RAPID COMMUNICATION



First discovery of adventive populations of *Trissolcus japonicus* in Europe

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Abstract

The brown marmorated stink bug, *Halyomorpha halys* (Stål), native to East Asia, emerged as an invasive pest in Europe in the 2000s. In its native range, *Trissolcus japonicus* (Ashmead) is the dominant egg parasitoid of *H. halys*, and thus it has been considered for classical biological control in countries invaded by the pest. A survey of native egg parasitoids conducted in 2017 and 2018 with frozen, sentinel egg masses of *H. halys* revealed that *T. japonicus* was already present in apple orchards in the Canton Ticino, Switzerland. *Trissolcus japonicus* was recovered in both years and from three different sites. In total, 17 egg masses were recovered from which 42 adult parasitoids emerged. A genetic analysis using the barcode mitochondrial DNA confirmed the morphological identification of *T. japonicus* and evidenced a best match of the "Ticino populations" to Japanese populations, but the pathways of entry remain unknown.

Keywords Biological control · Egg parasitoids · *Halyomorpha halys* · Scelionidae

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Key message

- Surveys for egg parasitoids of *Halyomorpha halys* were conducted in south-eastern Switzerland.
- For the first time, the Asian parasitoid *T. japonicus* was recovered from sentinel *H. halys* egg masses in Europe.
- Trissolcus japonicus is established in Switzerland and was found in two consecutive years at three different sites.
- Parasitism levels by T. japonicus are currently low, with a maximum of 2%.

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is native to East Asia (China, Japan, and the Korean peninsula) and has emerged as an invasive pest in North America and Europe in the 1990s and 2000s, respectively (Hoebeke and Carter 2003; Haye et al. 2015). It is highly polyphagous, feeding on over 170 plant species in at least 12 families (Rice et al. 2014; Leskey and Nielsen 2018), causing economic losses to a wide variety of crops in invaded areas, and having a particularly devastating



economic impact in tree fruit (e.g. apples, peaches, pears), and hazelnuts in the USA, Italy, and Georgia (United States Apple Association 2010; Maistrello et al. 2017; Bosco et al. 2018). As the application of broad-spectrum insecticides is the most widely used strategy for managing *H. halys* in Europe, more environmentally friendly and self-sustaining control measures, such as classical or augmentative biological control, are urgently needed for an area-wide control of *H. halys*.

Throughout its native range, *H. halys* is heavily attacked by a wide variety of hymenopteran egg parasitoids in the genera *Trissolcus* Ashmead, *Telenomus* Haliday (Scelionidae), *Ooencyrtus* Ashmead (Encyrtidae) and *Anastatus* Motschulsky (Eupelmidae) (reviewed in Lee et al. 2013). Surveys in north-eastern China have shown that the dominant parasitoid of *H. halys* is *Trissolcus japonicus* (Ashmead), with parasitism levels often ranging from 50 to 90% (Qiu et al. 2007; Yang et al. 2009; Zhang et al. 2017) and thus, this species has been considered as a classical biological control agent in invaded areas in North America and Europe.

The native geographic range of *T. japonicus* widely overlaps with that of *H. halys*, including Japan, China, Taiwan, and the Republic of Korea (Qiu et al. 2007; Yang et al. 2009; Zhang et al. 2017). Bioclimatic envelope models predict that *T. japonicus* could establish in *H. halys* infested areas in Europe and North America (Avila and Charles 2018), and in fact adventive populations have already been discovered in the USA [Beltsville, Maryland in 2014 (Talamas et al. 2015); Vancouver, Washington in 2015 (Milnes et al. 2016); Portland, Oregon in 2016 (Hedstrom et al. 2017)] and have since been reported in 10 states (Morrison et al. 2018). The pathway(s) of entry for *T. japonicus* in North America remains unknown, and is presumed to be the same as for the introduction of *H. halys* (Talamas et al. 2015).

In Europe, several native species have been reared from sentinel H. halys egg masses, including Anastatus bifasciatus (Geoffroy) (from both viable and frozen eggs), Trissolcus cultratus (Mayr) (frozen eggs) (Haye et al. 2015; Abram et al. 2017), Ooencyrtus telenomicida (Vassiliev) (frozen eggs) (Roversi et al. 2016), and some Trissolcus and Telenomus spp. (viable eggs) (LT, FT, unpublished data). Most of the native European Trissolcus species have been reported to oviposit in *H. halys* eggs, but their offspring are unable to develop, and thus, the exotic host is considered as evolutionary trap for native scelionid parasitoids (Schlaepfer et al. 2005; Haye et al. 2015). This phenomenon has also been observed in North American scelionids (Abram et al. 2014). Surveys for egg parasitoids in Europe have been restricted to western Switzerland (Haye et al. 2015) and northern Italy (Roversi et al. 2016; Costi 2018) and to date no adventive populations of T. japonicus have been detected. Halyomorpha halys has been introduced several times into Europe,

with evidence of multiple invasions from different source populations (Gariepy et al. 2015; Cesari et al. 2018). As *T. japonicus* may use the same pathways of entry as its host *H. halys*, we considered that this species may eventually arrive in Europe as well. Therefore, the exposure of sentinel *H. halys* egg masses was extended to include the Ticino in south-eastern Switzerland to ensure more complete coverage of the areas where populations of *H. halys* are established.

Materials and methods

Exposure of sentinel eggs

In 2017, a total of 226 H. halys sentinel egg masses were exposed between August and September in a conventionally managed apple orchard in the municipality of Bellinzona, Canton of Ticino, Switzerland (site 1, 46°09'42.1"N 8°58′12.2″E). Sentinel egg masses used for exposure were collected from host plants and mesh parts of cages of the laboratory rearing at CABI in Delémont, Switzerland, without the use of water when they were less than 24 h old. They were immediately frozen at − 80 °C for a maximum of 1 month and thawed no more than 2 days before the exposure. Rearing methods for H. halys have been described in detail in Stahl et al. (2018). All eggs were counted before field exposure, and only egg masses consisting of at least 20 eggs were used for exposure. Egg masses were glued directly on the underside of tree leaves 50 cm to 180 cm above ground, using 'Cementit' (merz + benteli AG, Niederwangen, Switzerland). Leaves of various host plants taken from the *H. halys* laboratory rearing cages were placed next to the egg masses and fixed with twist ties to potentially increase the chance of parasitism by presenting an array of chemical cues. Exposure lengths varied between 4 and 6 days. After field exposure, recovered eggs were counted and predation by chewing and sucking predators was assessed under a Leica routine stereo microscope M50 with a magnification of up to 40× (see Morrison et al. 2016). All egg masses were then kept at 26 °C and monitored for parasitoid emergence for the following 6 weeks. Newly emerged parasitoids were provided with fresh (unfrozen) egg masses to test whether their offspring could complete development. All parasitoids that emerged from egg masses, both exposed in the field and in the laboratory, were stored in 99% ethanol.

In 2018, surveys for parasitoids were extended, and a total of 710 *H. halys* egg masses were exposed between May and August at five sites around Bellinzona, including the same site as in 2017 (site 1) and four additional sites in the same municipality (Table 1). The new sites included two conventionally managed mixed fruit orchards (apple, persimmon, pear, cherry, plum) (site 2, 46°09'34.2"N 8°56'03.3"E, and site 3, 46°09'57.1"N 8°56'37.9"E), one



Table 1 Parasitism and predation of frozen H. halys egg masses exposed at various sites in the Canton Ticino, Switzerland, in 2017 and 2018

Exposure date	Location	Egg masses (eggs) exposed	Egg masses (eggs) recovered ^a	Egg masses (eggs) attacked by chew- ing predators	Egg masses (eggs) attacked by suck- ing predators	Egg masses (eggs) parasitized ^b	% Parasitism (total)	% Parasit- ism by <i>T.</i> <i>japonicus</i>
2017								
3–10 Aug	1	25 (637)	20 (416)	12 (49)	2(2)	1 (16)	3.9	0
10-17 Aug	1	146 (3767)	141 (3004)	95 (546)	6 (18)	17 (77)	2.6	0.2
28 Aug–4 Sep 2018	1	55 (1387)	53 (774)	48 (391)	0 (0)	1 (5)	0.7	0.7
17– 22 May	1	20 (541)	19 (427)	10 (103)	0 (0)	1(1)	0.2	0
	2	20 (543)	15 (320)	2 (8)	0 (0)	0 (0)	0	0
	3	20 (531)	19 (434)	4 (7)	0 (0)	0 (0)	0	0
	4	20 (540)	17 (373)	9 (60)	0 (0)	1 (16)	4.3	0
	5	20 (496)	16 (328)	7 (32)	1(1)	3 (13)	4.0	0
	Total	100 (2651)	86 (1882)	32 (210)	1(1)	5 (30)	1.6	0
20–25 Jun	1	50 (1343)	46 (1127)	11 (42)	0 (0)	1 (4)	0.4	0
	2	20 (526)	11 (172)	8 (80)	0 (0)	0 (0)	0	0
	3	20 (542)	13 (226)	14 (170)	0 (0)	2 (38)	16.8	0
	4	20 (535)	10 (227)	13 (175)	0 (0)	1 (12)	5.3	0
	5	20 (550)	12 (231)	6 (80)	0 (0)	3 (20)	8.7	0
	total	130 (3496)	93 (2018)	52 (547)	0 (0)	7 (74)	3.7	0
19–25 Jul	1	75 (1985)	64 (1555)	12 (46)	6 (36)	0 (0)	0	0
	2	20 (508)	16 (355)	13 (42)	0 (0)	3 (37)	10.4	0
	3	20 (500)	21 (202)	9 (94)	0 (0)	4 (35)	17.3	0
	4	20 (496)	6 (118)	14 (234)	0 (0)	1 (7)	5.9	0
	5	20 (501)	7 (129)	11 (137)	0 (0)	1 (16)	12.4	0
	Total	155 (3990)	104 (2373)	59 (553)	6 (36)	9 (95)	4.0	0
25–30 Jul	1	140 (3717)	137 (3489)	12 (19)	2(2)	7 (38)	1.1	0.06
	6	30 (757)	27 (612)	14 (85)	0 (0)	0 (0)	0	0
	Total	170 (4474)	164 (4101)	26 (104)	2(2)	7 (38)	0.9	0.06
30 Jul–4 Aug	6	98 (2507)	85 (1797)	38 (246)	0 (0)	12 (27)	1.5	0.2
08–13 Aug	1	105 (2697)	95 (2125)	48 (292)	3 (4)	2 (21)	1.0	0
13–17 Aug	2	20 (516)	18 (383)	16 (107)	0 (0)	0 (0)	0	0
	3	20 (537)	17 (335)	11 (161)	0 (0)	0 (0)	0	0
	4	20 (517)	16 (290)	12 (152)	1 (10)	1 (6)	2.1	2.1
	5	20 (541)	10 (240)	7 (115)	0 (0)	0 (0)	0	0
	6	63 (1677)	57 (1356)	28 (146)	5 (18)	11 (22)	1.6	1.3
	Total	185 (4808)	118 (2604)	74 (681)	6 (28)	11 (22)	0.8	0.5

^aEgg masses that contained at least one egg that had not been attacked by either chewing or sucking predators after recollection

conventional orchard with apple and pear trees (site 5, 46°13′12.4″N 9°03′11.4″E), and one private garden with peach trees (site 4, 46°09′30.0″N 8°59′21.0″E). Once per month, 15–135 frozen egg masses were exposed at every site on apple or peach trees and five additional egg masses were exposed in the closest available natural site nearby (shrubs, hedges, etc.). Additionally, egg masses (in total 191) were exposed three times in an organic apple orchard located near the Italian border in

the municipality of Manno, Canton of Ticino, Switzerland (site 6, 46°01′52.8″N 8°55′20.4″E) (Table 1).

Morphological identification of parasitoids

Ethanol-stored specimens were dried and glued on cardpoints for morphological analyses. A Leitz large-field stereo microscope TS with magnification up to 160 × and a spot light Leica CLS 150X were used for morphological diagnosis. For Scelionidae, *Telenomus* species were determined



^bParasitism measured by parasitoid offspring emergence of recovered eggs

using the keys of Kozlov and Kononova (1983) and Johnson (1984), and *Trissolcus* species were identified using the keys by Talamas et al. (2017). Moreover, *Trissolcus* specimens were compared with pictures of holotype and paratypes in Hymenoptera Online (HOL) and in Talamas et al. (2017). *Anastatus* individuals were identified using the key by Askew and Nieves-Aldrey (2004). All the specimens used for morphological analysis were deposited in the Natural History Museum of Bern, Switzerland (NMBE) and Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA) (ESM1).

Molecular confirmation

Morphological identification of *T. japonicus* was confirmed molecularly at the DISAFA as a routine procedure. In total, two males and three females of *T. japonicus* from the Ticino and, as a positive control, one female from the CABI colony (China) were processed (ESM1). Genomic DNA was extracted according to Gariepy et al. (2014), and the barcode region of the cytochrome oxidase I (COI) gene was amplified using universal PCR primers for insects [LCO-1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO-2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994)]. The PCR was performed in a 50 µl reaction volume: 2 µl of DNA, 37.9 µl molecular grade water, 5 µl 10X Qiagen PCR buffer, 3 µl dNTPs (25 mM each), 1.5 μl MgCl₂, 0.2 μl of each primer (0.3 μM each), 0.2 µl Taq DNA Polymerase (Qiagen, Hilden, Germany). Thermocycling conditions were optimized to shorten reaction times and included initial denaturation at 94 °C for 300 s, followed by 35 cycles of 94 °C for 30 s, annealing at 52 °C for 45 s and extension at 72 °C for 60 s; then further 600 s at 72 °C for final extension. All PCR products were purified using a commercial kit (QIAquick PCR Purification Kit, Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions and sent for sequencing in one direction using the forward primer to an external service (Genechron S.r.l., Rome, Italy).

The same sequence of 612 bp in length was obtained in all samples and compared with sequences present in the GenBank database by similarity search using the Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLASTn) confirming the taxonomy of all morphologically identified specimens. All residual DNA is archived at DISAFA.

Genetic matching with Asian populations of *Trissolcus japonicus*

We used the barcode approach to identify and exclude Asian T. japonicus populations that were highly divergent from the "Ticino population." Mounted specimens (three males and three females) recovered from three parasitized egg masses from Ticino were shipped to EBCL along with 10 females from the laboratory colony kept in the quarantine at CABI Switzerland, which originated from the Beijing Province, China (ESM1). Genomic DNA from all specimens was extracted according to Gariepy et al. (2014), except those from mounted specimens which were non-destructively isolated as described in Giantsis et al. (2015). Amplification of all barcode sequences and their analysis were done as described in Ganjisaffar et al. (2018). All sequences of 674 bp in length generated from this study are deposited in the GenBank (Table 2), and all residual DNA extracts are archived at EBCL (Table 2). Of note, when this study was initiated, only 24 barcodes were deposited in the GenBank database. Twenty-three of the 24 are from Japan and most are published by Matsuo et al. (2014). For this study, we had access to a not yet published EBCL database of 127 barcodes of T. japonicus mostly from laboratory colonies derived from natural populations collected in China, Korea, Japan and USA (EBCL custom database). Searches for sequence similarity against this database confirmed the close similarity of the "Ticino sequence" with those of Japanese origin. Therefore, the 26 sequences generated from this study were aligned with 10 "Japanese T. japonicus" sequences from this custom database and 22 sequences retrieved from GenBank following the procedure described in Ganjisaffar et al. (2018). Two sequences from GenBank were not considered in our data set as the sequences were not of adequate length. The final alignment of 58 barcode sequences of 423 bp in length revealed a total of six haplotypes. The phylogenetic relationships among these haplotypes were depicted using statistical parsimony in TCS as implemented in PopART (Leigh and Bryant 2015). This approach also enabled us to display the geographical distribution of all haplotypes analysed.

Results

Exposure of sentinel eggs

Between August and September 2017 and from May to August 2018, more than 48,000 sentinel frozen *H. halys* eggs were exposed at six sites in the Canton Ticino to retrieve native egg parasitoids (Table 1). Overall



Table 2 Sampling information, GenBank Accession Numbers and haplotypes for the T. japonicus included in this study (*)

Collection code and sex	Country	Region	Year of collection, name of collector ^b	Host	GenBank Accession Number	Barcode haplo- type
GBIFCH 00543446, ♀	Switzerland	Ticino	2017, JS	Halyomorpha halys	MH919753*	H1
GBIFCH 00543447, ♀	Switzerland	Ticino	2017, JS	H. halys	MH919754*	H1
GBIFCH 00543448, ♀	Switzerland	Ticino	2017, JS	H. halys	MH919755*	H1
GBIFCH 00543449, ♂	Switzerland	Ticino	2017, JS	H. halys	MH919756*	H1
GBIFCH 00543450, ♂	Switzerland	Ticino	2017, JS	H. halys	MH919757*	H1
GBIFCH 00543451, ♂	Switzerland	Ticino	2017, JS	H. halys	MH919758*	H1
CABI colony, $\c (n=10)$	China	Beijing	2018, TH	H. halys	MH919759*	H2
Tsp1 EBCL ^a , na ^c	Japan	Tsukuba	2012, KH	na	MH919743	H1
Tsp77, EBCL ^a na	Japan	Tsukuba	2012, KH	na	MH919744	Н3
Tsp78, EBCL ^a na	Japan	Tsukuba	2012, KH	na	MH919745	Н3
Tsp79, EBCL ^a na	Japan	Tsukuba	2012, KH	na	MH919746	Н3
Tsp88, EBCL ^a na	Japan	Tsukuba	2012, KH	na	MH919747	Н3
Tsp90, EBCL ^a na	Japan	Tsukuba	2012, KH	na	MH919748	Н3
Tsp91, EBCL ^a na	Japan	Tsukuba	2012, KH	na	MH919749	Н3
Tsp93, EBCL ^a na	Japan	Tsukuba	2012, KH	na	MH919750	Н3
Tsp223, EBCL ^a ♀	Japan	Kanagawa	2015, KH	H. halys	MH919751	Н3
Tsp226 EBCL ^a ,♀	Japan	Kanagawa	2015, KH	Plautia stali	MH919752	Н3
<i>na</i> , ♀	Japan	Kanagawa	2012, TM	P. stali	AB847131-32,36 ^d	H4
<i>na</i> , ♀	Japan	Fukuoka	2012, KM	P. stali	AB847144-145 ^d	H5
na,♀	Japan	Fukuoka	2012, KM	H. halys	AB908179-182 ^d	H5
na, na	Japan	na	na	na	AB894834-35, AB894838-39 ^e	H5
<i>na</i> , ♀	Japan	Fukuoka	2012, KM	P. stali	AB847129,130, 137,143,146 ^d	Н6
na, na	Japan	na	na	na	AB894836,837,840,841 ^e	Н6

^aEBCL DNA collection

parasitism was highly variable among sites and exposure dates, ranging from 0 to 17.3% (Table 1). Predation by sucking or chewing predators ranged from 0 to 2.2% and from 1.6 to 66.5%, respectively (Table 1).

Morphological identification of parasitoids

Based on morphological analyses, *Trissolcus* individuals were identified as *T. cultratus*, *T. semistriatus* (Nees van Esenbeck) [syn. *Trissolcus grandis* (Thomson); Talamas et al. 2017], and the exotic *T. japonicus* (Fig. 1). In particular, *T. japonicus* was identified according to the following characters: vertex with hyperoccipital carina uniform and robust; area between hyperoccipital carena and medial ocellus coriaceous; longitudinal groove below preocellar pit; clypeus with 4 setae; orbital furrow expanded with medial margin well defined at intersection with malar sulcus; genal carina absent; mesoscutum with distinct notauli and

without median mesoscutal carina; median lobe of mesoscutum without oblique rugulae; mesopleuron with episternal foveae forming a continuous line of cells from dorsal apex of postacetabular sulcus to mesopleural pit; laterotergite I without setae; T1 without sublateral setae; T2 with striae present throughout anterior half of tergite (Talamas et al. 2017). Other parasitoids were identified as *A. bifasciatus* and *Telenomus turesis* Walker (Fig. 1). In total, 12 *T. japonicus* adults were reared from three egg masses exposed at two dates in August 2017 in an apple orchard near Bellinzona, Canton of Ticino, Switzerland (site 1). In 2018, 17 individuals were reared from 7 egg masses at three different sites (site 1, 4, 6) (Table 1).

Molecular confirmation

The morphological identification of *T. japonicus* was confirmed by the molecular analysis performed by DISAFA. A



^bName of collectors: KH, Kim Hoelmer (USDA-ARS), TM, Toshiharu Mita, KM, Kazunori Matsuo, JS, Judith Stahl (CABI), TH, Tim Haye (CABI)

^cNot available

^dMatsuo et al. (2014)

^eMatsuo and Hirose unpublished

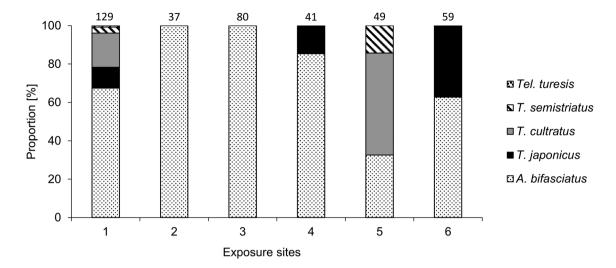


Fig. 1 Species composition of parasitoids reared from sentinel egg masses of *Halyomorpha halys* exposed at six sites in the Canton Ticino, Switzerland, in 2017 and 2018. Numbers of exposure sites

correspond with locations listed in Table 1; total number of emerged parasitoids per site is displayed above bars

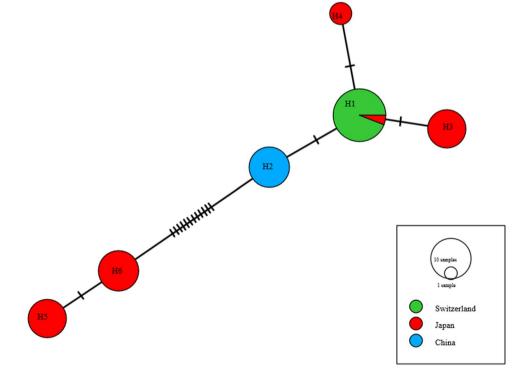
BLAST search showed the best similarity score (100%) of our barcode sequence (612 bp in length) with *T. japonicus* (Accession No. AB971832).

Genetic matching with Asian populations of *Trissolcus japonicus*

The six analysed specimens recovered from the field yielded a single haplotype, H1. This haplotype was also

found in Tsukuba in Japan in 2012 (Fig. 2, Table 2) and is distant by one mutation from the two haplotypes (H3 and H4) uncovered in Tsukuba and in Kanagawa in Japan (Fig. 2). The 10 specimens from the CABI colony yielded a single haplotype (H2) which is one mutation away from H1, indicating that the "Ticino populations" of *T. japonicus* did not escape accidentally from the CABI colony.

Fig. 2 Barcode haplotype network of the 58 *T. japonicus* analysed in this study. Each circle corresponds to one haplotype; circle size gives the proportion of individuals belonging to the haplotype. The colour inside each circle represents the geographical origin. Numbers correspond to the haplotype numbers. Hatch marks symbolize the number of mutations between haplotypes. The H2 haplotype from Beijing, China, represents the CABI colony





Discussion

Parasitism and predation of sentinel *H. halys* eggs by native European egg parasitoids and predators in southwestern Switzerland were generally low, which is consistent with previous studies in Europe and North America (e.g. Jones et al. 2014; Haye et al. 2015; Abram et al. 2017; Dieckhoff et al. 2017). Parasitoids recorded in the present study included two species, *T. cultratus* and *A. bifasciatus*, which have been recorded in earlier surveys in western and northern Switzerland (Haye et al. 2015), whereas *T. semistriatus*, *Tel. turesis*, and the exotic *T. japonicus* have not been previously reported from *H. halys* in Europe. To our best knowledge, this is also the first record of *T. japonicus* parasitizing *H. halys* eggs in apple orchards.

Recently, an increasing number of unintended introductions of biological control agents have been recorded (Servick 2018); however, it remains difficult to trace the origin of such introductions. Presumably, the majority are accidentally transported from their native range along with the pest. The actual pathways of entry for T. japonicus are unknown, but it is likely that either plants carrying parasitized egg masses of Asian Pentatomidae (H. halys or other hosts, see Zhang et al. 2017) or diapausing adults were introduced. As a result of population size bottlenecks and genetic drift in small founding populations as likely experienced by the T. japonicus "Ticino population," the level of haplotype diversity is reduced to a unique haplotype. Our haplotype analysis found a best match of the "Ticino populations" to Japanese populations so far, possibly indicating an introduction from Japan. Although our custom database is quite representative of the geographic distribution of T. japonicus, we cannot entirely exclude that the haplotype H1 may also be present in other parts of T. japonicus' native range, e.g. China or Korea, but has thus far remained unsampled. Tracing the source of introduction necessitates obtaining meaningful population structure information (Gariepy et al. 2015), requiring sometimes to use more than one locus. To add better resolution, a comprehensive phylogeographic study with the barcode is currently being undertaken, as well as with the microsatellite loci recently developed de novo for T. japonicus. Whether Switzerland was the actual country of introduction or whether T. japonicus was accidentally introduced into the climatically highly suitable northern Italy (Avila and Charles 2018) and is now spreading northwards into Switzerland remains unclear. However, considering that in 2016 Italy imported goods of more than twice the value from the region of China, Japan and the Republic of Korea than Switzerland (World Bank 2018), an introduction into Italy seems more likely, but future surveys in both regions may help to clarify the invasion pathways.

Field and laboratory studies in China and Japan showed that the host range of T. japonicus is not restricted to H. halys (Ryu and Hirashima 1984; Matsuo et al. 2016; Yang et al. 2009; Zhang et al. 2017), and fundamental host range studies conducted in Europe suggest that some native European Pentatomidae are suitable hosts for development (TH, LT, unpublished data). Accordingly, T. japonicus has the potential to directly impact native non-target stink bug species. However, the extent to which attacks may result in significant reductions in native stink bug populations will depend on various factors, such as habitat overlap, competition with native egg parasitoids, size and suitability of non-target host species. The adventive establishment of T. japonicus in Switzerland provides the opportunity to study the establishment and spread of this species in Europe, as well as to assess potential risks to native biodiversity under natural conditions. In particular, it will enable us to determine whether results from host range studies in Asia (Zhang et al. 2017) are able to predict the impact of this parasitoid outside of its native range.

It is too early to evaluate the impact of adventive T. japonicus populations on invasive H. halvs populations in Switzerland as it has likely only recently arrived. However, based on the very high parasitism levels of H. halys eggs observed in Asia, its establishment may have the potential to reduce invasive stink bug densities below economic thresholds. Current T. japonicus populations are likely very low, which may explain why in both years it was only recovered in the second half of summer. In China, T. japonicus parasitizes H. halys eggs over the course of the entire season, from May to September, but parasitism is usually highest in August (Zhang et al. 2017). Continued studies exposing sentinel eggs and collecting natural egg masses will be necessary to determine the current distribution and spread of T. japonicus and to evaluate how egg mortality may affect populations of *H. halys* and non-target pentatomids in the near future.

Authors' contribution

JS, CM and TH conceived and designed research. LT, FT, MP and MCB identified the parasitoids. KH provided parasitoids from Asia for molecular analysis. All authors contributed to writing the manuscript and approved the final version.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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