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DATA PAPER

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Two Asian egg parasitoids of *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae) emerge in northern Italy: *Trissolcus mitsukurii* (Ashmead) and *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae)

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Abstract

Halyomorpha halys (Stål) is a severe agricultural pest that is spreading worldwide from its original distribution in Asia. Egg parasitoids from Asia, which play a key role in the population dynamics of *H. halys*, are following its host along global pathways. We present the first records of *Trissolcus mitsukurii* in Europe, and of *Trissolcus japonicus* in Italy. Both discoveries were made in northern Italy, where *H. halys* is widely present and has reached extremely high population densities in some areas. Given the availability of their host, the distributions and populations of these exotic egg parasitoids are expected to expand, even in the absence of human intervention.

Keywords

Egg parasitoid, brown marmorated stink bug, exotic species

Introduction

The invasive stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), also known as the brown marmorated stink bug, is now a cosmopolitan pest. From its native range in East Asia, it first became established in North America in the mid-1990s (Hoebeke and Carter 2003), followed by Europe in the mid-2000s (Wermelinger et al. 2008), and is now established in South America (Faúndez and Rider 2017). It has been intercepted numerous times in New Zealand but has not yet established a permanent population there (Ormsby 2018). *Halyomorpha halys* is a highly polyphagous pest, and severe damages are recorded on forest, urban and agricultural trees, and on the fruits and seeds of crops and horticultural plants (Lee et al. 2013, Leskey and Nielsen 2018). Worldwide, the control of *H. halys* currently relies mainly on pesticides (Kuhar and Kamminga 2017). However, because only broad-spectrum pesticides are effective, beneficial insects are also killed and integrated pest management programs are negatively impacted. Thus, much attention has been given to alternative methods for a long-term solution, including biological control. Among natural enemies of *H. halys*, considerable emphasis has been placed on egg parasitoids, which appear to be the most effective enemies in the native Asian range (Yang et al. 2009, Lee 2015). Several studies explored the ability of parasitoids to attack *H. halys* eggs, both in its native distribution and in the newly invaded areas worldwide (Haye et al. 2015, Talamas et al. 2015, Roversi et al. 2016, Herlihy et al. 2016, Abram et al. 2017, Dieckhoff et al. 2017). Presently, only Asian native species of egg parasitoids appear to be promising candidates as biological control agents, primarily *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) (Zhang et al. 2017).

The spread of *H. halys* has also provided an invasion opportunity for its parasitoids. In 2015 *T. japonicus*, commonly known as the samurai wasp, was detected in the eastern United States (Talamas et al. 2015), followed shortly by discovery of a second, independently established population in the Pacific Northwest (Milnes et al. 2016). In 2017 and again in 2018, *T. japonicus* was recorded in Switzerland, its first reported recovery in Europe (Stahl et al. 2018). We present here the discovery of two adventive populations of exotic parasitoids, *T. mitsukurii* (Ashmead) and *T. japonicus*, that are parasitizing *H. halys* eggs in the wild in northern Italy.

Methods

Field survey and recovery of *Trissolcus mitsukurii* in northeastern Italy

During the 2018 brown marmorated stink bug monitoring campaign in fruit orchards in the Region of Friuli-Venezia-Giulia (northeastern Italy), personnel of ERSA on August 7, and jointly with personnel of CREA on August 8, noted the presence of darkly colored *H. halys* egg masses, which is an indicator of parasitism (see Results for site descriptions). During the surveys, egg masses of other stink bug

species were also collected when found. A portion of the egg masses collected in the field were reared in climatic chambers (26 °C, 65%RH, 16:8 L:D) until adult parasitoids emerged. For further study, adults were kept alive in glass tubes and provided with pure honey droplets as food. The remaining field collected egg masses were reared in a laboratory room in Petri dishes until parasitoids emerged and specimens were stored in ethanol for further studies. All emerged specimens were counted, identified to species and sexed.

Field survey and recovery of *Trissolcus japonicus* in northwestern Italy

During routine research activities of CREA personnel on July 27 and August 3, 2018, several *H. halys* egg masses with dark coloration were observed on *Acer campestris* L. trees in a parking lot near Lodi, a site close to the town of Milan (see Results for site description). All collected egg masses were reared in climatic chambers as described previously, and adult parasitoids were kept alive in rearing tubes with pure honey for further studies. Emerged specimens were counted, identified to species and sexed.

Reared specimens of *Trissolcus* were identified using the key to Palearctic *Trissolcus* provided in Talamas et al. (2017). Image Z-stacks were taken with a Canon EOS 80D camera attached to an Olympus BX51 compound scope and a Macropod imaging system from Macroscopic Solutions and were rendered using Helicon Focus (Helisoft). Voucher specimens are deposited in the Florida State Collection of Arthropods (Gainesville, FL), the USDA-ARS European Biological Control Laboratory (EBCL) (Montpellier, France), and CREA-DC (Florence, Italy). Collection data associated with a subset of these specimens are deposited in the Hymenoptera Online Database (hol.osu.edu). Morphological terminology follows Mikó et al (2007).

Character annotations

ats	postacetabular sulcus (Fig. 7)
cs	clypeal setae (Fig. 9)
eps	episternal foveae (Fig. 7)
hoc	hyperoccipital carina (Figs 3, 6, 8)
lo	lateral ocellus (Figs 3, 6)
lpt1	lateral setal patch on mediotergite of T1 (Fig. 3)
mp	mesopleural pit (Fig. 7)
ms	malar sulcus (Figs 4, 7)
mtps	metapleural sulcus (Fig. 2)
not	notauli (Fig. 8)
of	orbital furrow (Figs 4, 7)
pof	preocellar furrow (Fig. 9)
slt1	setal patch on laterotergite of T1 (Fig. 3)

Molecular analysis

DNA extraction, PCR amplification and sequencing

Following their morphological examination, all specimens were preserved in 95% ethanol and shipped to EBCL. Tables 3, 4 list the specimens and voucher information included in the analysis. Prior to DNA extraction, individual specimens were bathed three times at room temperature in molecular grade water for five minutes. Genomic DNA was nondestructively isolated from the entire specimen using the Qiagen DNeasy kit (Hilden, Germany) as published in Taekul et al. (2014) with minor modification. In Step 7, the elution buffer, warmed to 55 °C, was allowed to sit on the membrane during 15 min before centrifugation. The collected flow-through was reloaded onto the spin column to increase the DNA yield. A negative control (no insect tissue) was included in each extraction to detect potential contamination. The barcode region of the mitochondrial Cytochrome Oxidase Subunit I (*COI*) was amplified using the universal barcoding primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Amplification and barcode editing and analysis were done as described in Ganjisaffar et al. (2018). All sequences generated from this study and those from our custom barcode database are deposited in Genbank (Tables 3 and 4) and all residual DNAs are archived at EBCL (Tables 3 and 4). The sequences obtained were compared with sequences present in Genbank by similarity search using the Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLASTn).

Although tracing the source of the Italian populations was not the scope of the present study, we used this barcode approach to provide better insights into the mitochondrial diversity of these two *Trissolcus* species, identify and exclude Asian populations that were highly divergent from Italian populations, and tentatively find similar populations. Of note, *T. mitsukurii* barcodes are poorly represented in Genbank database and BOLD as there is only one record published by Mita et al. (2014). For this study we had access to an unpublished EBCL database of 12 barcodes of *T. mitsukurii* collected in China, Korea and Japan and obtained within the framework of an ongoing USDA biocontrol program. Two sequences generated from the present study were aligned using Clustal W with the sequence from Mita et al. (2014) and the 12 sequences of our custom database.

For *T. japonicus*, the five sequences generated from this study were aligned with Clustal W with all barcode sequences retrieved from Genbank and BOLD (which contained some sequences not present in Genbank). Only sequences from Asian and Swiss samples were included in the dataset. For both taxa, the phylogenetic relationships among haplotypes were depicted using statistical parsimony in TCS as implemented in PopART (Leigh and Bryant 2015). This approach enabled us to display the geographical distribution of all haplotypes.

Field surveys

Trissolcus mitsukurii recoveries in northeastern Italy

Site descriptions for recovery sites are given in Table 1. A total of 31 *H. halys* egg masses were collected at three survey sites in northeastern Italy on August 7–8, 2018 (Fig. 1, Table 2). Of these, 4 egg masses were not parasitized, and nymphs hatched at a rate of 98.23%; 25 egg masses were parasitized by *T. mitsukurii*; and 2 egg masses were parasitized by *Anastatus bifasciatus* (Geoffroy) (Eupelmidae) and a species of Pteromalidae. From the 25 egg masses parasitized by *T. mitsukurii,* a total of 424 adults emerged of which 91.75% were females. Depending on locality, the parasitism rate of eggs within single egg masses ranged from 32.14% to 100% and number of parasitized egg masses out of the total number of egg masses detected ranged among sites from 50.00% to 84.21%.

During surveys at the Codroipo site, an egg mass of a predatory stink bug belonging to the subfamily of Asopinae was collected which was also parasitized by *T. mitsukurii*. From this egg mass, 83.87% of the eggs (31 eggs in total) were parasitized (emergence rate 96.15%; 80.00% females); only one egg of the cluster produced a stink bug nymph, and four apparently unparasitized eggs did not hatch.

Trissolcus japonicus findings in northwestern Italy

The recovery site of *T. japonicus* in northwestern Italy was located at 45.3031N, 9.4794E (Fig. 1). Parasitized egg masses were found on *Acer campestris* L. trees in a parking lot surrounded by a multi-host patchy landscape (crop fields, uncultivated fields, hedgerows), and industrial and urban areas with ornamental plants.

Table 1. Descriptions of *Trissolcus mitsukurii* recovery sites in northeastern Italy.

Locality	Coordinates	Main culture (plant species and management)	Surrouding cultures	Surrounding environment
Cordenons site 1	46.0089N, 12.6824E	Kiwi orchard (*Actinidia chinensis*), organic farming	Vineyards, maize and soybean crops managed by integrated pest management	Hedgerows, apple and kiwi orchards, vineyards, maize and soybean fields
Cordenons site 2	46.0082N, 12.6713E	Hedgerow (*Robinia pseudoacacia*)	Apple orchard, vineyards, maize and soybean crops managed by integrated pest management	Hedgerows, apple and kiwi orchards, vineyards, maize and soybean fields
Codroipo	45.9675N, 13.0251E	Kiwi orchard (*Actinidia deliciosa*), integrated pest management	Apple and pear orchards, vineyards, maize and soybean crops managed by integrated pest management	Hedgerows, apple and pear orchards, vineyards, maize and soybean fields

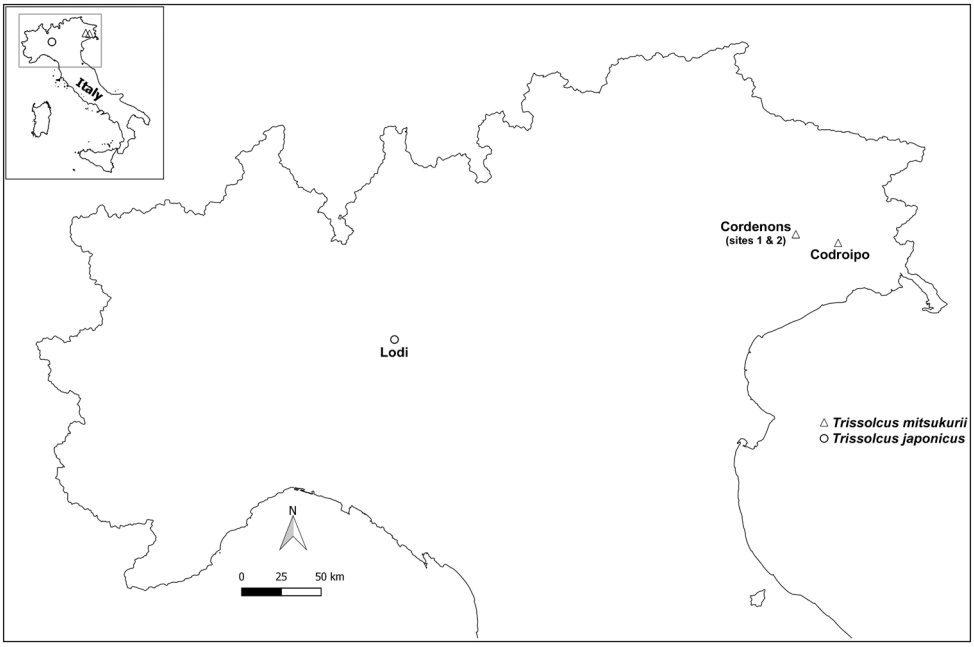


Figure 1. Recovery sites of *Trissolcus mitsukurii* and *Trissolcus japonicus* in northern Italy.

Table 2. Parasitism of *Halyomorpha halys* eggs by *Trissolcus mitsukurii* collected in field surveys of August 7–8, 2018, in northeastern Italy.

Site	n. of parasitized egg masses/total egg masses detected	Mean n. of eggs/egg mass [a]	Parasitized egg masses				Unparasitized egg masses
			Mean % of parasitized eggs/egg mass [a]	Mean emergence rate (%)[a]	Mean sex ratio (% of females) [a]	Mean % of hatched:unhatched eggs/egg mass	Hatching rate (n. of egg masses)
Cordenons site 1	16:19	28.00 (27–29)	87.04 (32.14–100)	94.22 (57.89–100)	92.31 (88.99–100)	0.00:12.74	100% (3)
Cordenons site 2	1:2*	21.50 (15–28)	100	100	92.86	0.00:0.00	not assessed (0)
Codroipo	8:10*	28.00 (27–29)	92.89 (78.57–100)	90.30 (65.38–100)	90.44 (84.21–95.45)	0.00:3.59	100% (1)

* one egg mass was entirely parasitized by other parasitoids. [a] minimum and maximum values in parentheses

Field collections were made on July 27 and August 3, 2018, and a total of 45 *H. halys* egg masses were collected (with a mean of 25.77 eggs/egg mass). On July 27, only one egg mass was detected and was found to be successfully parasitized by *T. japonicus*, with the emergence of 8 specimens, all of which were males. Among the 44 egg masses collected on August 3, 21 egg masses were not parasitized and eggs hatched into nymphs with a mean rate of 82.44%, and 22 egg masses were parasitized by *A. bifasciatus*. Only

Table 3. Sampling Information, GenBank Accession numbers and haplotypes for *Trissolcus mitsukurii* included in this study.

Collection code and Sex	Country	Site	Year of Collection, Name of Collector [b]	Host	GenBank Accession Number	Barcode Haplotype (617bp)	Isolate (EBCL)
FSCA00033071, ♀	Italy	Cordenons, Friuli-Venezia-Giulia	2018, IB, LB & GM	*Halyomorpha halys*	MK097189 (this study)	H5	Tsp270
FSCA00033072, ♀	Italy	Cordenons, Friuli-Venezia-Giulia	2018, IB, LB & GM	*H. halys*	MK097190 (this study)	H5	Tsp269
Tm1-EBCL[a], ♂	Japan	Tsukuba, (NARO)[c]	2007, KH	*H. halys*	MK097191 (this study)	H1	Tm1
USNMENT01197989, ♀	South Korea	Gochang	2015, KH	unidentified host eggs (not *H. halys*)	MK097192 (this study)	H3	Tsp202
USNMENT01197242, ♀	South Korea	Jeju	2012, ET& IM	na	MK097193 (this study)	H4	Tsp233
USNMENT01197243, ♀	South Korea	Jeju	2012, ET& IM	na	MK097194 (this study)	H4	Tsp234
USNMENT01197244, ♀	South Korea	Jeju	2012, ET& IM	na	MK097195 (this study)	H4	Tsp235
USNMENT00977533, ♀	China	Yunnan Prov., Kunming	2013, KH	*Erthesina fullo*	MK097196 (this study)	H2	Tsp39
USNMENT01059335, ♀	China	Yunnan Prov., Kunming	2013, KH	*E. fullo*	MK097197 (this study)	H2	Tsp60
USNMENT01197294, ♀	Japan	Tsukuba, (NARO)[c]	2012, KH	*H. halys*	MK097198 (this study)	H1	Tsp149
USNMENT01197295, ♀	Japan	Tsukuba, (NARO)[c]	2012, KH	*H. halys*	MK097199 (this study)	H1	Tsp150
Tsp151- EBCL[a], na	Japan	Tsukuba, (NARO)[c]	2012, KH	*H. halys*	MK097200 (this study)	H1	Tsp151
Tsp152- EBCL[a], na	Japan	Tsukuba, (NARO)[c]	2012, KH	*H. halys*	MK097201 (this study)	H1	Tsp152
Tsp153- EBCL[a], na	Japan	Tsukuba, (NARO)[c]	2012, KH	*H. halys*	MK097202 (this study)	H1	Tsp153
na, na	Japan	Fukuoka	na, na	*Nezara viridula*	AB971831 (Mita et al. 2014)	H1	

[a] EBCL DNA collection. [b] name of collectors: IB: Iris Bernardinelli, LB: Luca Benvenuto, GM: Giorgio Malossini, KH: Kim Hoelmer, ET: Elijah Talamas, IM: Istvan Mikó. [c] NARO: National Agriculture and Food Research Organization.

one egg mass collected on August 3 was parasitized by *T. japonicus*. All eggs that were apparently parasitized (35.71% of eggs in the mass) produced adult parasitoids (9 females and 2 males), 46.43% of eggs were unhatched and 17.86% of the eggs hatched into stink bug nymphs. Ninety percent of the emerged *T. japonicus* were females.

Taxonomy

Trissolcus mitsukurii

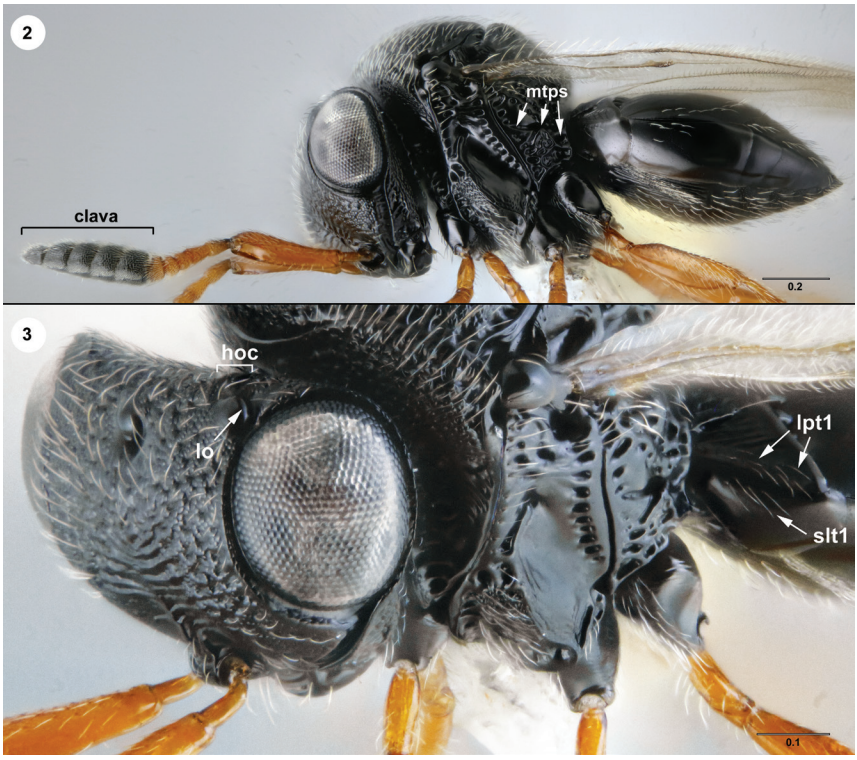
Trissolcus mitsukurii is a straightforward species to identify and is separated early in the key to Palearctic *Trissolcus* (Talamas et al. 2017). We thus do not consider it necessary to produce an updated key to European *Trissolcus* that includes this species, and instead

Table 4. Sampling Information, GenBank Accession numbers and haplotype for *Trissolcus japonicus* included in this study.

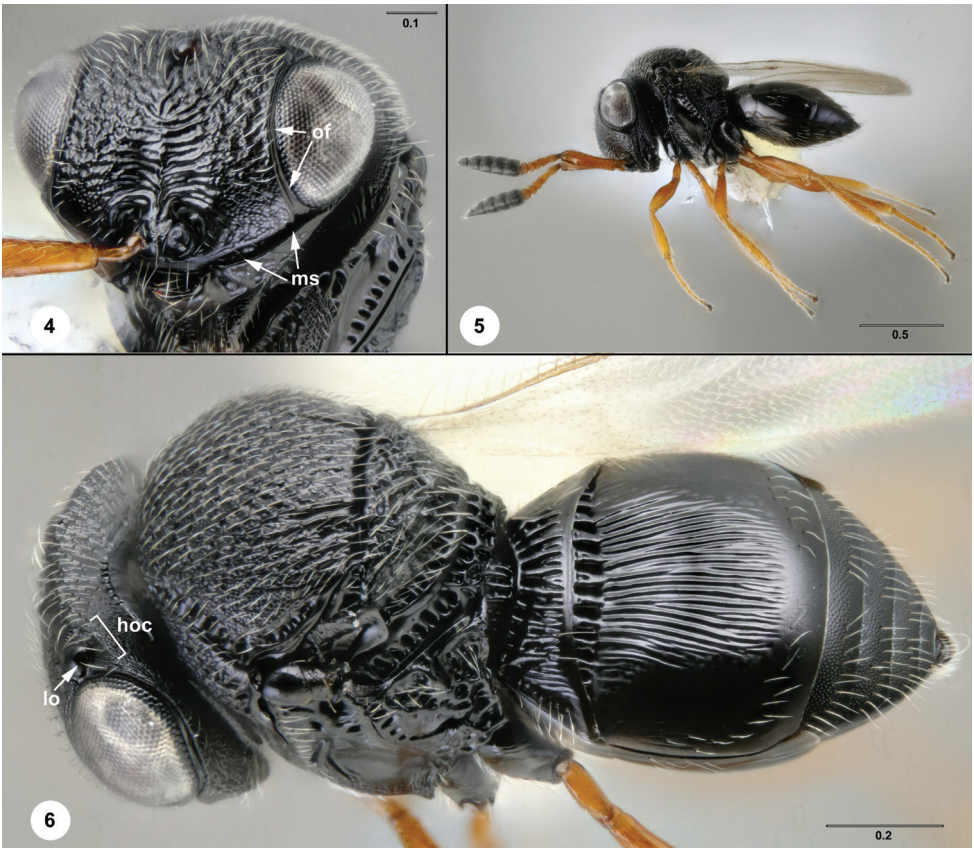
Collection code and Sex	Country	Site	Year of Collection, Name of Collector [b]	Host	GenBank Accession Number/ Bold Accession number	Barcode Haplotype (373bp)	Isolate (EBCL)
FSCA 00033060, ♀	Italy	Lodi, Lombardy	2018, PFR	*Halyomorpha halys*	MK097184 (this study)	H1	Tj406
FSCA 00033065, ♀	Italy	Lodi, Lombardy	2018, PFR	*H. halys*	MK097185 (this study)	H1	Tj408
FSCA0033097, ♂	Italy	Lodi, Lombardy	2018, PFR	*H. halys*	MK097186 (this study)	H1	Tj421
FSCA0033098, ♂	Italy	Lodi, Lombardy	2018, PFR	*H. halys*	MK097187 (this study)	H1	Tj422
FSCA0033096, ♂	Italy	Lodi, Lombardy	2018, PFR	*H. halys*	MK097188 (this study)	H1	Tj423
GBIFCH00543446, ♀	Switzerland	Ticino	2017, JS	*H. halys*	MH919753 (Stahl et al., 2018)	H1	Tj388
GBIFCH00543447, ♀	Switzerland	Ticino	2017, JS	*H. halys*	MH919754 (Stahl et al., 2018)	H1	Tj389
GBIFCH00543448, ♀	Switzerland	Ticino	2017, JS	*H. halys*	MH919755 (Stahl et al., 2018)	H1	Tj390
GBIFCH00543449, ♂	Switzerland	Ticino	2017, JS	*H. halys*	MH919756 (Stahl et al., 2018)	H1	Tj391
GBIFCH00543450, ♂	Switzerland	Ticino	2017, JS	*H. halys*	MH919757 (Stahl et al., 2018)	H1	Tj392
GBIFCH00543451, ♂	Switzerland	Ticino	2017, JS	*H. halys*	MH919758 (Stahl et al., 2018)	H1	Tj393
Tsp77- EBCL[a], na	Japan	Tsukuba (NARO)[c]	2012, KH	*H. halys*	MH919744 (Bon et al., unpublished)	H1	Tsp77
Tsp78- EBCL[a], na	Japan	Tsukuba (NARO)[c]	2012, KH	*H. halys*	MH919745 (Bon et al., unpublished)	H1	Tsp78
Tsp79- EBCL[a], na	Japan	Tsukuba (NARO)[c]	2012, KH	*H. halys*	MH919746 (Bon et al., unpublished)	H1	Tsp79
Tsp88- EBCL[a], na	Japan	Tsukuba (NARO)[c]	2012, KH	*H. halys*	MH919747 (Bon et al., unpublished)	H1	Tsp88
Tsp90-EBCL[a], na	Japan	Tsukuba (NARO)[c]	2012, KH	*H. halys*	MH919748 (Bon et al., unpublished)	H1	Tsp90
Tsp91-EBCL[a], na	Japan	Tsukuba (NARO)[c]	2012, KH	*H. halys*	MH919749 (Bon et al., unpublished)	H1	Tsp91
Tsp93- EBCL[a], na	Japan	Tsukuba (NARO)[c]	2012, KH	*H. halys*	MH919750 (Bon et al., unpublished)	H1	Tsp93
Tsp226-EBCL[a], ♀	Japan	Kanagawa	2015, KH	*Plautia stali*	MH919752 (Bon et al., unpublished)	H1	Tsp226
na, ♀	Japan	Kanagawa	2012, TM	*P. stali*	AB847131-32,36 (Matsuo et al., 2014)	H1	
na, ♀	Japan	Fukuoka	2012, KM	*P. stali*	AB847144-145 (Matsuo et al., 2014)	H2	
na, ♀	Japan	Fukuoka	2012, KM	*H. halys*	AB908179-182 (Matsuo et al., 2014)	H2	
na, na	Japan	na	na, na	na	AB894834-35, AB894838-39 (Matsuo, K. and Hirose,Y., unpublished	H2	
na, ♀	Japan	Fukuoka	2012, KM	*P. stali*	AB847129,130, 137,143,146 (Matsuo et al., 2014)	H3	
na, na	Japan	na	na, na	na	AB894836,837,840,841 (Matsuo, K. & Hirose,Y. (unpublished),	H3	
na, na	Japan	Kanagawa	na, na	*P. stali*	AB971832 (Mita et al., 2014)	H1	
na, na	China			*H. halys*	KF303518.1 (Gariepy et al., 2014)	H7	
USNMENT01059340, ♀	China	Langfang	2012, KH	*E. fullo*	/ NSCEL009-18 (Gariepy unpublished)	H4	Tsp61
USNMENT01197300, ♀	China	Kunming	2014, KH	*E. fullo*	/NSCEL010-18 Gariepy unpublished)	H5	Tsp155
Tsp1-EBCL[a], na	Japan	Tsukuba (NARO)[c]	2012, KH	*H. halys*	/NSCEL011-18	H1	Tsp1

Collection code and Sex	Country	Site	Year of Collection, Name of Collector [b]	Host	GenBank Accession Number/ Bold Accession number	Barcode Haplotype (373bp)	Isolate (EBCL)
USNMENT00977534, ♀	S. Korea	Jirisan Park	2013, KH	*H. halys*	/NSCEL012-18 Gariepy unpublished)	H6	Tsp53
Tj1-EBCL[a], na	China	Hebei	2012, KH	*H. halys*	/NSCEL013-18 Gariepy unpublished)	H7	Tj1
Trj2-EBCL[a], na	China	Hebei	2012, KH	*H. halys*	/NSCEL014-18 Gariepy unpublished)	H7	Trj2
USNMENT01197806, ♀	Japan	Kanagawa	2015, KH	*H. halys*	/NSCEL017-18 Gariepy unpublished)	H1	Tsp223
USNMENT01197320, ♀	South Korea	Seoul	2014, KH	*H. halys*	/NSCEL018-18 Gariepy unpublished)	H7	Tsp175
na, na	China	Hebei	2012, TH	*H. halys*	/PPENT028-12 Gariepy unpublished)	H7	
na, na	China	Hebei	2012, TH	*H. halys*	/PPENT029-12 Gariepy unpublished)	H7	
na, na	China	Hebei	2012, TH	*H. halys*	/PPENT030-12 Gariepy unpublished)	H7	
na, na	China	Hebei	2012, TH	*H. halys*	/PPENT031-12 Gariepy unpublished)	H7	
na, na	China	Hebei	2012, TH	*H. halys*	/PPENT032-12 Gariepy unpublished)	H7	

[a] EBCL DNA collection. [b] name of collector: PFR: Pio Federico Roversi, JS: Judith Stahl, KH: Kim Hoelmer, KM: Kazunori Matsuo, TM: Toshiharu Mita, TH: Tim Haye. [c] NARO: National Agriculture and Food Research Organization.



Figures 2–3. *Trissolcus mitsukurii*, female (FSCA 00033025) **2** head, mesosoma, metasoma, lateral view **3** head, mesosoma, metasoma, anterolateral view. Scale bars in millimeters.



Figures 4–6. *Trissolcus mitsukurii*, female (FSCA 00033025) **4** head, anterior view **5** habitus, lateral view **6** head, mesosoma, metasoma, dorsolateral view. Scale bars in millimeters.

we provide the following diagnosis, with references to illustrations, that will be helpful for distinguishing *T. mitsukurii* from the European fauna: the clava of the female antenna is 5-merous, and the clavomeres are distinctly larger and darker than preceding antennomeres (Fig. 2); the orbital furrow is expanded where it intersects the malar sulcus (Fig. 4); the hyperoccipital carina is often present directly posterior to the lateral ocellus, but is absent between the ocelli (Figs 3, 6); the metapleuron is without setae below the metapleural sulcus (Fig. 3); a setal patch is present on the first laterotergite of the metasoma (slt1, Fig. 3). Care should be taken not to confuse the setal patch of the laterotergite with the setal patch on the mediotergite (lpt1, Fig. 3).

Trissolcus japonicus

The key to European *Trissolcus* in Talamas et al. (2017) included *T. japonicus* to provide identification of this species given the possibility that it might become estab-

Figures 7–10. *Trissolcus japonicus* **7** female (FSCA 00033063), head, mesosoma, metasoma, ventrolateral view **8** female (FSCA 00033063), head, mesosoma, metasoma, dorsolateral view **9** female (FSCA 00033063) head, anterior view **10** male (FSCA 00033095), habitus, lateral view. Scale bars in millimeters.

lished in Europe. The specimens from northern Italy are fully congruent with the concept of this species presented by Talamas et al. (2017). *Trissolcus japonicus* can be separated from other species of European *Trissolcus* by the following diagnosis: four clypeal setae are present below the antennal insertions (Fig. 9); microsculpture is pre-

sent throughout the frons (Fig. 9); the orbital furrow is expanded at its intersection with the malar sulcus (Fig. 7); the hyperoccipital carina is complete (Fig. 8); episternal foveae extend from the postacetabular sulcus to the mesopleural pit (Fig. 7); and the mesoscutum is without oblique rugae between the notauli (Fig. 8). The preocellar furrow, which extends ventrally from the median ocellus (Fig. 9), is a useful character for confirming the identity of *T. japonicus*, but it is not always present, and exhibits the greatest variability in males.

Anastatus bifasciatus

Specimens of *A. bifasciatus* were identified by GSP using the keys of Kalina (1981) and Askew and Nieves-Aldrey (2014) and the identification was confirmed by Dr. Lucian Fusu (University of Iasi, Romania) who compared them with authoritatively identified specimens.

Molecular identification

Trissolcus mitsukurii

The two voucher specimens recovered from the field in Cordenons (site 1) in the region of Friuli-Venezia-Giula yielded a similar barcode sequence of 666-bp in length. A BLAST search showed the best similarity score (99%) of this barcode sequence with *T. mitsukurii* (Accession No. AB971831). From the final alignment of 617-bp of 15 *T. mitsukurii* barcodes, a total of five haplotypes (denoted H1-H5) were recovered (Table 3). The haplotype H5 found in Italy is a new haplotype, as it did not match any haplotype found so far in Asia (Fig. 11, Table 3). From the network analysis, H5 differed by seven substitutions from the two closest haplotypes H1 and H2 from Japan and China respectively (Fig. 11).

Trissolcus japonicus

The five voucher specimens recovered from the field in Lodi yielded a unique barcode sequence of 666-bp in length. A BLAST search showed the best similarity score (100%) of this barcode sequence with *T. japonicus* (Accession No. AB971832). From the final alignment of 373-bp of 60 *T. japonicus* barcodes, a total of seven haplotypes (denoted H1 to H7) were recovered (Table 4). The haplotype H1 found in Italy was also found in Tsukuba and Kanagawa in Japan, and in Switzerland (Fig. 12, Table 4). H1 is also the predominant haplotype found in the specimens collected in Japan (45%). Samples from China were the most diverse, displaying 4 haplotypes (H4, H5, H6, H7), although they represent only 22% of the total sampling. From the network analysis, H7 from China and South Korea differed only by one substitution from the haplotype

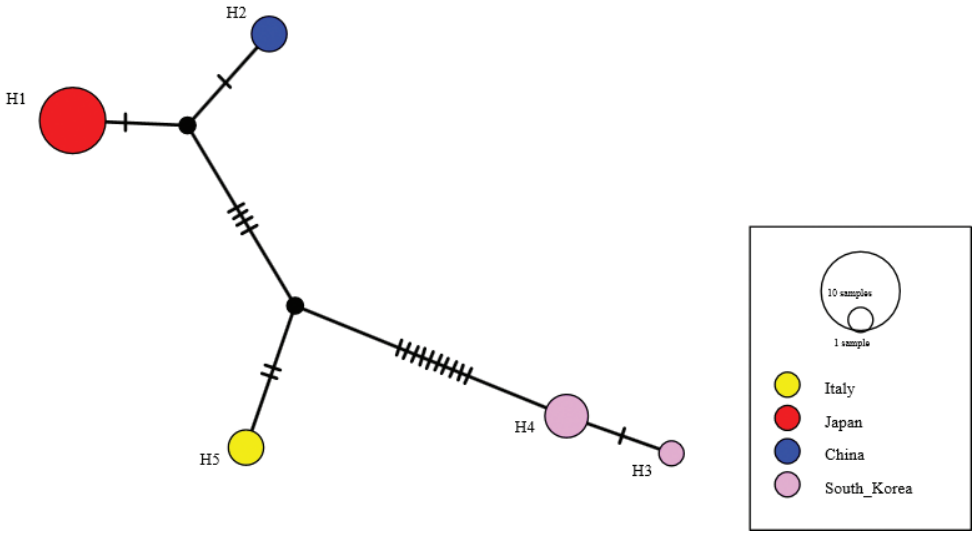


Figure 11. *COI* haplotype network of the *Trissolcus mitsukurii* analyzed in this study. Each circle corresponds to one haplotype; circle size gives the proportion of individuals belonging to the haplotype. The color inside each circle represents the geographical origin. Numbers correspond to the haplotype numbers. Hatch marks symbolize the number of mutations between haplotypes.

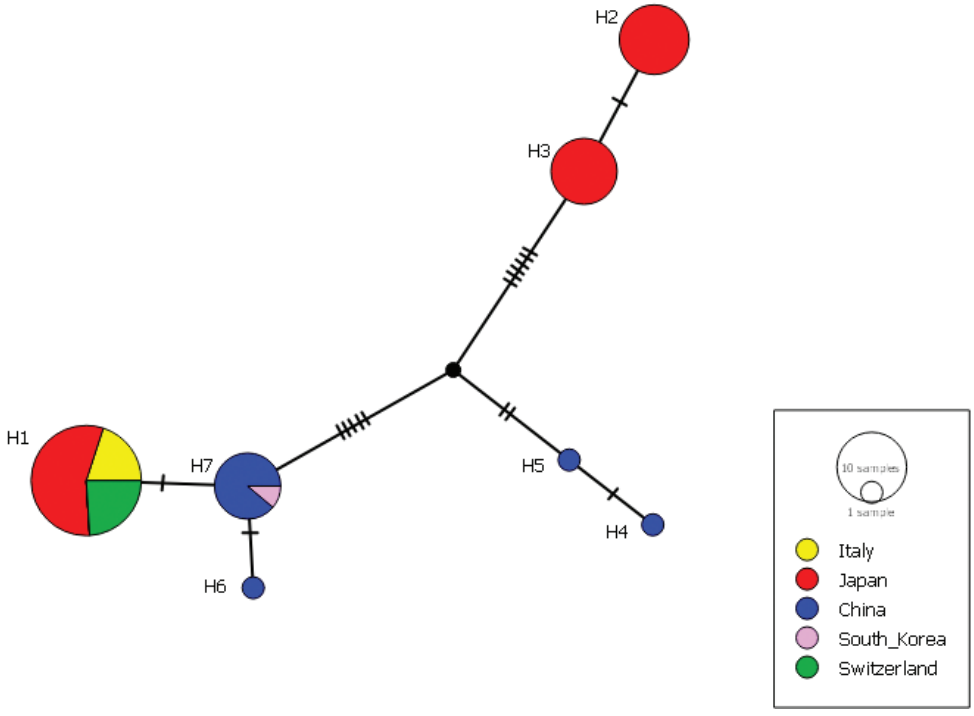


Figure 12. *COI* haplotype network of the *Trissolcus japonicus* analyzed in this study. Each circle corresponds to one haplotype; circle size gives the proportion of individuals belonging to the haplotype. The color inside each circle represents the geographical origin. Numbers correspond to the haplotype numbers. Hatch marks symbolise the number of mutations between haplotypes.

H1 (Fig. 12). Although our haplotype analysis evidenced a best match of the Italian populations to Japanese and Swiss populations so far, we cannot entirely exclude that the haplotype H1 may also be present in unsampled populations of other parts of Asia where *T. japonicus* is present. Tracing the source of an introduction depends on the availability of information about population structure and may require analysis of more than one locus. To this end, a more comprehensive phylogeography study is underway which includes the *COI* barcode and microsatellite loci recently developed de novo in *T. japonicus*.

Discussion

Since the first detection of adventive *T. japonicus* in 2015, additional recoveries in the USA have shown that the adventive populations have established and are spreading. Given that *T. japonicus* occurs throughout the range of *H. halys* in its native range of eastern Asia, one potential outcome of the discovery of *T. japonicus* in Italy is that it will also establish and spread wherever *H. halys* has established in this region. Its recent discovery in the Ticino region of Switzerland (Stahl et al. 2018) lends support to this possibility.

Trissolcus mitsukurii is widespread in Asia, and its distribution extends to the southern limit of eastern Australia (Johnson 1991). Barcode sequences of Australian specimens of *T. mitsukurii* are not yet available and this region must be considered as a possible source of the Italian population. Of note, *T. mitsukurii* was introduced into Hawaii in 1966 as a biological control agent of *Nezara viridula* (L.) but apparently did not become established (Davis and Krauss 1967). This species has otherwise not been reported as an adventive parasitoid outside of its native range, but it is conceivable that its distribution will follow that of *H. halys*, as has occurred with *T. japonicus*. Continued surveys throughout the region will be needed to document their establishment and dispersal, in addition to determining their impact on *H. halys* populations and their interaction with native natural enemies in the region.

The phenomenon of parasitoids following in the footsteps of their invasive hosts has become a growing trend, particularly with species of *Trissolcus* Ashmead. In addition to discoveries of *T. japonicus* and *T. mitsukurii* in the invaded range of *H. halys*, an adventive population of *T. hyalinipennis* Rajmohana & Narendran was found in California, USA, parasitizing eggs of the invasive bagrada bug, *Bagrada hilaris* (Burmeister) (Ganjisaffar et al. 2018). In each of these cases, the discovery was made through the cooperative effort of scientists working in the disciplines of biological control, taxonomy, and biological diversity, highlighting the synergy and necessity of collaboration.

Expansive ranges have recently been documented in platygastroid wasps that are not known to be of agricultural significance (Masner et al. 2009, Oliveira and Schoeninnger 2017, Popovici et al. 2018), but are charismatic and thus more easily recognized. As parasitoids are increasingly examined in the context of the world fauna, we expect to discover many more widespread species.

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