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First detection of *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) in southwestern France

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Abstract

We report the first detection of *Trissolcus japonicus* in southwestern France. A total of almost 3000 sentinel and 700 field-laid egg masses of *Halyomorpha halys* were exposed or collected in the administrative region of Nouvelle-Aquitaine in 2022 and 2023. A total of 12 and 44 specimens of *T. japonicus* emerged from one and two egg masses in 2022 and 2023, respectively. Morphologic analysis confirmed the identification of this egg parasitoid, while molecular analysis of COI matched the haplotype of the French population to the population of *T. japonicus* released in Italy. *Trissolcus japonicus* may have arrived in Nouvelle-Aquitaine from introduction pathways similary to *T. mitsukurii*, detected in 2020, although these pathways cannot be clearly defined. The co-occurrence of these two major parasitoids of BMSB should now be surveyed as possible competition can arise. In the frame of the biological control program currently running in France against BMSB, the use of one or two parasitoid species is also considered.

Keywords

Biological control, brown marmorated stink bug, DNA barcoding, exotic species

Introduction

Herbivorous stink bugs are a good example of increasing economic loss by invasive insects; many of these species are agricultural pests in their native and invasive range (McPherson 2018; Conti et al. 2021). The Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is one of the main invasive stink bug species in recent decades. Native to eastern Asia, BMSB arrived in North America in the mid-1990's (Leskey and Nielsen 2018) and in Europe in the 2000's where it spread quickly in Switzerland, Georgia, Italy and, more recently, France (Bosco et al. 2018; Kereselidze et al. 2022). Like many invasive Pentatomidae species, BMSB is highly polyphagous, feeding on more than one hundred host plants (Lee et al. 2013; Bergmann et al. 2016) from wild and ornamental to cultivated species, including peach, peer, apple, grape and hazelnut. Thus, it can incur considerable damages on a wide range of agricultural systems, like in northern Italy (Maistrello et al. 2017; Bosco et al. 2018) where outbreaks in 2019 caused more than € 356 million damage on pear, peach, and nectarine productions, with up to 80–100% yield losses (Centro Servizi Ortofrutticoli 2019). In hazelnut orchards, the absence of insecticide treatment can lead to 23–40% of damaged hazelnuts (de Benedetta et al. 2023). In France, the presence of BMSB is increasing since first identified in 2012, and this species represents an important risk for many French crops, according to the French Agency for Food, Environmental, and Occupational Health and Safety (Haye et al. 2014). In the case of hazelnut orchards, average bug damage increased continuously from 0.2% in 2015 to 2% in 2018, with peaks that reached 14% and up to 30% in 2022 (unpublished data, Unicoque).To control BMSB, the use of broad-spectrum insecticides can no longer be considered due to the ecological and sanitary risks, the growing societal demand that results in the progressive suppression of authorized chemicals, and the possibility of resistance in BMSB populations, as has happened in other pentatomid species (Sosa-Gomez et al. 2001). Classical biological control (CBC) appears as a possible alternative strategy to the use of insecticides. CBC aims at restoring ecosystem balance by introducing exotic natural enemies to better control an invasive pest, which often lacks efficient natural enemies in its invasive range. In Europe for instance, BMSB can be parasitized by parasitoid wasps in the genera *Trissolcus* Ashmead, *Telenomus* Haliday, *Ooencyrtus* Ashmead or *Anastatus* Motchoulsky, but parasitism often leads to egg abortion, incomplete development of parasitoids or low parasitism rates (Haye et al. 2016; Roversi et al. 2016). Among the natural enemies of the BMSB in its native range, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) is considered as one of the most promising candidates for CBC (Yang et al. 2009; Zhong et al. 2017). Numerous studies have been conducted to assess the parasitoid's efficiency and biosafety as part of the requirements for its legal introduction in the USA (Hedstrom et al. 2017), Canada (Abram et al. 2019), New Zealand (Charles et al. 2019), and Europe (Haye et al. 2020). *Trissolcus mitsukurii* (Ashmead) is also known as a natural enemy of the BMSB in Japan

(Arakawa and Namura 2002) and is considered as a valid candidate for its control in Italy together with *T. japonicus* (Sabbatini Pevereri et al. 2020). Since *T. mitsukurii* is absent in North America, Europe (especially Italy) has been the only known place where the control of BMSB by both parasitoid species could be studied (Moraglio et al. 2020).

Legislative restrictions in the USA and Europe may be a limiting factor for the introduction of exotic species by focusing on perceived risks for non-targets organisms rather than the benefits of pest reduction (van Lenteren et al. 2006; Rondoni et al. 2021). However, unintentional introduction of exotic natural enemies is likely to happen more often than expected (Mason et al. 2017). Parasitoids often move along the same routes as their hosts, and this phenomenon can lead to unintentional biological control as defined by Beers et al. (2022) and has been documented several times, for instance in the USA, Germany and Italy: *Leptopilina japonica* Novković and Kimura (Hymenoptera: Figitidae) against *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) (Puppato et al. 2020; Beers et al. 2022; Martin et al. 2023); as well as Scelionidae species, *Paratelenomus saccharalis* (Hym. Platygastridae) (Dodd) (Gardner et al. 2013) against *Megacopta cribraria* (F.) (Hem. Plataspidae), with *Trissolcus hyalinipennis* Rajmohana and Narendran and *Gryon aetherium* Talamas (Ganjisaffar et al. 2018; Hogg et al. 2021) against *Bagrada hilaris* (Burmeister) (Hem. Pentatomidae). Fortuitious populations of *T. japonicus* were found in the USA (Talamas et al. 2015; Milnes et al. 2019), Canada (Abram et al. 2019), Switzerland, Italy (Sabbatini Peverieri et al. 2018; Stahl et al. 2019; Moraglio et al. 2020), Germany (Dieckhoff et al. 2021) and Slovenia (Rot et al. 2021), while *T. mitsukurii* was detected in Italy (Sabbatini Peverieri et al. 2018), France (Bout et al. 2021) and Serbia (Konjević et al. 2024).

The discovery of adventive populations of *T. japonicus* in the USA and Italy probably led to lower constraints for releases (Talamas et al. 2015). In France, the discovery of an adventive population of *T. mitsukurii* (Bout et al. 2021) led to the obtainment of a release permit that opened the way to a biological control program against BMSB in 2022. The detection of *T. japonicus* in France should help in the same way and represents a unique opportunity to study possible interactions of both parasitoid species. Despite surveys conducted in southeastern and southwestern France since 2017 and multiple releases in northern Italy since 2020, *T. japonicus* has not been detected yet in France. Meanwhile, the geographical proximity with the Piedmont region (Italy) where *T. japonicus* is released and the important commercial and human connection between northern Italy and southeastern France could lead to unintentional dispersion of this parasitoid. This dispersion is also expected to reach southwestern France where an adventive population of *T. mitsukurii* already established, likely from the Italian population (Bout et al. 2021). In the region Nouvelle-Aquitaine (France), where the current study took place, releases of *T. mitsukurii* were initiated in 2022 in the frame of the project RIPPOSTE and field monitoring was conducted with a focus on *T. mitsukurii* and *T. japonicus*.

Materials and methods

Field surveys

Exposure of BMSB sentinel egg masses and collection of field-laid egg masses of Pentatomidae and Coreidae were used to assess the diversity of egg parasitoids in France. The field surveys took place in 2022 and 2023 and covered 11 sampling sites in Nouvelle-Aquitaine (southwestern France) in three departments: Lot-et-Garonne, Dordogne and Gironde. In this sampling area, opportunistic collection of egg masses from *H. halys* and other pentatomid species on localities that are not part of the 11 chosen sites were also included in the samples.

Laboratory colony of the BMSB

The monitoring of egg parasitoids of BMSB requires the use of sentinel egg masses that can be produced in laboratory conditions. To obtain these egg masses, a laboratory colony of BMSB was constituted from adult individuals collected in Cancon (France) during fall 2021 using pheromone traps (Trece®) and transferred to laboratory conditions (T: 25 ± 2 °C, RH: 50 ± 10%, L:D: 16:8 h). They were reared in nylon net cages (47.5 × 47.5 × 47.5 cm, Bugdorm®, MegaView Science Co., Ltd., Taichung, Taiwan) containing fresh beans and apples, complemented with fresh maize and hazelnuts. Egg masses were collected daily and stored under the same laboratory conditions until hatching of the first instars. These were transferred to new cages so that each cage was homogeneous in developmental stage.

Field parasitoid monitoring

Sentinel egg masses were obtained from fresh BMSB egg masses (< 24 h) collected from the laboratory colony and were directly exposed in the field or stored in a fridge (c.a. 8 °C) for up to 2 days. Frozen eggs were not used as they can be less detectable or acceptable for egg parasitoids and lead to sampling bias (Jones et al. 2014). Each egg mass was glued on a cardboard piece (1 × 3 cm) and stapled to the under- side of the leaves of a wide variety of host plants, mostly woody trees (e.g., *Prunus* spp., *Acer* spp., *Catalpa bigninoides* Walt., *Paulownia tomentosa* (Thunb.), and *Corylus avellana* L.). Sentinel egg masses were removed from the field three days after deployment. They were exposed in the field every two weeks on each monitored site from June 10th to September 16th in 2022 and every week from April 28th to September 15th in 2023. Field-laid egg masses from BMSB and other heteropteran species (Pentatomidae, Coreidae) were collected in the sampling area in N-A from May 18th to September 16th, 2022, and from May 10th to September 8th, 2023. During the surveys, egg masses of stink bug species were visually located on various host plants and collected by hand. All egg masses, sentinel and field-laid, were kept in the laboratory under controlled conditions (22 ± 1 °C; RH 50 ± 5%; L:D: 16:8 h) until the emergence of bug nymphs or adult parasitoids. Egg masses were individually stored in Petri dishes and checked daily for emergence since the first observation of external indication of parasitoid development (grey or black coloration of the egg). Emerged parasitoids were stored in 70% ethanol while non-emerged eggs were dissected under a stereomicroscope (Nikon SMZ 1270) to determine whether they were parasitized or not (presence of an identifiable larvae, nymph, or adult parasitoid). When the egg content was not identifiable, the egg was considered as non-parasitized.

Molecular analysis

DNA-barcoding characterization consisted in a double identification based on the Cytochrome Oxydase I (COI) amplification and sequencing, and morphological identification of the corresponding voucher (exoskeleton of the specimen). Molecular analyses were performed as describe in Bout et al. (2021) for *T. mitsukurii* identification. Extraction of DNA was performed using the DNA kit extraction MA150E – QuickExtract – DNA, following company specifications. This non-destructive method allowed the vouchers to remain intact for morphological identifications. PCR amplifications were performed on a portion of the Cytochrome Oxydase I subunit (COI) locus using the LCO-HCO primer: HCO2198 (5'-TAAA CTT CAG GGT GAC CAA AAA ATC A-3'), LCO1490 (5'- GGTC AAC AAA TCA TAA AGA TAT TGG-3') (Folmer et al. 1994), allowing amplification of an approximately 600–700 bp portion of DNA on this locus. The product was sent to Genewiz (Leipzig, Germany) for a double, single read sequencing with the HCO2198 primer. All residual DNAs are archived at INRAE Sophia-Antipolis (France). Correction, annotation and alignment were performed manually using BioEdit Geneious R10 software. The comparison of nucleotide sequences with sequences available in the NCBI database (GenBank) was performed using Blastn (Altschul et al. 1990) with standard settings. From GenBank (NCBI), all COI sequences (72) clearly identified as *T. japonicus* species were downloaded. Two sequences from CREA-Italy were added to the study and compiled with the ten sequences obtained from French population of *T. japonicus* collected in 2022 (7 sequences) and 2023 (3 sequences) (Table 2), resulting in a final table of 84 sequences (Suppl. material 2). Analysis of sequences data was done with the MegaX software (Tamura et al. 2013), using the neighbor joining (NJ) method (Saitou et al. 1987), with bootstrap values based on 500 replications. Nucleotide distances in NJ trees were estimated by the Kimura's two-parameters method (Kimura, 1980). Some sequences were significantly shorter than the others. Hence, analyses exclude the 22 shorter sequences and work only on the common part of the 62 other sequences (fragment of 575 pb). A fast alignment file was used with DnaSP6 to create all haplotypes of *T. japonicus* sequences. The resulting nexus file was analyzed with PopArt software (Otago University, Dunedin, New Zeland) to organize haplotype networks.

Morphological identification

All parasitoid wasps emerged were morphologically identified using a Wild M5 stereomicroscope with the appropriate taxonomic key. Species of Scelionidae were identified using Kozlov and Kononova (1983), Javahery (1968), Moraglio et al. (2021), Talamas et al. (2017), Tortorici et al. (2019). Eupelmidae wasps were identified using Askew and Nieves-Aldrey (2004) and Peng et al. (2020). Images of the specimens were taken using a Canon 90D camera (Canon Inc., Tokyo, Japan) equipped with extension tube, 10× and 20× LWD microscope lenses mounted on a macro-rail. The final pictures were processed with Zerene Stacker (PMax algorithm, Zerene Systems LLC, Richland, WA, USA) and with Photoshop (Adobe Systems Inc, USA). The specimens used for morphological analysis were deposited in the collection of the Dipartimento di Scienze Agrarie, Forestali e Alimentari, University of Turin, Italy.

Results

Parasitism on BMSB egg masses

Various species of native egg parasitoids belonging to Scelionidae (*Trissolcus* spp. and *Telenomus* spp.), Eupelmidae (*Anastatus bifasciatus* (Geoffroy)), and Encyrtidae (*Ooencyrtus* spp.) emerged from egg masses of BMSB collected during the surveys, as well as sentinel egg masses (data not shown). The parasitism rate of sentinel egg masses was lower than naturally-laid ones in 2022 and 2023 (Table 1). In early August 2022, one parasitized egg mass of BMSB laid on *Ailanthus altissima* L. was collected in Castillonnès (N-A, France) and produced 12 specimens of *T. japonicus*. In early July and late August 2023 respectively, two sentinel egg masses were found parasitized by *T. japonicus* in Sainte Foy la grande (N-A, France; 42 km far from Castillonnès) and produced together 44 specimens. However, no specimen was further recovered from 2023 survey in Castillonnès.

Morphological identification

Females and males of *T. japonicus* specimens detected in Castillonnès and Sainte Foy la grande had typical morphological features of this species as described by Talamas

Table 1. Parasitism rates of sentinel and naturally laid egg masses of BMSB in 2022 and 2023 in Nouvelle-Aquitaine (France). (1) All parasitism occurrences; (2) *Trissolcus japonicus* occurrences.

Monitoring period (in Nouvelle-Aquitaine, France)	Type of egg mass	Number of collected egg masses	Number of parasitized egg masses	(1) % of egg masses parasitized	(2) % of egg masses parasitized
June–September 2022	< 24 h sentinel	1169	40	3.4	0
May–September 2022	Natural	155	26	19.2	0.6
April–September 2023	< 24 h sentinel	1822	83	4.6	0.1
May–September 2023	Natural	181	18	9.9	0

et al. (2017): a uniform, well-defined hyperoccipital carina on the vertex between lateral ocelli (Figs 1B, C, 2) and females had a clypeus with four setae and well-defined episternal foveae that extend from the postacetabular sulcus to the mesopleural pit (Fig. 1A). In addition, the absence of rugae on the mesoscutum and the absence of a smooth area below the median ocellus (Fig. 1A, B) confirm that it is neither of the Palearctic species closest to *T. japonicus*, *Trissolcus kozlovi* Rjachovskij and *Trissolcus plautiae* (Watanabe).

Molecular identification

Molecular characterization of the specimens collected in Nouvelle-Aquitaine, resulted in one specific haplotype: haplotype Hap02 (Fig. 3; Suppl. material 2). These sequences match with *T. japonicus* cluster (Fig. 3; Suppl. material 1). The haplotype analyses highlights that these specimens are the same haplotype that the Asian

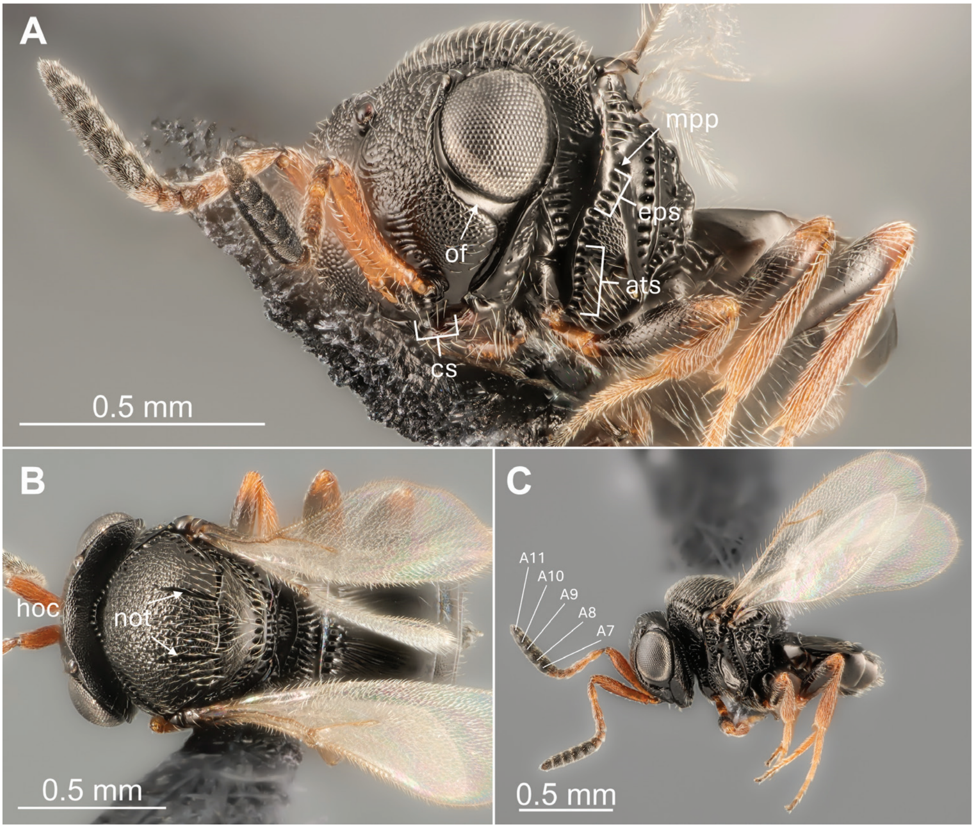

Figure 1. *Trissolcus japonicus* female, voucher n° DISAFA-FT HYM651 **A** head and mesopleuron **B** habitus in dorsal **C** habitus in lateral view; of = orbital furrow; cs = clypeal seteae; eps = episternal foveae; ats = postacetabular sulcus; mpp = mesopleural pit; hoc = hyperoccipital carina; A7–A11 = Antenomeres 7–11 (clavomeres).

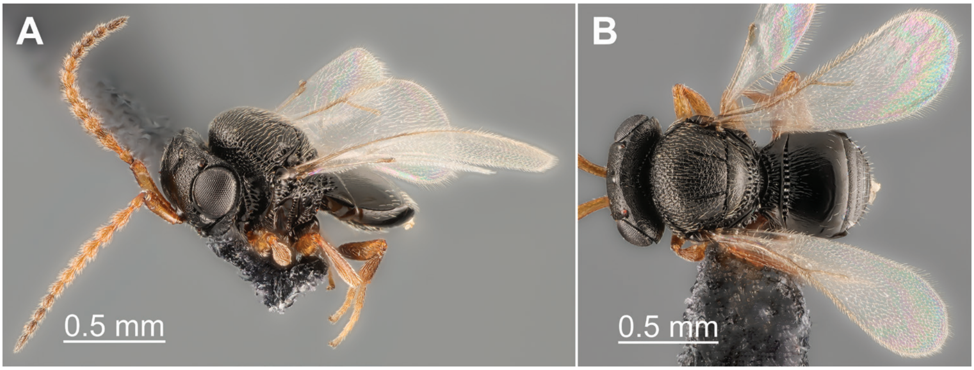

Figure 2. *Trissolcus japonicus* male, voucher n° DISAFA-FT HYM652 **A** habitus in latero-dorsal view **B** habitus in dorsal view.

Table 2. GenBank accession number and sample information for COI sequences of French *Trissolcus japonicus* presented in this study.

Collection Code	Department country	Year of collection	GPS coordinates (DMS)	GenBank accession number
ISA45651	Lot et Garonne, France	2022	44.65474, 0.5883531	PP766189
ISA45652	Lot et Garonne, France	2022	44.65474, 0.5883531	PP766190
ISA45653	Lot et Garonne, France	2022	44.65474, 0.5883531	PP766191
ISA45654	Lot et Garonne, France	2022	44.65474, 0.5883531	PP766192
ISA45671	Lot et Garonne, France	2022	44.65474, 0.5883531	PP766193
ISA45672	Lot et Garonne, France	2022	44.65474, 0.5883531	PP766194
ISA45673	Lot et Garonne, France	2022	44.65474, 0.5883531	PP766195
ISA47499	Gironde, France	2023	44.8405337, 0.212578	PP766196
ISA47500	Gironde, France	2023	44.8405337, 0.212578	PP766197
ISA47501	Gironde, France	2023	44.8405337, 0.212578	PP766198

strain labelled “Beijing USDA”, recently released in Italy for the official CBC program (Suppl. material 2). In addition, translation of all COI sequences into protein resulted in homogeneous protein sequences, with no stop codons. The few changes in amino acid composition observed are between amino acids of the same structural group (Suppl. material 3). Two sequences, corresponding to haplotype Hap01, show a change of amino acid at position 92 *i.e.* Alanine instead of Serine, which belong to two different structural groups (Alanine being the simplest amino acid, while Serine which derived from alanine, has an alcohol function).

Discussion

The sampling of egg parasitoids of BMSB using natural and fresh sentinel egg masses has been effective for the detection of exotic *Trissolcus* species in several countries including France (Talamas et al. 2015; Milnes et al. 2019; Stahl et al. 2019; Moraglio et al. 2020; Bout et al. 2021; Dieckhoff et al. 2021). In Nouvelle-Aquitaine, where

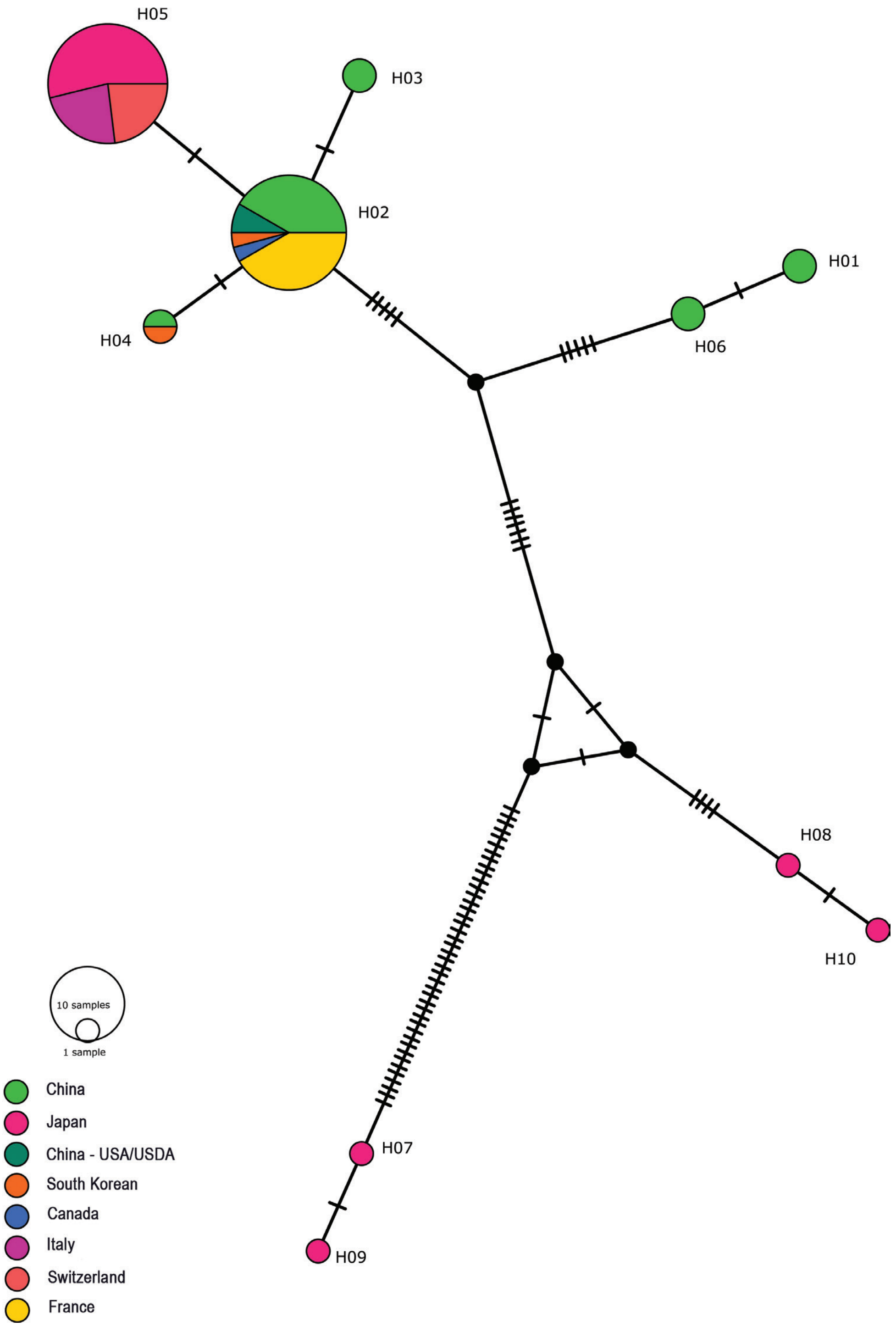

Figure 3. Haplotype network obtained from the 62 COI sequences (575 bp) of *Trissolcus japonicus*. Countries of origin were indicated by colors. POP ART program (Leigh and Bryant 2015).

T. mitsukurii was previously collected from natural egg masses, *T. japonicus* was equally collected from natural and fresh sentinel egg masses. This indicates that both are physiologically suitable for the parasitoid, although the low general parasitism rate on sentinel egg masses suggests they are more difficult to detect or exploit. Regarding the geographic range of our sampling, we likely detected the first non-intentional introduction of *T. japonicus* in France. A migration of the two exotic species *T. japonicus* and *T. mitsukurii* in southwestern France has been expected as (i) they are widespread in Italy and Switzerland, and (ii) the growing density of BMSB provides an invasion opportunity for its native parasitoids (Zapponi et al. 2020). The shared distribution of *T. mitsukurii* and *T. japonicus* in Asia, as well as their co-occurrence in Italy (Zapponi et al. 2020; Falagiarda et al. 2023), reveals similar ecological preferences. French climatic conditions, as those of Europe, are favorable for widespread establishment of *T. japonicus* following the presence of BMSB (Avila and Charles 2018). The detection of *T. japonicus* in Nouvelle-Aquitaine also matches with its modelized distribution proposed by Tortorici et al. (2023). On the other hand, *T. japonicus* has not been collected in the French region of Alpes-Maritimes which is close to Italy, or the regions close to Switzerland as no sampling was performed along an East-West gradient. Thus, it cannot be concluded whether the presence of *T. japonicus* in Nouvelle-Aquitaine results from human activities or natural dispersion, although it is likely to have used similar dispersal ways than *T. mitsukurii*.

Very low recorded parasitism levels are characteristic of the initial detections in other areas where adventive *T. japonicus* populations have since been confirmed and spread (Talamas et al. 2015; Abram et al. 2019; Milnes et al. 2019). Detections of *T. japonicus* and *T. mitsukurii* in France always occurred in urban areas or in proximity to ornamental trees. This was also the case in the USA and Italy (Hedstrom et al. 2017; Sabbatinni Peverieri et al. 2018; Lowenstein et al. 2019). While *T. mitsukurii* was first discovered in France peri-urban area BMSB egg masses laid on cherry laurel, *T. japonicus* was collected in a residential area where BMSB established at high densities on preferred host plants *A. altissima* and *C. bigninoides.* In North America, BMSB is highly associated with *A. altissima*, as well as in Italy (Zapponi et al. 2020; Dyer et al. 2022) so that these tree species are relevant sampling spot (Quinn et al. 2019).

All of the samples of *T. japonicus* collected between 2022 and 2023 in Nouvelle-Aquitaine present the same haplotype Hap02. This haplotype corresponds to strains of *T. japonicus* present in China previously collected by USDA and introduced in Europe *i.e.* Cabi quarantine (Switzerland) and CREA-DC (Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Difesa e Certificazione, Italy). In the same way as for *T. mitsukurii* (Bout et al. 2021) the most probable origin of the French population of *T. japonicus* appears to be Italy, likely from material or plants moved from Italy to France which would support the common introduction way. The collection of specimens between 2022 and 2023 would indicate that this population of *T. japonicus* is established, although it was collected on two different sites. Hence, the question of multiple introductions remains. Molecular sequences used in this study are of good quality: translation of sequences into proteins are preserved and homogeneous, without stop codon. These sequences reflect a genetic polymorphism present within the species. The two sequences representing the haplotype Hap01, with a more significant amino acid replacement at position 92, could reflect a DNA amplification (or correction) error. These sequences could probably be part of the haplotype Hap06.

Conclusion

The detection of *T. japonicus* in 2023, even on a different site than 2022, encourages new prospects and may indicate establishment of the parasitoid. How populations of *T. japonicus* will manage to establish and spread will depend on their ability to survive and reproduce after winter, as well as finding suitable hosts when temperatures rise. In Oregon (USA), *T. japonicus* was able to survive average winter lows of −3 °C (Lowenstein et al. 2019) and it could establish in Italy despite winter reaching -5 °C (Falagiarda et al. 2023). With similar thermal conditions in southwestern France, it is likely to overcome winter, which is currently under investigation. Regarding possible hosts, in addition to BMSB, surveys should continue to include non-target host species. In Italy, surveys one year after releases of *T. japonicus* indicated that *Pentatoma rufipes* (L.), *Graphosoma lineatum* (L.) and *Palomena prasina* (L.) were parasitized (up to 26% parasitized eggs for *G. lineatum*) (Falagiarda et al. 2023).The monitoring of non-target pentatomid species also provides data on egg parasitoid diversity and prevalence, with some of species like *A. bifasciatus* or *Trissolcus belenus* (Walker) able to apply a "natural" regulation of BMSB. Finally, the co-occurrence of *T. japonicus* and *T. mitsukurii* should be investigated both in terms of parasitism rate and geographical dispersion, since monitoring in Italy highlighted that the natural spread of *T. japonicus* was of lower importance than *T. mitsukurii* (Falagiarda et al; 2023).

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References

Abram PK, Talamas EJ, Acheampong S, Mason PG, Gariepy TD (2019) First detection of the samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae), in Canada. Journal of Hymenoptera Research 68: 29–36. https://doi.org/10.3897/jhr.68.32203

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

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). Entomological Science 5: 215–218.

Askew RR, Nieves-Aldrey JL (2004) Further observations on Eupelminae (Hymenoptera, Chalcidoidea, Eupelmidae) in the Iberian Peninsula and Canary Islands, including descriptions of new species. Graellsia 60: 27–39. https://doi.org/10.3989/graellsia.2004.v60.i1.191

Avila GA, Charles JG (2018) Modelling the potential geographic distribution of *Trissolcus japonicus*: a biological control agent of the brown marmorated stink bug, *Halyomorpha halys*. BioControl 63: 505–518. https://doi.org/10.1007/s10526-018-9866-8

Beers EH, Beal D, Smytheman P, Abram PK, Schmidt-Jeffris R, Moretti E, Daane KM, Looney C, Lue C-H, Buffington M (2022) First records of adventive populations of the parasitoids *Ganaspis brasiliensis* and *Leptopilina japonica* in the United States. Journal of Hymenoptera Research 91: 11–25. https://doi.org/10.3897/jhr.91.82812

Bergmann EJ, Venugopal PD, Martinson HM, Raupp MJ, Shrewsbury PM (2016) Host Plant Use by the Invasive *Halyomorpha halys* (Stål) on Woody Ornamental Trees and Shrubs. PLoS ONE 11: e0149975. https://doi.org/10.1371/journal.pone.0149975

Bosco L, Moraglio ST, Tavella L (2018) *Halyomorpha halys*, a serious threat for hazelnut in newly invaded areas. Journal of Pest Science 91: 661–670. https://doi.org/10.1007/s10340-017-0937-x

Bout A, Tortorici F, Hamidi R, Warot S, Tavella L, Thomas M (2021) First Detection of the Adventive Egg Parasitoid of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) *Trissolcus mitsukurii* (Ashmead) (Hymenoptera: Scelionidae) in France. Insects 12: 761. https://doi.org/10.3390/insects12090761

Charles JG, Avila GA, Hoelmer KA, Hunt S, Gardner-Gee R, MacDonald F, Davis V (2019) Experimental assessment of the biosafety of *Trissolcus japonicus* in New Zealand, prior to the anticipated arrival of the invasive pest *Halyomorpha halys*. BioControl 64: 367–379. https://doi.org/10.1007/s10526-019-09949-x

Conti E, Avila G, Barratt B, Cingolani F, Colazza S, Guarino S, Hoelmer K, Laumann RA, Maistrello L, Martel G, Peri E, Rodriguez-Saona C, Rondoni G, Rostás M, Roversi PF, Sforza RFH, Tavella L, Wajnberg E (2021) Biological control of invasive stink bugs: review of global state and future prospects. Entomologia Experimentalis et Applicata 169: 28–51. https://doi.org/10.1111/eea.12967

CSO Italy - Centro Servizi Ortofrutticoli (2019) Estimation of damage from Brown Marmorated Stink Bug and pathologies related to climate change. www.csoservizi.com de Benedetta F, Giaccone M, Pica F, Lisanti MT, Vinale F, Turrà D, Giacca GM, Bernardo U (2023) Fruit Phenology of Two Hazelnut Cultivars and Incidence of Damage by *Halyomorpha halys* in Treated and Untreated Hazel Groves. Horticulturae 9: 727. https://doi.org/10.3390/horticulturae9060727

Dieckhoff C, Wenz S, Renninger M, Reißig A, Rauleder H, Zebitz CPW, Reetz J, Zimmermann O (2021) Add Germany to the List—Adventive Population of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) Emerges in Germany. Insects 12: 414. https://doi.org/10.3390/insects12050414

Dyer JE, Talamas EJ, Leskey TC, Bergh JC (2022) Influence of Trap Location in the Tree Canopy on Captures of Adventive *Trissolcus japonicus* (Hymenoptera: Scelionidae). Journal of Economic Entomology 115: 904–908. https://doi.org/10.1093/jee/toac039

Falagiarda M, Carnio V, Chiesa SG, Pignalosa A, Anfora G, Angeli G, Ioriatti C, Mazzoni V, Schmidt S, Zapponi L (2023) Factors influencing short term parasitoid establishment and efficacy for the biological control of *Halyomorpha halys* with the samurai wasp *Trissolcus japonicus*. Pest Management Science 79(7): 2397–2414. https://doi.org/10.1002/ps.7423

Folmer O, Black M, Hoeh W; Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochon-drial cytochrome c oxydase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Ganjisaffar F, Talamas EJ, Bon M-C, Brown BV, Gonzalez L, Perring TM (2018) *Trissolcus hyalinipennis* Rajmohana andamp; Narendran (Hymenoptera, Scelionidae), a parasitoid of *Bagrada hilaris* (Burmeister) (Hemiptera, Pentatomidae), emerges in North America. Journal of Hymenoptera Research 65: 111–130. https://doi.org/10.3897/jhr.65.25620

Gardner WA, Blount JL, Golec JR, Jones WA, Hu XP, Talamas EJ, Evans RM, Dong X, Ray Jr CH, Buntin GD, Gerardo NM, Couret J (2016) Discovery of *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastridae), an Egg Parasitoid of *Megacopta cribraria* F. (Hemiptera: Plataspidae) in its Expanded North American Range. Journal of Entomological Science 48: 355–359. https://doi.org/10.18474/0749-8004-48.4.355

Haye T, Hoelmer K, Rossi J-P, Streito J-C, Tassus X (2014) Analyse de risque phytosanitaire express *Halyomorpha halys* - la punaise diabolique. In Avis de l'Anses, Rapport D'expertise Collective. Edition Scientifique, Maisons Alfort, 2014. http://www.anses.fr/sites/default/files/documents/SVEG2013sa0093Ra.pdf

Haye T, Fischer S, Zhang J, Gariepy T (2015) Can native egg parasitoids adopt the invasive brown marmorated stink bug, *Halyomorpha halys* (Heteroptera: Pentatomidae), in Europe? Journal of Pest Science 88(4): 693–705. https://doi.org/10.1007/s10340-015-0671-1

Haye T, Moraglio ST, Stahl J, Visentin S, Gregorio T, Tavella L (2020) Fundamental host range of *Trissolcus japonicus* in Europe. Journal of Pest Science 93: 171–182. https://doi.org/10.1007/s10340-019-01127-3

Hedstrom C, Lowenstein D, Andrews H, Bai B, Wiman N (2017) Pentatomid host suitability and the discovery of introduced populations of *Trissolcus japonicus* in Oregon. Journal of Pest Science 90: 1169–1179. https://doi.org/10.1007/s10340-017-0892-6

Hogg BN, Hougardy E, Talamas E (2021) Adventive *Gryon aetherium* Talamas (Hymenoptera, Scelionidae) associated with eggs of *Bagrada hilaris* (Burmeister) (Hemiptera, Pentatomidae) in the USA. Journal of Hymenoptera Research 87: 481–492. https://doi.org/10.3897/jhr.87.73778

Javahery M (1968) The egg parasite complex of British Pentatomoidea (Hemiptera): Taxonomy of Telenominae (Hymenoptera: Scelionidae). Transactions of the Royal Entomological Society of London 120: 417–436. https://doi.org/10.1111/j.1365-2311.1968.tb00345.x

Jones AL, Jennings DE, Hooks CRR, Shrewsbury PM (2014) Sentinel eggs underestimate rates of parasitism of the exotic brown marmorated stink bug, *Halyomorpha halys*. Biological Control 78: 61–66. https://doi.org/10.1016/j.biocontrol.2014.07.011

Kereselidze M, Pilarska D, Guntadze N, Linde A (2022) *Halyomorpha halys* Stål, (Hemiptera:Pentatomidae) feeding effects on some agricultural fruits in Georgia. Turkish Journal of Zoology 46: 298–303. https://doi.org/10.55730/1300-0179.3058

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581

Konjević A, Tavella L, Tortorici F (2024) The first records of *Trissolcus japonicus* (Ashmead) and *Trissolcus mitsukurii* (Ashmead) (Hymenoptera, Scelionidae), alien egg parasitoids of *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae) in Serbia. Biology 13: 316. https://doi.org/10.3390/biology13050316

Kozlov MA, Kononova SV (1983) Telenominae of the Fauna of the USSR. Nauka: Leningrad, 1983.

Lee D-H, Short BD, Joseph SV, Bergh JC, Leskey TC (2013) Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. Environmental Entomology 42: 627–641. https://doi.org/10.1603/EN13006

Leskey TC, Nielsen AL (2018) Impact of the invasive brown marmorated stink bug in north america and europe: History, biology, ecology, and management. Annual Review of Entomology 63: 599–618. https://doi.org/10.1146/annurev-ento-020117-043226

Lowenstein DM, Andrews H, Hilton RJ, Kaiser C, Wiman NG (2019) Establishment in an introduced range: Dispersal capacity and winter survival of *Trissolcus japonicus*, an adventive egg parasitoid. Insects 10: 443. https://doi.org/10.3390/insects10120443

Maistrello L, Vaccari G, Caruso S, Costi E, Bortolini S, Macavei L, Foca G, Ulrici A, Bortolotti PP, Nannini R, Casoli L, Fornaciari M, Mazzoli GL, Dioli P (2017) Monitoring of the invasive *Halyomorpha halys*, a new key pest of fruit orchards in northern Italy. Journal of Pest Science 90: 1231–1244. https://doi.org/10.1007/s10340-017-0896-2

Martin J, Vogel J, Peters RS, Herz A (2023) First record of *Leptopilina japonica* Novković & Kimura, 2011 (Hymenoptera: Figitidae) in Germany, a parasitoid of the Spotted Wing Drosophila *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae). Journal of Applied Entomology 147: 1067–1073. https://doi.org/10.1111/jen.13182

Mason PG, Olfert OO, Haye T, Gariepy TD, Abram PK, Gillespie DR (2017) Risks and benefits of accidental introductions of biological control agents in Canada. In: Mason PG, Gillespie DR, Vincent C (Eds) Proceedings of the 5th International Symposium on Biological Control of Arthropods, Langkawi, Malaysia, September 11–15, 2017. CABI, UK, 6–8. https://doi.org/10.1079/9781786394118.0006

McPherson JE (2018) Invasive Stink Bugs and Related Species (Pentatomoidea): Biology, Higher Systematics, Semiochemistry, and Management. CRC Press, 864 pp. https://doi.org/10.1201/9781315371221

Milnes JM, Beers EH (2019) *Trissolcus japonicus* (Hymenoptera: Scelionidae) Causes Low Levels of Parasitism in Three North American Pentatomids Under Field Conditions. Journal of Insect Science 19: 15. https://doi.org/10.1093/jisesa/iez074

Moraglio ST, Tortorici F, Pansa MG, Castelli G, Pontini M, Scovero S, Visentin S, Tavella L (2020) A 3-year survey on parasitism of *Halyomorpha halys* by egg parasitoids in northern Italy. Journal of Pest Science 93: 183–194. https://doi.org/10.1007/s10340-019-01136-2

Moraglio ST, Tortorici F, Visentin S, Pansa MG, Tavella L (2021) *Trissolcus kozlovi* in North Italy: Host Specificity and Augmentative Releases against Halyomorpha halys in Hazelnut Orchards. Insects 12: 464. https://doi.org/10.3390/insects12050464

Peng L, Gibson G, Tang L, Xiang J (2020) Review of the species of *Anastatus* (Hymenoptera: Eupelmidae) known from China, with description of two new species with brachypterous females. Zootaxa 4767: 351–401. https://doi.org/10.11646/zootaxa.4767.3.1

Puppato S, Grassi A, Pedrazzoli F, De Cristofaro A, Ioriatti C (2020) First Report of *Leptopilina japonica* in Europe. Insects 11: 611. https://doi.org/10.3390/insects11090611

Quinn NF, Talamas EJ, Leskey TC, Bergh JC (2019) Sampling Methods for Adventive *Trissolcus japonicus* (Hymenoptera: Scelionidae) in a Wild Tree Host of *Halyomorpha halys* (Hemiptera: Pentatomidae). Journal of Economic Entomology 112: 1997–2000. https://doi.org/10.1093/jee/toz107

Rondoni G, Borges I, Collatz J, Conti E, Costamagna AC, Dumont F, Evans EW, Grez AA, Howe AG, Lucas E, Maison haute J-É, Onofre Soares A, Zaviezo T, Cock MJW (2021) Exotic ladybirds for biological control of herbivorous insects – a review. Entomologia Experimentalis et Applicata 169: 6–27. https://doi.org/10.1111/eea.12963

Rot M, Maistrello L, Costi E, Bernardinelli I, Malossini G, Benvenuto L, Trdan S (2021) Native and Non-Native Egg Parasitoids Associated with Brown Marmorated Stink Bug (*Halyomorpha halys* [Stål, 1855]; Hemiptera: Pentatomidae) in Western Slovenia. Insects 12: 505. https://doi.org/10.3390/insects12060505

Roversi P, Binazzi F, Marianelli L, Costi E, Maistrello L, Sabbatini, PG (2016) Searching for native egg-parasitoids of the invasive alien species *Halyomorpha halys* Stål (Heteroptera Pentatomidae) in Southern Europe. Journal of Zoology 99: 1–8. https://doi.org/10.19263/redia-99.16.01

Sabbatini Peverieri G, Dieckhoff C, Giovannini L, Marianelli L, Roversi PF, Hoelmer K (2020) Rearing *Trissolcus japonicus* and *Trissolcus mitsukurii* for Biological Control of *Halyomorpha halys*. Insects 11: 787. https://doi.org/10.3390/insects11110787

Sabbatini Peverieri G, Talamas E, Bon MC, Marianelli L, Bernardinelli I, Malossini G, Benvenuto L, Roversi PF, Hoelmer K (2018) Two Asian egg parasitoids of *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae) emerge in northern Italy: *Trissolcus mitsukurii* (Ashmead) and *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae). Journal of Hymenoptera Research 67: 37–53. https://doi.org/10.3897/jhr.67.30883

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sosa-Gomez DR, Corso IC, Morales L (2001) Insecticide resistance to endosulfan, monocrotophos and metamidophos in the neotropical brown stink bug, *Euschistus heros* (F.). Neotropical Entomology 30: 317–320. https://doi.org/10.1590/S1519-566X2001000200017

Stahl J, Tortorici F, Pontini M, Bon M-C, Hoelmer K, Marazzi C, Tavella L, Haye T (2019) First discovery of adventive populations of *Trissolcus japonicus* in Europe. Journal of Pest Science 92: 371–379. https://doi.org/10.1007/s10340-018-1061-2

Talamas EJ, Buffington ML, Hoelmer K (2017) Revision of Palearctic *Trissolcus* Ashmead (Hymenoptera, Scelionidae). Journal of Hymenoptera Research 56: 3–185. https://doi.org/10.3897/jhr.56.10158

Talamas EJ, Herlihy MV, Dieckhoff C, Hoelmer KA, Buffington ML, Bon M-C, Weber DC (2015) *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. Journal of Hymenoptera Research 43: 119–128. https://doi.org/10.3897/JHR.43.4661

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Tortorici F, Bombi P, Loru L, Mele A, Moraglio ST, Scaccini D, Pozzebon A, Pantaleoni RA, Tavella L (2023) *Halyomorpha halys* and its egg parasitoids *Trissolcus japonicus* and *T. mitsukurii*: the geographic dimension of the interaction. NeoBiota 85: 197–221. https://doi.org/10.3897/neobiota.85.102501

Tortorici F, Talamas EJ, Moraglio ST, Pansa MG, Asadi-Farfar M, Tavella L, Caleca V (2019) A morphological, biological and molecular approach reveals four cryptic species of *Trissolcus* Ashmead (Hymenoptera, Scelionidae), egg parasitoids of Pentatomidae (Hemiptera). Journal of Hymenoptera Research 73: 153–200. https://doi.org/10.3897/jhr.73.39052

van Lenteren JC, Bale J, Bigler F, Hokkanen HMT, Loomans AJM (2006) Assessing risks of releasing exotic biological control agents of arthropod pests. Annual Review of Entomology 51: 609–634. https://doi.org/10.1146/annurev.ento.51.110104.151129

Yang Z-Q, Yao Y-X, Qiu L-F, Li Z-X (2009) A new species of *Trissolcus* (Hymenoptera: Scelionidae) parasitizing eggs of *Halyomorpha halys* (Heteroptera: Pentatomidae) in China with comments on its biology. Annals of the Entomological Society of America 102: 39–47. https://doi.org/10.1603/008.102.0104

Zapponi L, Bon M, Fouani J, Anfora G, Schmidt S, Falagiarda M (2020) Assemblage of the egg parasitoids of the invasive stink bug *Halyomorpha halys*: Insights on Plant Host Associations. Insects 11: 588. https://doi.org/10.3390/insects11090588

Zhong Y-Z, Zhang J-P, Ren L-L, Tang R, Zhan H-X, Chen G-H, Zhang F (2017) Behavioral responses of the egg parasitoid *Trissolcus japonicus* to volatiles from adults of its stink bug host, *Halyomorpha halys*. Journal of Pest Science 90: 1097–1105. https://doi.org/10.1007/s10340-017-0884-6

Supplementary material 1

Phylogenetic tree based on molecular clustering of worldwid *Trissolcus japonicus* vouchers including French samples

Authors: Guillaume Martel, Alexandre Bout, Francesco Tortorici, Rachid Hamidi, Luciana Tavella, Maud Thomas

Data type: pdf

Link: https://doi.org/10.3897/jhr.97.132433.suppl1

Supplementary material 2

Sequences references of *Trissolcus japonicus* vouchers used to build the Haplotype network

Authors: Guillaume Martel, Alexandre Bout, Francesco Tortorici, Rachid Hamidi, Luciana Tavella, Maud Thomas

Data type: xlsx

Link: https://doi.org/10.3897/jhr.97.132433.suppl2

Supplementary material 3

COI proteine translation from *Trissolcus japonicus* sequences used in the study

Authors: Guillaume Martel, Alexandre Bout, Francesco Tortorici, Rachid Hamidi, Luciana Tavella, Maud Thomas

Data type: pdf

Link: https://doi.org/10.3897/jhr.97.132433.suppl3