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First detection of the samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae), in Canada

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Abstract

We report the first detection of *Trissolcus japonicus*, an exotic Asian egg parasitoid and the primary candidate for classical biological control of the invasive brown marmorated stink bug, *Halyomorpha halys*, in Canada. Twenty-eight *Trissolcus japonicus* emerged from an *H. halys* egg mass from a site heavily infested by *H. halys* in Chilliwack, British Columbia, in 2018. This egg mass was deployed and retrieved as part of ongoing sentinel egg mass surveys for natural enemies of *H. halys* from 2017–2018 in coastal and interior British Columbia (total of 1,496 egg clusters at 16 sites). The identification of *T. japonicus* was based on biology (high levels of successful emergence from *H. halys* eggs), morphology, and mitochondrial DNA sequences. *Trissolcus japonicus* was not detected at any other survey sites in 2017–2018; however, three species of indigenous egg parasitoids were found attending or emerging from *H. halys* egg masses at low levels (<4%) at several sites. The origin of the detected *T. japonicus*, the extent of its establishment in British Columbia, and its ultimate impact on *H. halys* populations remain to be determined. Nonetheless, the detection of this exotic biological control agent in Canada concurrently with regulatory review of its intentional importation and release is emblematic of the current uncertainty around regulatory control on the movement of biological control agents across borders.

Keywords
adventive establishment, classical biological control, brown marmorated stink bug, *Halyomorpha halys*

Introduction

Classical (= importation) biological control of invasive pests, where natural enemies are imported and intentionally introduced from a pest's area of origin, involves years of research to assess risks and benefits of proposed introductions, followed by regulatory approval (Bigler et al. 2006, Cock et al. 2016, Heimpel and Cock 2018). However, there is increasing recognition that unintentional introductions of natural enemies are probably common, introducing a high level of uncertainty to the regulatory process for biological control introductions (Mason et al. 2017a).

The samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), has become a prominent case study for the establishment of a candidate biological control agent outside of its native range prior to a decision by regulatory authorities on the appropriateness of release (Servick 2018). Its host, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), also native to Asia, is an invasive alien pest that has caused extensive economic damage and increases in insecticide use in a wide range of crops in areas of the United States and Europe where it has become established (Leskey and Nielsen 2018). *Halyomorpha halys* is also a nuisance pest in human dwellings. A classical biological control program for *H. halys* was initiated in the USA and *T. japonicus* was identified as the most promising candidate for introduction based on high parasitism rates of *H. halys* (typically ~60–90%) in Asia (Qiu et al. 2007, Yang et al. 2009, Zhang et al. 2017). However, in 2014, while non-target host range testing was still underway, a population of this parasitoid was recovered in nature from sentinel egg masses in Maryland, USA, indicating that it had been introduced accidentally (Talamas et al. 2015a). Other adventive populations were found in Oregon and Washington State (USA) in 2015 (Milnes et al. 2016, Hedstrom et al. 2017). More adventive populations of *T. japonicus* in both the Pacific Northwest and the northeastern USA continue to be discovered (stopbmsb.org), and intentional redistributions are now taking place within some states (K.A. Hoelmer, personal communication). Unexpectedly, in 2017 and 2018, adventive populations of *T. japonicus* were also detected in Switzerland (Stahl et al. 2018) and Italy (Sabbatini et al. 2018), suggesting that like its host, *T. japonicus* is becoming a "global invader".

Trissolcus japonicus has not been detected previously in Canada, where *H. halys* populations have established recently (Gariepy et al. 2014, Abram et al. 2017a). Thus, intentional introduction of *T. japonicus* to control *H. halys* populations in Canada would require regulatory approval based on review of a petition for release (Mason et al. 2017b). Here we report the detection of *T. japonicus* in British Columbia, Canada, representing another remarkable instance where this parasitoid has arrived in a country before a regulatory decision has been made regarding the appropriateness of its introduction.

From May to September in each of 2017 and 2018, a total of 1,496 *H. halys* sentinel egg masses (= 41,351 eggs) were set out at 16 field sites in coastal and interior British Columbia where large, established breeding populations of *H. halys* are present (Table 1). Eggs were then retrieved to measure parasitism levels and parasitoid species composition. All sentinel sites were in urban, suburban, and backyard settings with mixed woody and herbaceous vegetation. *Halyomorpha halys* egg masses, laid on Reemay® polyester fabric (Avintiv, USA), were collected from *H. halys* laboratory colonies within 24 hours of being laid and either placed in the field the same day or stored at 10 °C to delay development for up to a week before they were deployed. The fabric substrate holding the eggs was stapled to the undersides of the leaves of wide variety of host plants infested by *H. halys*, mostly woody trees (e.g. *Prunus* spp., *Davidia* spp., *Acer* spp., *Gleditsia* spp., *Ailanthus* spp., *Sorbus* spp.) and shrubs (e.g., *Rubus* sp., *Mahonia* spp., *Symphoricarpos* spp., *Rosa* spp.). Sentinel egg masses were retrieved from the field within four days, before the emergence of *H. halys* nymphs. Parasitoids found attending egg masses at recovery (indicating post-oviposition brood guarding behavior; see Abram et al. 2014, Cornelius et al. 2016) were also collected. Egg masses were then kept in Petri dishes (50 mm diameter, 9 mm depth) under ambient laboratory conditions to assess parasitoid emergence. Attending and emerging egg parasitoids were preserved in 95% EtOH, then point-mounted and identified to species using the key of Talamas et al. (2015b). Finally, all egg masses were dissected under a stereomicroscope to verify that non-emerged eggs did not contain parasitoids.

While three species (26 total individuals) of indigenous egg parasitoids [*Trissolcus euschisti* (Ashmead), *Trissolcus cosmopeplae* (Gahan), and *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae)] were found attending *H. halys* sentinel egg masses upon recovery, successful emergence of parasitoids from *H. halys* eggs was rare (Table 1). Of these species, only *T. euschisti* successfully emerged. Less than one fourth of the eggs in each of these masses were parasitized successfully (average of 22.5 ± 1.4%; mean ± SE, n = 6), and emerging parasitoids produced few or no offspring when subsequently offered *H. halys* eggs in the laboratory. These findings are consistent with past surveys and laboratory trials in other areas of North America and Europe showing that attack of *H. halys* egg masses by indigenous egg parasitoids is probably common (Gariepy et al. 2018), but their offspring are usually unable to complete development successfully (Abram et al. 2014, Haye et al. 2015, reviewed in Abram et al. 2017b). In contrast, all 28 eggs (100%) of one egg cluster deployed at a site highly infested by *H. halys* in Chilliwack, BC on August 23, 2018 were parasitized, and emerging offspring completely parasitized a number of *H. halys* egg masses offered in the laboratory with >90% successful offspring emergence.

Specimens were identified to species using the key to Nearctic *Trissolcus* by Talamas et al. (2015b) and are fully congruent with the concept of *T. japonicus* presented by Talamas et al. (2015b, 2017). Specifically, the presence of 4 clypeal setae (Fig. 1A) and well-defined episternal foveae that extend from the postacetabular sulcus to the mesopleural pit (Fig. 1B) unambiguously separate *T. japonicus* from the Nearctic fauna. Additionally, the absence of rugae on the mesoscutum (Fig. 1C) and the absence of

Table 1. Locations of field sites for sentinel egg mass surveys, the number of sentinel *H. halys* egg masses set out and retrieved, and the parasitoid species found attending and emerging from *H. halys* egg masses.

Site name (GPS coordinates)	Year(s) surveyed[a]	Total # sentinel egg clusters (total # eggs)	% egg clusters with parasitoid emergence[b]	Parasitoid species emerging from eggs (% of parasitized egg clusters)	Parasitoid species found attending egg clusters (total number)[c]
Chilliwack #1 (49.158°N, -122.003°W)	2017, 2018	313 (8,642)	0.64%	*T. euschisti* (50%); *T. japonicus* (50%)	*T. euschisti* (4); *T. podisi* (3)
Chilliwack #2 (49.159°N, -121.997°W)	2017	55 (1,426)	0.00%	–	*T. euschisti* (1)
Chilliwack #3 (49.192°N, -121.931°W)	2018	186 (5,182)	0.00%	–	–
Rosedale (49.184°N, -121.800°W)	2017	63 (1,647)	0.00%	–	*T. podisi* (1)
Abbotsford (49.003°N, -122.264°W)	2017, 2018	217 (6,004)	0.00%	–	*T. euschisti* (2); *T. podisi*(1)
Langley (49.122°N, -122.657°W)	2017	10 (308)	0.00%	–	–
Kelowna #1 (49.885°N, -119.485°W)	2018	76 (2,128)	1.31%	*T. euschisti* (100%)	*T. euschisti* (1)
Kelowna #2 (49.880°N, -119.485°W)	2018	78 (2,172)	1.28%	*T. euschisti* (100%)	*T. euschisti* (4)
Kelowna #3 (49.872°N, -119.490°W)	2018	76 (2,123)	0.00%	–	–
Kelowna #4 (49.885°N, -119.457°W)	2018	76 (2,096)	0.00%	–	*T. euschisti*(1)
Kelowna #5 (49.882°N, -119.484°W)	2018	75 (2,086)	0.00%	–	*T. euschisti* (3); *T. cosmopeplae* (1)[d]
Kelowna #6 (49.869°N,-119.486°W)	2018	66 (1,845)	1.51%	*T. euschisti* (100%)	*T. euschisti*(1)
Kelowna #7 (49.894°N, -119.405°W)	2018	60 (1,684)	3.33%	*T. euschisti* (100%)	*T. euschisti* (2)
Kelowna #8 (49.879°N, -119.484°W)	2018	60 (1,692)	0.00%	–	*T. podisi* (1)
Kelowna #9 (49.881°N, -119.484°W)	2018	60 (1,662)	0.00%	–	–
Kelowna #10 (49.868°N, -119.494°W)	2018	25 (654)	0.00%	–	–
TOTAL		1,496 (41,351)	0.47%	–	–

[a] For sites that were surveyed in both years, results are pooled.
[b] Percentage of egg clusters from which at least one parasitoid emerged.
[c] Attending parasitoids were found resting on top of the egg cluster at the time of collection, probably indicating post-oviposition brood guarding behaviour.

[d] This represents the first published record of *T. cosmopeplae* in British Columbia

a smooth area below the median ocellus (Fig. 1A) confirm that it is neither of the Palearctic species closest to *T. japonicus*, *T. kozlovi* and *T. plautiae* (Talamas et al. 2017). Voucher specimens are deposited in the Florida State Collection of Arthropods and the Canadian National Collection of Insects (Table 2). The collection data, including host associations, for all voucher specimens are deposited in the Hymenoptera Online Database (hol.osu.edu).

DNA was extracted from 5 specimens using a chelex DNA extraction protocol, and the universal primers LCO-1490 and HCO-2198 (Folmer et al. 1994) were used for amplification and sequencing of the DNA barcode region of the Cytochrome Oxidase I (COI) gene (as described by Gariepy et al. 2014). All 5 specimens yielded identical COI sequences of 643-bp in length, and a representative sequence was submitted to GenBank (Accession number: MK188349) and uploaded to the North American Scelionidae DNA barcode database (project NSCEL) available on the Barcode of Life Datasystems (BOLD, http://www.boldsystems.org). In comparison to public sequences available in the NSCEL database, these specimens shared 100% se-

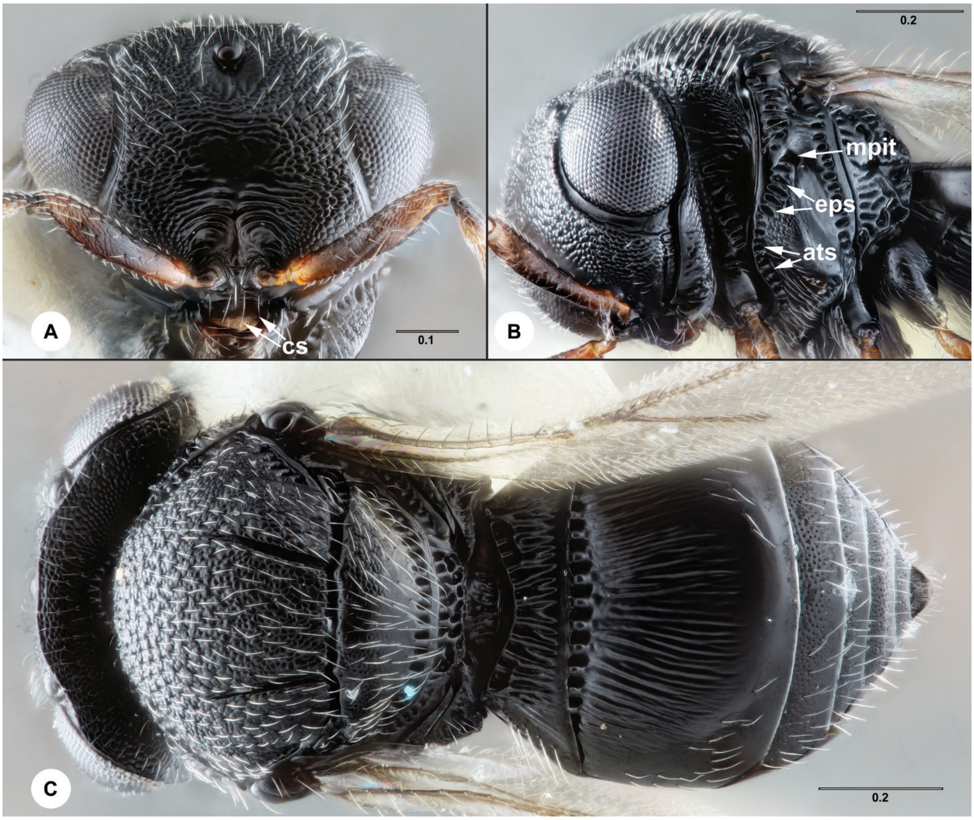

Figure 1. *Trissolcus japonicus* female (FSCA 00033107) from Chilliwack, British Columbia: **A** head, anterior view, cs: clypeal setae **B** head and mesosoma, anterolateral view, ats: postacetabular sulcus, eps: episternal foveae, mpit: mesopleural pit **C** head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

quence similarity with voucher *T. japonicus* collected from established populations in the USA, and shared 99–100% sequence similarity with voucher specimens collected in Asia. The small amount of variation among *T. japonicus* specimens is likely due to intraspecific variation between individuals collected from geographically distinct regions (e.g., Stahl et al. 2018).

This detection of *T. japonicus* in Canada occurred while a petition for the release of this biological control agent was under review by the Canadian Food Inspection Agency (CFIA), the national regulatory authority in Canada. We are not aware of any historical cases where this has occurred, in Canada or elsewhere, and its implications for the prospects of intentionally importing and releasing *T. japonicus* in Canada remain to be seen. It is important to note that because *T. japonicus* has been detected only at a single site in one year, we cannot yet definitively conclude that this species is established in Canada. However, given the relative proximity (<400km) of the closest known established populations in Washington State (Milnes et al. 2016, stopbmsb.org), it is plausible that this detection is indicative of a range expansion of adventive

Table 2. Collecting unit identifiers and institutions where voucher specimens are deposited.

Species	Collecting Unit Identifier	Institution
Trissolcus cosmopeplae	FSCA 00033197–FSCA 00033201	Canadian National Collection of Insects
	FSCA 00033202–FSCA 00033206	Florida State Collection of Arthropods
Trissolcus euschisti	FSCA 00033177–FSCA 00033181	Canadian National Collection of Insects
	FSCA 00033182–FSCA 00033186	Florida State Collection of Arthropods
Trissolcus japonicus	FSCA 00033110–FSCA 00033111	Canadian National Collection of Insects
	FSCA 00033107–FSCA 00033109	Florida State Collection of Arthropods
Telenomus podisi	FSCA 00033187–FSCA 00033191	Canadian National Collection of Insects
	FSCA 00033192–FSCA 00033196	Florida State Collection of Arthropods

T. japonicus populations, and that the parasitoid is in the early phases of establishment. Very low parasitism levels, as we observed here, were also characteristic of the initial detections in other areas where adventive *T. japonicus* populations have since been confirmed and are spreading (Talamas et al. 2015a, Milnes et al. 2016). In addition, conservative climate suitability modeling has predicted that several areas of southern Canada, including British Columbia, are suitable for *T. japonicus* establishment, survival, and reproduction (Avila and Charles 2018). Continuing field surveys and extensive phylogeographic analyses using microsatellite DNA markers are underway to track the establishment and biological control impact of *T. japonicus* in Canada, and to reconstruct potential pathways of introduction.

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