Hymenoptera: Scelionidae) attacking eggs of pyralid pests (Lepidoptera) in Africa: a review and guide to identification. Bull Entomol Res 80:57–71
- Ryu J, Hirashima Y (1984) Taxonomic studies on the genus *Trissolcus* Ashmead (Hymenoptera: Scelionidae) of Japan and Korea. J Fac Agric Kyushu Univ 29:35–58
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651–702
- Sugiura N, Furukawa T, Takizaki Y, Misumi M (2007) Effect of stink bugs feeding on fruits in the young fruit stage on the fruit quality of satsuma-mandarin at harvest in Amakusa, Kumamoto. Kyushu Pl Prot Res 53:86–89 (in Japanese)
- Tachikawa T, Miyahara M, Yamada K (1977) Hymenopterous parasites of the eggs of *Plautia stali* Scott (Hemiptera: Pentatomidae) in Japan. Trans Shikoku Entomol Soc 13:131–132
- Talamas EJ, Buffington M, Hoelmer K (2013) New synonymy of *Trissolcus halyomorphae* Yang. J Hym Res 33:113–117
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol Biol Evol 24:1596–1599
- The Japanese Society of Applied Entomology and Zoology (2006) Major insect and other pests of economic plants in Japan. Japan Plant Protection Association, Tokyo 387 pp
- Toyama M, Mishihiro K (2010) Effect of temperature on the life history of *Trissolcus plautiae* (Hymenoptera: Scelionidae), an egg-parasitoid wasp of the fruit-piercing stink bugs (Heteroptera: Pentatomidae). Bull Natl Ins Fruit Tree Sci 11:17–23 (in Japanese with English summary)
- Tsutsumi T (2004) A method for the control of brown winged green bug, *Plautia crossota stali* by an autodissemination system using an entomopathogenic fungus *Beauveria bassiana* combined with aggregation pheromone. Plant Prot 58:377–380 (in Japanese)
- Watanabe C (1954) Discovery of four new species of Telenominae (Hymenoptera: Proctotrupoidea), egg-parasites of pentatomid and plataspid bugs, in Shikoku, Japan. Tran Shikoku Entomol Soc 4:17–22
- Yamada K, Miyahara M (1979) Studies on the ecology and control of pentatomid bugs attacking fruits. IV Parasitoids of the brown-winged green bug, *Plautia stali* (Hemiptera: Pentatomidae). Proc Assoc Pl Prot Kyushu 25:147–150 (in Japanese)
- Yang ZQ, Yao YX, Qiu LF, Li ZX (2009) A new species of *Trissolcus* (Hymenoptera: Scelionidae) parasitizing eggs of *Halyomorpha halys* (Heteroptera: Pentatomidae) in China with comments on its biology. Ann Entomol Soc Am 102:39–47
- Yara K, Sasawaki T, Kunimi Y (2010) Hybridization between introduced *Torymus sinensis* (Hymenoptera: Torymidae) and indigenous *T. beneficus* (late-spring strain), parasitoids of the Asian chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae). Biol Control 54:14–18