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Trissolcus hyalinipennis Rajmohana & Narendran (Hymenoptera, Scelionidae), a parasitoid of *Bagrada hilaris* (Burmeister) (Hemiptera, Pentatomidae), emerges in North America

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Abstract

Trissolcus hyalinipennis Rajmohana & Narendran is an Old World egg parasitoid of *Bagrada hilaris* (Burmeister). Its potential as a classical biological control agent in the United States has been under evaluation in quarantine facilities since 2014. A survey of resident egg parasitoids using fresh sentinel *B. hilaris* eggs in Riverside, California, revealed that *T. hyalinipennis* is present in the wild. Four cards with parasitized eggs were recovered, from which one yielded a single live *T. hyalinipennis* and two unidentified dead wasps (Scelionidae), and three yielded twenty live *Trissolcus basalis* (Wollaston) and one dead wasp. Subsequently, samples from Burbank, California, collected with a Malaise trap as part of the BioSCAN project, yielded five females of *T. hyalinipennis*. It is presumed that the introduction of *T. hyalinipennis* to this area was accidental. Surveys will be continued to evaluate the establishment of *T. hyalinipennis* as well as the presence of other resident parasitoid species.

Keywords
bagrada bug, painted bug, biological control, egg parasitoid, sentinel eggs

Introduction

The invasive stink bug, *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), is native to Africa, Asia, and the Middle East (Howard 1907, Husain 1924). In the United States, it was first discovered in Los Angeles County, California, in 2008 (Arakelian 2008), and since then its range has expanded into other regions of southern California (Reed et al. 2013), the central coast of California (Joseph et al. 2016), Arizona (Palumbo and Natwick 2010, Palumbo et al. 2016), New Mexico (Bundy et al. 2012), Texas (Vitanza 2012), Nevada (Perring et al. 2013), Utah (Reed et al. 2013) and Hawaii (Matsunaga 2014). It also has been reported from Mexico (Sánchez-Peña 2014) and Chile (Faúndez et al. 2016). The species feeds on various wild and cultivated plants, but it is a severe pest of cole crops in the genus *Brassica* L. within its native and invaded range (Howard 1907, Obopile et al. 2008, Abrol 2009, Perring et al. 2013, Reed et al. 2013, Palumbo et al. 2016). In the southwestern deserts of the United States, cole crops are planted in late August and early September, and the first *B. hilaris* population peak occurs from September to October. During the winter, when wild cruciferous weeds such as London rocket (*Sisymbrium irio* L.), shepherd's purse (*Capsella bursa-pastoris* (L.)), shortpod mustard (*Hirschfeldia incana* (L.)), and Sahara mustard (*Brassica tournefortii* Gouan) grow, *B. hilaris* populations peak again on these weed species (Reed et al. 2013).

There are several strategies that contribute to the integrated management of *B. hilaris* (Palumbo et al. 2016, Bundy et al. 2018), with chemical control being the anchor of these tactics. While there are known predators and parasitoids that attack *B. hilaris* (Bundy et al. 2018), there are no established biological control programs in practice. In Pakistan, *B. hilaris* is only a sporadic pest, likely due to a suite of natural enemies. Therefore, in 2014, three species of parasitoid wasps were collected from *B. hilaris* eggs that had been laid on *Brassica* plant debris or eggs that were attached to sentinel cards: *Trissolcus* sp. (now identified by Elijah Talamas as *T. hyalinipennis* Rajmohana & Narendran), *Gryon* sp. (Platygastroidea: Scelionidae), and *Ooencyrtus* sp. (Chalcidoidea: Encyrtidae) (Mahmood et al. 2015). Live specimens of the three parasitoids were shipped to the USDA-ARS Stoneville Research Quarantine Facility in Stoneville, Mississippi, and their potential as classical biocontrol agents is being evaluated. In addition, surveys for resident egg parasitoids of *B. hilaris* have been initiated in northern, central, and southern California and Mexico using sentinel egg cards. This study presents results from southern California surveys in agricultural environments, as well as those from the BioSCAN survey of urban habitats, and reports the discovery of *Trissolcus hyalinipennis* for the first time in North America. We here provide documentation of this discovery and a means for others to distinguish *T. hyalinipennis* from other species of *Trissolcus* to facilitate additional survey work.

Materials and methods

All *B. hilaris* eggs used in the experiment came from a colony established in the fall of 2010 with adult bugs collected in Riverside (Riverside County), California. Insects were reared on a mixture of Brassicaceae seedlings (sweet alyssum, *Lobularia maritima* (L.) Desvaux; broccoli, *Brassica oleracea* L. variety Italica; canola, *Brassica napus* L.; and mustard greens, *Brassica juncea* (L.)) grown in 4-inch pots in insect cages (Bug-Dorm-2120, MegaView Science Co., Taiwan) in a greenhouse. The colony has been supplemented periodically with field collected insects to maintain genetic diversity. In preparation for the study, adult mating pairs were brought to the lab weekly and placed in round plastic containers (15 cm diameter × 6.5 cm height) with 2 screen openings (one on each side) for ventilation. White paper towels were cut in circles to fit the bottom of each container to absorb excess moisture and fecal material, and to provide a substrate for oviposition. Organic broccoli florets were provided as food and replaced every 24–48 h. Approximately 30 pairs were placed into each container and were maintained in an insectary room at 30±1 °C, 40–50% humidity and 14: 10 (L:D) photoperiod.

Survey sites

Two main host fields (alfalfa, *Medicago sativa* L. and mixed vegetables), located at the Agricultural Operations of the University of California, Riverside, were selected for the sampling surveys (Fig. 1). The alfalfa field was selected because it was a host for several stink bug species including *Nezara viridula* (Linnaeus), *Chlorochroa* sp., and *Thyanta* sp. during the spring and summer of 2017, making it a potential source of parasitoids that might parasitize *B. hilaris* eggs. The mixed vegetable field included bell pepper (*Capsicum annuum* L.), broccoli, cantaloupe (*Cucumis melo* L.), corn (*Zea mays* L.), okra (*Abelmoschus esculentus* L.), tomato (*Solanum lycopersicum* L.), and zucchini squash (*Cucurbita pepo* L.). Mid-way through the study (January 2018), a squash field (*Cucurbita mochata* L., cultivars black futsu and shishigatani) was selected when *B. hilaris* adults were found on shortpod mustard weeds within the field. In addition to these fields, sentinel egg cards were placed at other locations on the Agricultural Operations property where mustard weeds were found (Fig. 1).

Sentinel egg card preparation and deployment

Bagrada hilaris eggs (≤ 24 hours old) were collected and glued (Gorilla Super Glue Gel, The Gorilla Glue Co., Ohio, USA) on a 3 × 5 grid of squares on a weatherproof card so that each card contained 15 eggs (Fig. 2). For each location, two sentinel cards (a total of 30 eggs) were attached to the wire of a landscape flag (Fig. 3). The upper card

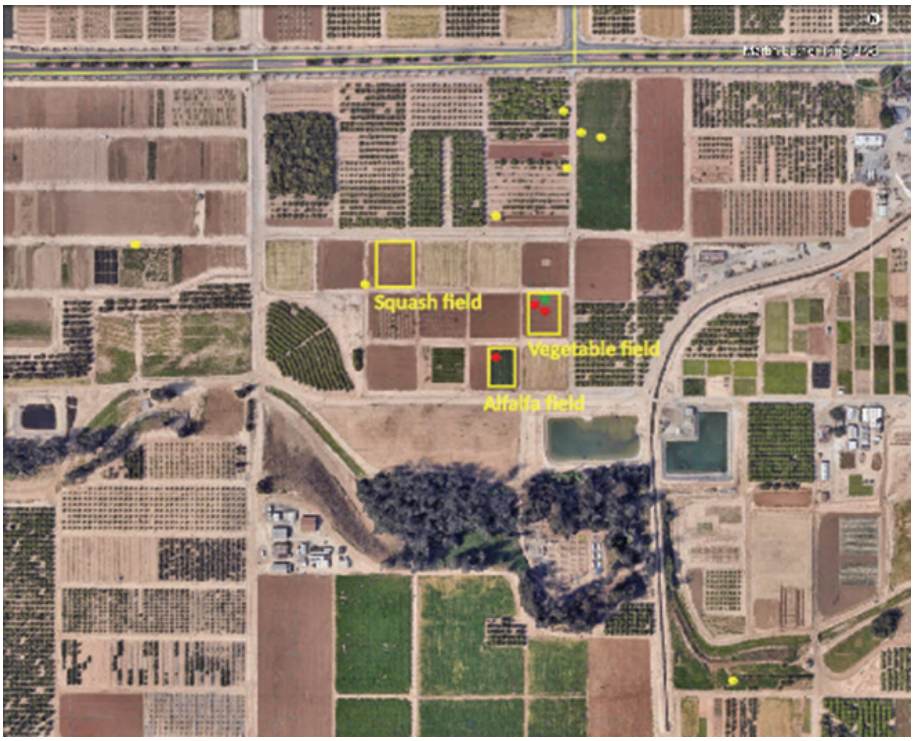


Figure 1. Locations at the Agricultural Operations of the University of California, Riverside that were selected to survey for resident egg parasitoids of *Bagrada hilaris*. Yellow squares show the host fields that included squash: Latitude 33°57'58.08"N, Longitude 117°20'35.37"W; alfalfa: Latitude 33°57'54.20"N, Longitude 117°20'27.01"W; and mixed vegetables: Latitude 33°57'56.32"N, Longitude 117°20'25.48"W. Yellow dots show other locations where mustard weeds were found and sentinel cards were placed. The green dot represents the location where *Trissolcus hyalinipennis* was recovered and the red dots represent the locations where *Trissolcus basalis* was recovered.

was taped to the flag wire, positioning it about 30 cm from the ground. The lower card was designed to be folded into a triangle through which the flag wire was used to secure it to the ground (Fig. 3). The number of cards used for each survey date varied from 16–20 depending on the availability of *B. hilaris* eggs. Sentinel egg cards were deployed in the survey sites starting in October 2017. Cards were left in the field for a maximum of 5 days, since *B. hilaris* eggs hatch after 5 days at similar temperatures (Reed et al. 2017). A total of 113 cards (1695 eggs) have been deployed to date. Surveys were conducted on October 21–25, 2017, November 3–8, 2017, January 5–7, 2018, January 12–16, 2018, January 19–23, 2018, and January 26–30 (Tab. 1). In the first 2 survey times, most of the eggs on the lower cards appeared to be eaten by predators (Fig. 4, Tab. 1) and the bug hatch rate was lower for the eggs glued on the lower cards than the upper cards (Tab. 1). Therefore, the surveys for January and beyond consisted only of upper cards.

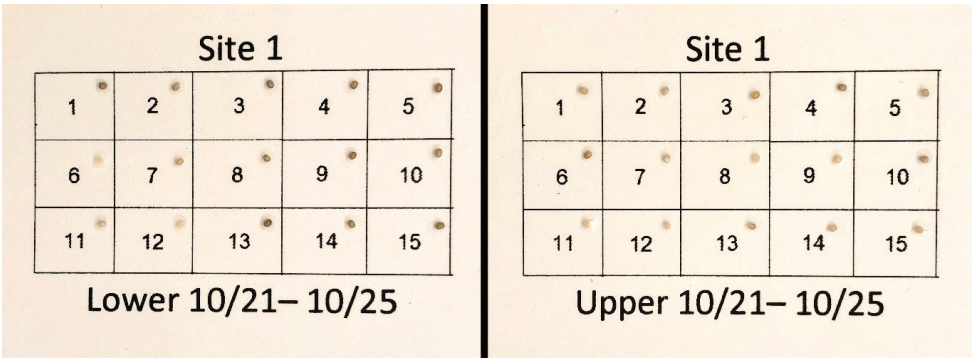


Figure 2. The sentinel card grids with fresh *Bagrada hilaris* eggs.

Figure 3. From left to right: The triangular and flat sentinel cards used in the study; the cards attached to a landscape flag wire in an alfalfa field; and in a vegetable field with broccoli and wild mustard.

Assessing eggs for parasitism

Once collected from the field, each grid was cut from the cards, placed in small Petri dishes (6 cm in diameter), and sealed with parafilm. The lid of the Petri dish had a 1-cm hole covered with a fine mesh screen for ventilation. The Petri dishes were maintained in the same insectary room that was used for the *B. hilaris* colony (30±1 °C, 40–50% humidity and 14:10 (L:D) photoperiod) and were checked daily for bug hatch or wasp emergence. Emerged wasps were transferred to vials containing 95% ethanol for identification.

BioSCAN Project

The Biodiversity Science: City & Nature (BioSCAN) is an urban biodiversity project of the Natural History Museum of Los Angeles County (LACM), which is attempting

Table 1. Results of the sentinel *Bagrada hilaris* egg cards that were deployed in two host fields.

Deployment dates	Host field	Card position	Number of Eggs					
			Initial number	Hatched	Parasitized	Predated	Missing/ Damaged	Dead
Oct 21–25 2017	Alfalfa	Upper/ Lower	75/ 75	12/ 3	0/ 0	0/ 57	0/ 0	63/ 15
	Vegetable	Upper/ Lower	75/ 75	2/ 2	3[a]/ 0	0/ 42	4/15	68/ 16
Nov 3–8 2017	Alfalfa	Upper/ Lower	75/ 75	17/ 0	0/ 0	8 / 61	2/ 0	48/ 14
	Vegetable	Upper/ Lower	75/ 75	12/ 2	0/ 0	0/ 42	1/ 15	62/ 16
Jan 5–7 2018	Alfalfa	Upper	75	19	0	0	0	56
	Vegetable	Upper	150	24	0	0	1	125
	Squash/Mustard	Upper	75	12	0	0	0	63
Jan 12–16 2018	Alfalfa	Upper	45	15	0	6	0	24
	Vegetable	Upper	150	35	II[b]	1	2	101
	Squash/Mustard	Upper	60	17	0	0	0	43
Jan 19–23 2018	Alfalfa	Upper	30	10	0	0	0	20
	Vegetable	Upper	150	36	0	6	4	104
	Mustard	Upper	60	10	0	3	5	42
Jan 26–30 2018	Alfalfa	Upper	45	7	9[b]	0	1	28
	Vegetable	Upper	150	30	I[b]	1	2	116
	Mustard	Upper	105	26	0	1	7	71

a: One *Trissolcus hyalinipennis* emerged; two other adult wasps were found dead in the eggs after dissection.
b: *Trissolcus basalis*. In the survey period of January 26–30, of 9 parasitized eggs from the alfalfa field, 8 *T. basalis* wasps emerged, and 1 dead adult wasp was found after egg dissection.
c: Sentinel egg cards were placed at other locations within the Agricultural Operations of the University of California, Riverside where shortpod mustard weeds were found.

to assess and understand the effects of urbanization on insects. Sampling has taken place across a large part of the urban areas of the Los Angeles Basin, the San Fernando Valley, and the San Gabriel Valley, a continuously urban area housing approximately 10 million people. In order to sample continuously in a variety of urban habitats, the project seeks volunteers ('citizen scientists') who agree to host a Malaise trap (lightweight Townes design) and weather station for an entire year. Specimens are captured and preserved in 95% ethanol, and sorted by staff and volunteers at the LACM.

Now entering its fourth year, the BioSCAN project has encountered an extraordinary diversity of unexpected species, including rare drosophilid flies (Grimaldi et al. 2015), at least one new mycetophilid fly (Kerr 2014), and 43 species of phorid flies new to science (Hartop et al. 2015, 2016). The project is ongoing, and analyses of the data on flies (Diptera) are progressing (Brown and Hartop 2016, Hartop et al. 2018). Yet, other insect groups have been given only cursory attention.

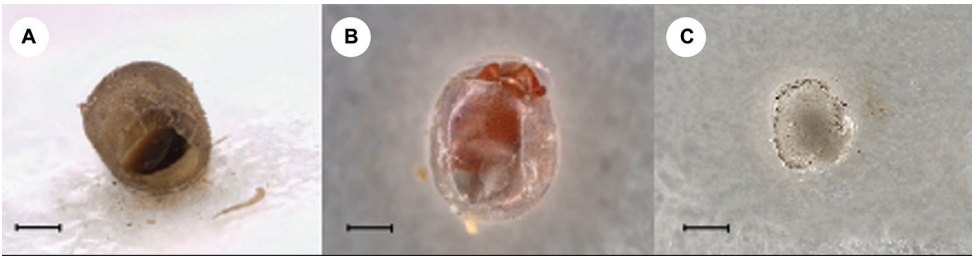


Figure 4. A *Bagrada hilaris* parasitized egg with the hole for wasp emergence **B** *Bagrada hilaris* egg hatching **C** *B. hilaris* egg showing signs that is had been eaten by a predator. Scale bars are 0.25 mm. Photo by Roger Burks.

Taxonomic methods

The recovered specimens of *T. hyalinipennis* were identified using recent treatments of *Trissolcus* in the Nearctic and Palearctic regions (Talamas et al. 2015a, Talamas et al. 2017). The DNA voucher specimen of *T. hyalinipennis* (DPI_FSCA 00010101) is deposited in the Florida State Collection of Arthropods (FSCA), Gainesville, FL. Specimens from the BioSCAN project are deposited in FSCA and the Los Angeles County Museum of Natural History. Vouchers of the specimens used to generate the DNA barcode database are deposited in FSCA, the National Museum of Natural History (USNM), Washington, DC, and the European Biological Control Laboratory (EBCL), Montpelier, France. Images of the specimens recovered in California, additional specimens of *T. hyalinipennis*, and all Nearctic and Palearctic species of *Trissolcus*, are available from the image database *Specimage* (specimage.osu.edu). Collection data associated with these specimens are deposited in the Hymenoptera Online Database (hol.osu.edu).

DNA extraction, PCR amplification and sequencing

Genomic DNA was nondestructively isolated from the whole specimen using the Qiagen DNeasy kit (Hilden, Germany) as described in Giantsis et al. (2016). 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'-TAAACTTCAGGGTGACCA-3') (Folmer et al. 1994). The PCR was performed in a 30 µl reaction volume: 2 µl of DNA, 3 µl of 10X Qiagen PCR buffer containing 15 mM $MgCl_2$, 0.9 µl of each primer (0.3 µM each), 0.6 µl of dNTPs (25 mM each), and 0.2 µl of 5 U/µl *Taq* DNA Polymerase (Qiagen, Hilden, Germany). The PCR conditions were as follows: 94°C for 3 min, followed by 40 cycles of 94 °C for 30 s, 52 °C for 1 min, 72 °C for 1 min with a final extension at 72 °C for 10 min. All PCR products were electrophoresed through agarose gel (1%), then sent to Genoscreen (Lille, France) for sequencing in both directions. Sequences were

Table 2. Sample information for the specimen and sequences included in the study.

Species	Collection code and Sex	Country, State	Year of collection	Locality	Host	Genbank accession number.
Trissolcus hyalinipennis	DPI_FSCA00010101 ♂	USA, California	2017	Riverside	*Bagrada hilaris*	(MG983475) This study
	PPl EBCL ♀	Pakistan, Punjab	2016	Toba Tek Singh		(MG983476)
	PP2 EBCL ♀	Pakistan, Punjab	2016	Toba Tek Singh		(MG983477)
	PP3 EBCL ♀	Pakistan, Punjab	2016	Toba Tek Singh		(MG983478)
	PP7 EBCL ♀	Pakistan, Punjab	2016	Toba Tek Singh		(MG983479)
	USNMENT01197282 ♀	Pakistan, Punjab	2014	Toba Tek Singh	*Podisus maculiventris*	(MG983480)
	USNMENT01197281 ♀	Pakistan, Punjab	2014	Toba Tek Singh		(MG983481)
Trissolcus erugatus	USNMENT01197260 ♀	USA, California	2015	Davis		(MG983482)
	USNMENT01197262 ♀	USA, California	2015	Davis		(MG983483)
	USNMENT01197263 ♀	USA, California	2015	Davis		(MG983484)
Telenomus podisi	OSUC 557747 *na*	USA, Ohio	2008	*na*	*na*	KR870964

The Genbank accession numbers *MG983476-84* were given to the above specimens that had been collected previously.

assembled and edited using Bioedit version 7 (Hall 1999). The consensus sequence was checked to detect frame-shift mutations and premature stop codons, indicating the presence of pseudogenes using the online ORFfinder available at: http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi. The sequences generated from this study and those from our custom barcode database are deposited in Genbank (Tab. 2). All residual DNAs are archived at the European Biological Control Laboratory (EBCL).

Sequence analysis

All sequences were aligned using ClustalW with default settings as implemented in Bioedit. Searches for sequence similarity against our custom database were performed using the online BLASTn available at: https://blast.ncbi.nlm.nih.gov. The pairwise nucleotide sequence distances among and within taxa were estimated using the Kimura 2-parameter model (K2P) of substitution using Mega 6 (Tamura et al. 2013). The best fitting evolution model was selected using Akaike information criterion (AIC) as implemented in jModelTest 2 (Darriba et al. 2012). Phylogenies then were inferred

under the optimal evolutionary model using Maximum Likelihood (ML) with Mega 6 and Bayesian inferences (BI) using MrBayes 3.2 (Ronquist et al. 2012). The nodes of confidence values (Bootstrap percentages, BP, and Posterior probabilities, PP) were mapped on the ML topology.

Results

The majority of sentinel eggs on the lower cards (76% and 81% in the alfalfa field, and 56% in the vegetable field) for the first two sampling periods were eaten by predators (Fig. 4, Tab. 1). Since these cards were placed on the ground, there was ample opportunity for generalist predators (ground beetles, ants, etc.) to eat these eggs. While we did not assess which predators may have been involved, we observed an abundance of argentine ants (*Linepithema humile* Mayr) in the area, suggesting their importance in this study. On the upper cards, most of the eggs (53–96%) died without hatching. We are not sure why this mortality was so high, but it may have been exposure to low night time temperatures during the survey periods. According to the meteorological data from the California Irrigation Management Information System (CIMIS) station in Riverside (UC Riverside #44), the minimum daily temperatures for our survey periods ranged from 3–21 °C. Reed et al. (2017) found that at a constant temperature of 20 °C, the egg hatchability was 40% and the first instar nymphs were not able to leave the surface of the egg, feed, and molt. Therefore, we expected that prolonged exposure of eggs to temperatures below 20 °C at night led to high egg mortality. Predation was rare on the upper cards, therefore our surveys in January 2018 consisted of upper cards only.

In our first survey period (October 21–25), 3 eggs on one of the upper cards in the vegetable field were parasitized. A single *T. hyalinipennis* male emerged from one of the eggs, while the other two parasitized eggs did not hatch. Dead adult wasps were found in those eggs after dissection, but due to the damage caused by extraction from the egg, we were unable to identify them. In the survey period of January 12–16, 11 eggs on a single card from the vegetable field were parasitized, and we recovered 11 wasps identified as *Trissolcus basalis* (Wollaston). In the survey period of January 26–30, nine eggs on a single card that had been placed in the alfalfa field were parasitized from which eight *T. basalis* wasps emerged and one dead adult wasp was found after egg dissection. Also, one *T. basalis* adult emerged from a parasitized egg on another card from the vegetable field in the same survey period (Tab. 1).

BioSCAN Survey

Five females of *T. hyalinipennis* were recovered from a backyard in Burbank California (34.165°N, -118.323°W). Samples were examined for five of the 18 sites for 2017, and additional recoveries are expected as the process of sorting and identifying material from this survey continues.

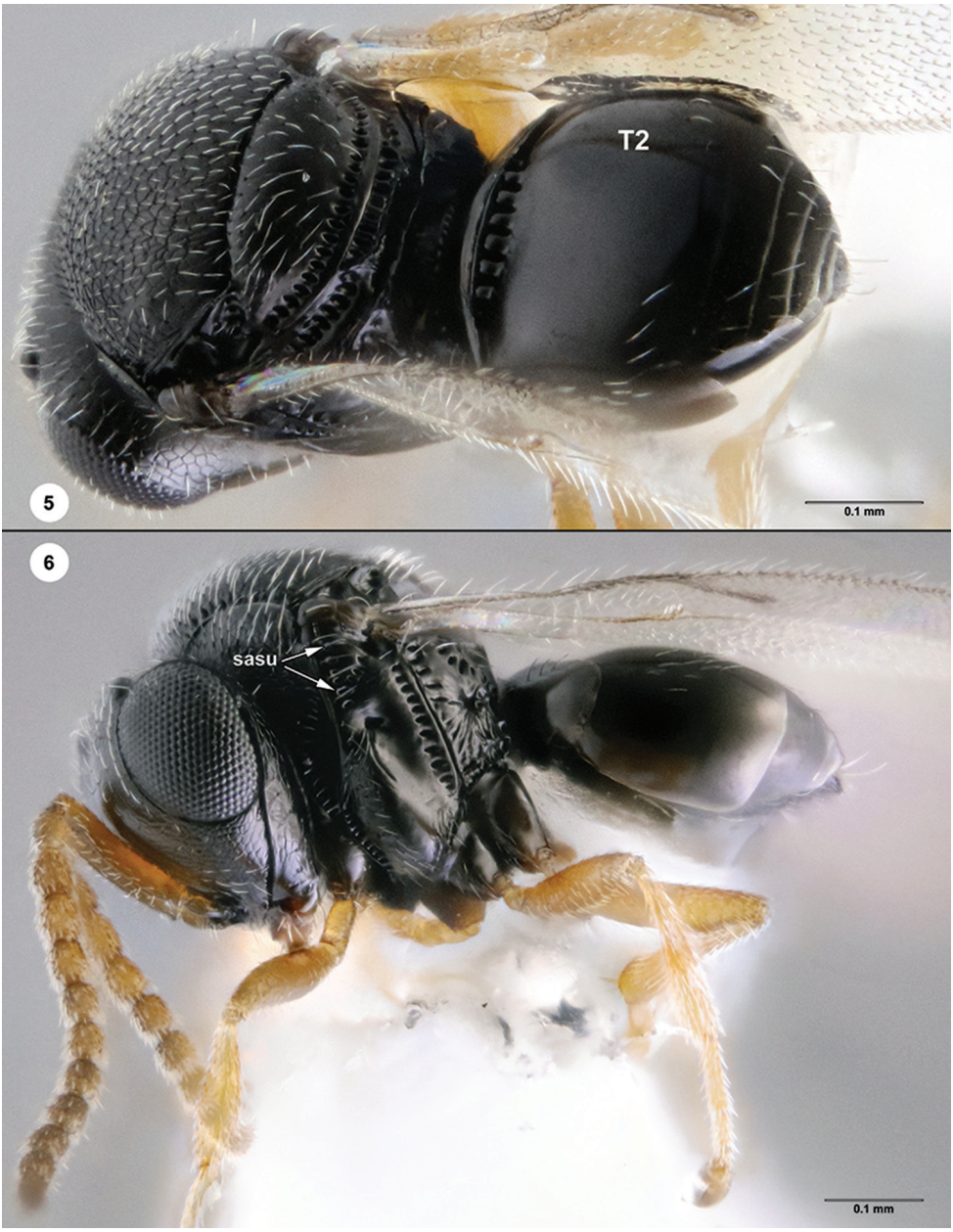
Taxonomy

Generic identification

The five-merous clava found in Nearctic *Trissolcus* was used by Talamas et al. (2015a) to help separate *Trissolcus* from *Telenomus,* in which the number of clavomeres is usually four, but can be five or six. The arrival of *T. hyalinipennis* into the Nearctic region significantly decreases the utility of this character because females of *T. hyalinipennis* have only four clavomeres. Talamas et al. (2017) provided a new character with potential to separate *Trissolcus* from *Telenomus*: the presence of subacropleural and prespecular sulci, which are clearly defined in *T. hyalinipennis* (Figs 6, 9). This character, however, may be difficult for non-taxonomists to interpret and it has yet to be thoroughly examined in *Telenomus*. In the context of species that are known to attack the eggs of stink bugs in North America, these genera can be separated much more easily by the shape of the second metasomal tergite (T2) in dorsal view, which is wider than long in *Trissolcus* (Figs 5, 10, 13–14) and longer than wide in *Telenomus* (Fig. 7).

Species identification

The four-merous clava in females of *T. hyalinipennis* (Fig. 8) enables them to be separated from the vast majority of other *Trissolcus*, which have a five-merous clava (Figures 15, 17). Only one other species of *Trissolcus*, *T. oobius* (Kozlov) from the Palearctic region, is known to also have a four-merous clava. Males do not display this feature, requiring reliance on other characters for identification. The specimens recovered in this study key to *T. erugatus* in the dichotomous key of Talamas et al. (2015a), which differs from *T. hyalinipennis* by the presence of episternal foveae (Fig. 15). The absence of episternal foveae in *T. hyalinipennis* is a useful character because these foveae are present in nearly all other species of Nearctic *Trissolcus*. During the course of this study, specimens of *T. basalis* were reared from sentinel eggs of *B. hilaris* in the same locality where *T. hyalinipennis* was discovered. These specimens are unusually small because of the small size of bagrada bug eggs. The episternal foveae tend to be small and shallow in typical specimens of *T. basalis* (Fig. 16), and the diminutive size of the specimens that emerged from bagrada bug eggs contributes to an even weaker expression of these foveae (Fig. 17). These small specimens of *T. basalis* also exhibit striation on T2 that is extremely faint to absent (Fig. 14), as is found in *T. hyalinipennis* (Figs 5, 10). Males, which lack the diagnostic antennal clava, are thus very similar in appearance to males of *T. hyalinipennis*. They can be separated efficiently based on the form of the netrion sulcus, which is dorsally complete in *T. hyalinipennis* (Figs 6, 8–9) and incomplete in *T. basalis* (Figs 16–17).



Figures 5–6. *Trissolcus hyalinipennis*, female (DPI_FSCA 00010101) **5** habitus, posterodorsal view **6** habitus, lateral view. sasu: subacropleural sulcus; T2: second metasomal tergite.

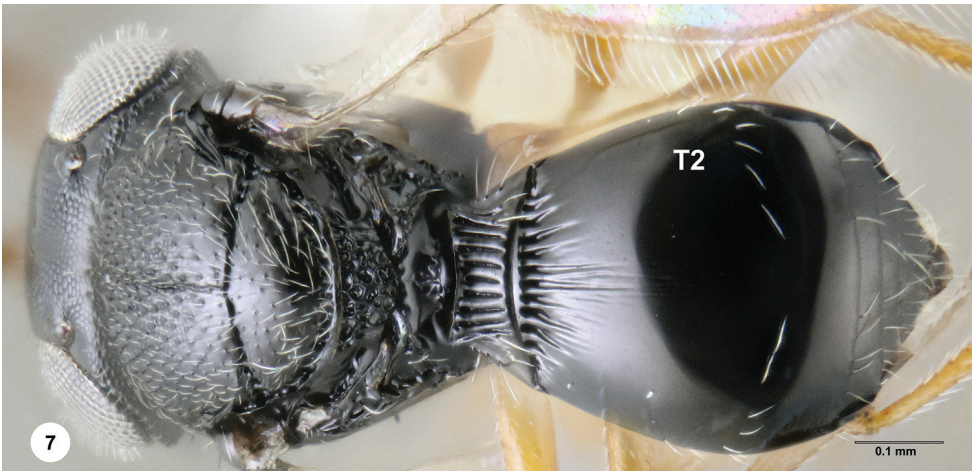
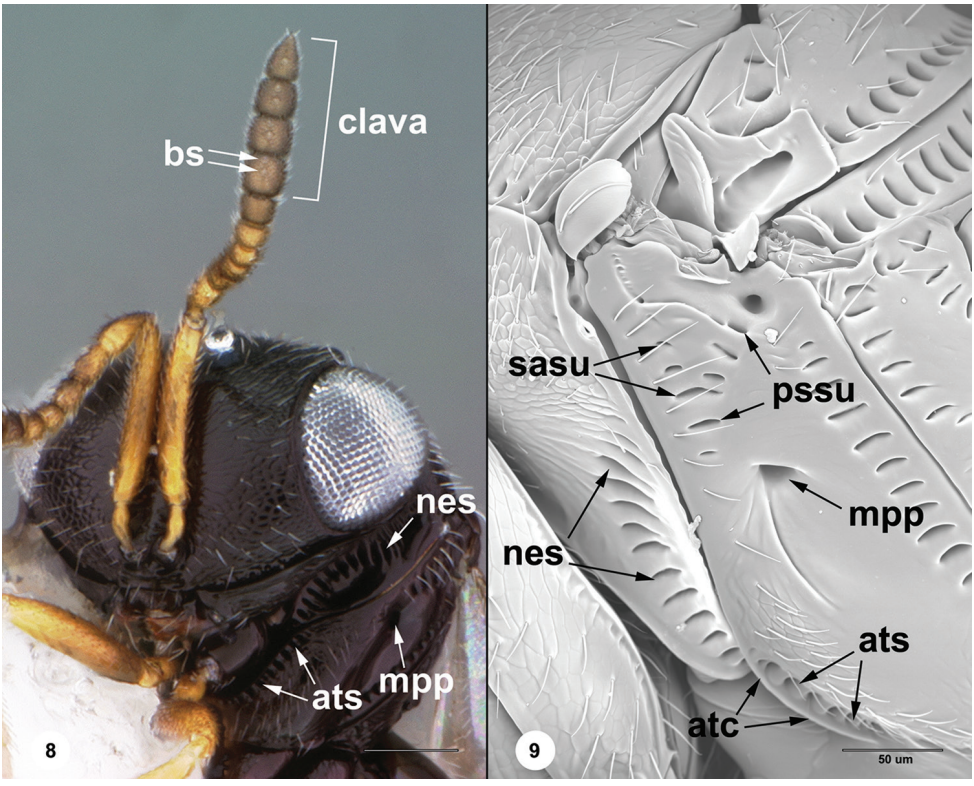
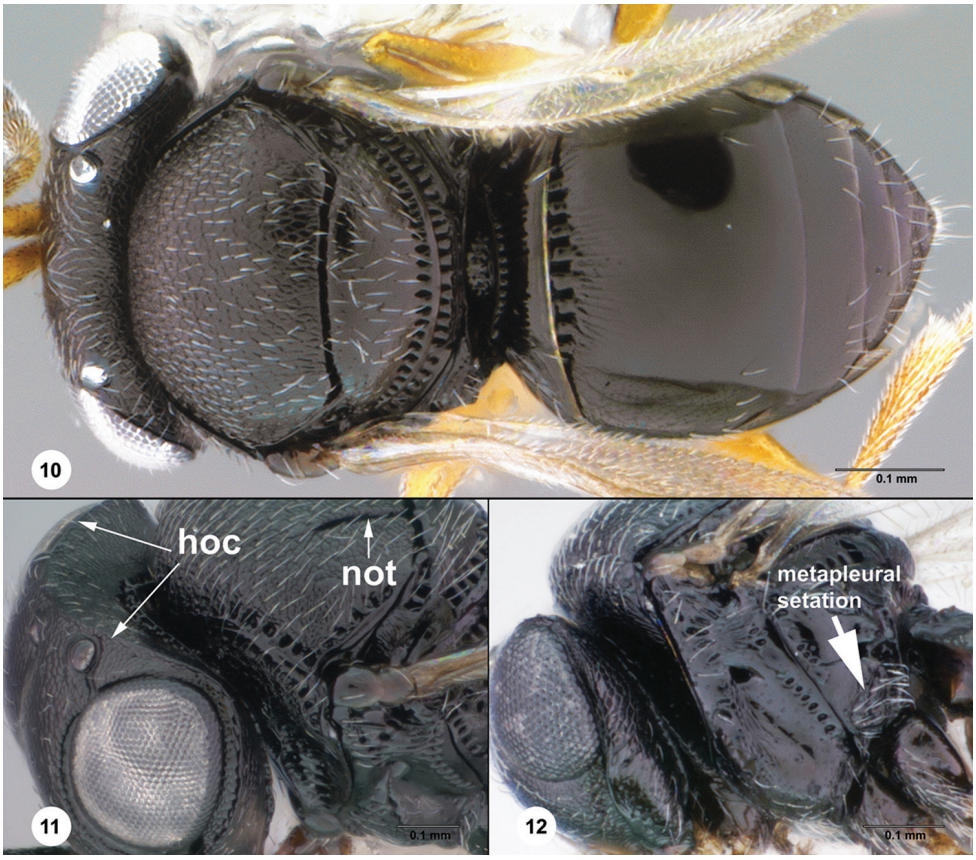


Figure 7. *Telenomus podisi*, female (DPI_FSCA 00010237), habitus, dorsal view. T2: second metasomal tergite.



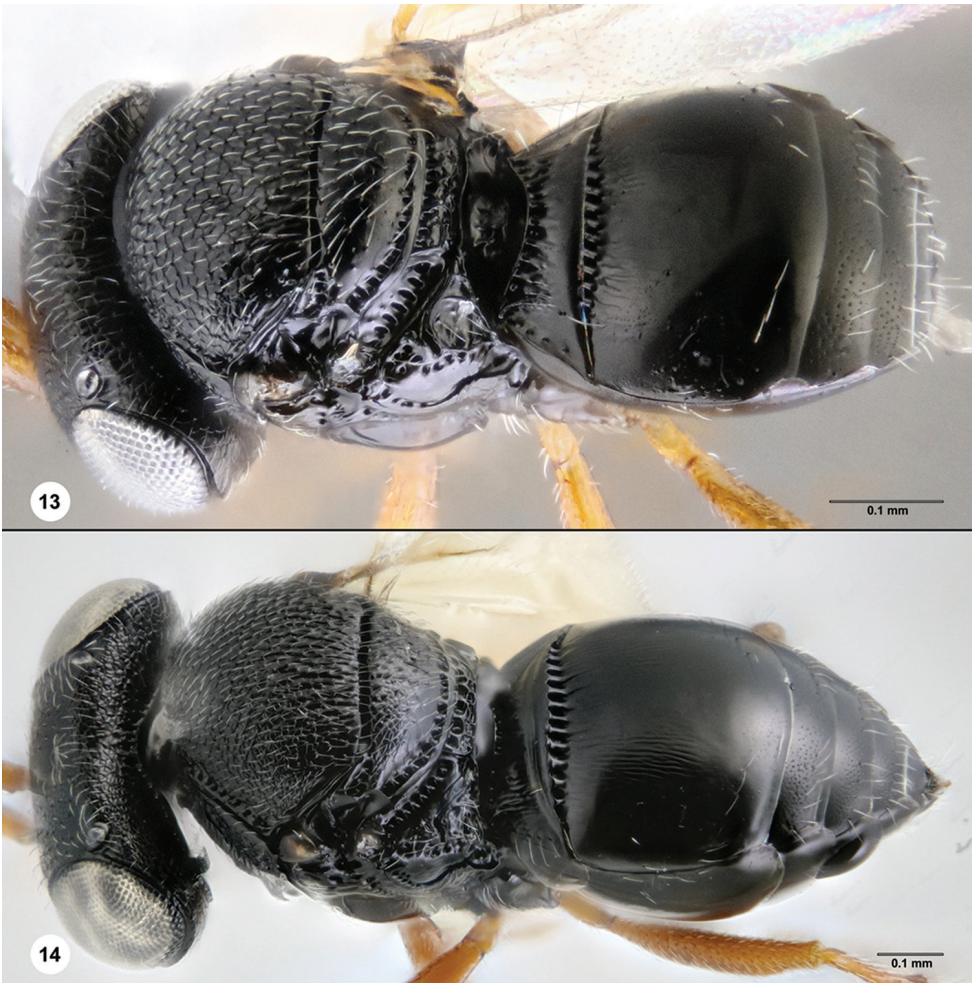
Figures 8–9. *Trissolcus hyalinipennis* **8** female (USNMENT01109060), head and mesosoma, anterolateral view **9** female (USNMENT01109061), mesosoma, lateral view. atc: acetabular carina; ats: postacetabular sulcus; bs: basiconic sensillum; nes: netrion sulcus; mpp: mesopleural pit; sasu: subacropleural sulcus.



Figures 10–12. 10 *Trissolcus hyalinipennis*, female (USNMENT01109062), habitus, dorsal view **11** *Trissolcus euschisti*, female (OSUC 334007), head and mesosoma, dorsolateral view **12** *Trissolcus thyantae*, (OSUC 76325), head and mesosoma, lateral view. hoc: hyperoccipital carina; not: notaulus; white arrow in Fig. 12 indicates posteroventral setose area of metapleuron.

Morphological diagnosis of *T. hyalinipennis*

The following characters found in *T. hyalinipennis* will separate both males and females from other *Trissolcus* in the Nearctic region: vertex without hyperoccipital carina (Fig. 10, compare to Fig. 11); frons without coarse sculpture (Fig. 8); female antennae with four clavomeres (Fig. 8, compare to Fig. 15, 17); netrion sulcus complete, extending dorsally to posterior margin of pronotum (Figs 6, 8–9, 15, compare to Figs 16–17); postacetabular sulcus comprised of discrete cells or punctures (Figs 6, 8–9, compare to Figs 16–17); anteroventral mesopleuron without episternal foveae (Figs 6, 8–9, compare to Figs 15–16); posteroventral metapleuron without setae (Fig. 6, compare to Fig. 12); mesoscutellum without coarse sculpture (Figs 5, 10); second metasomal tergite (T2) smooth and without prominent striae (Figs 5, 10).

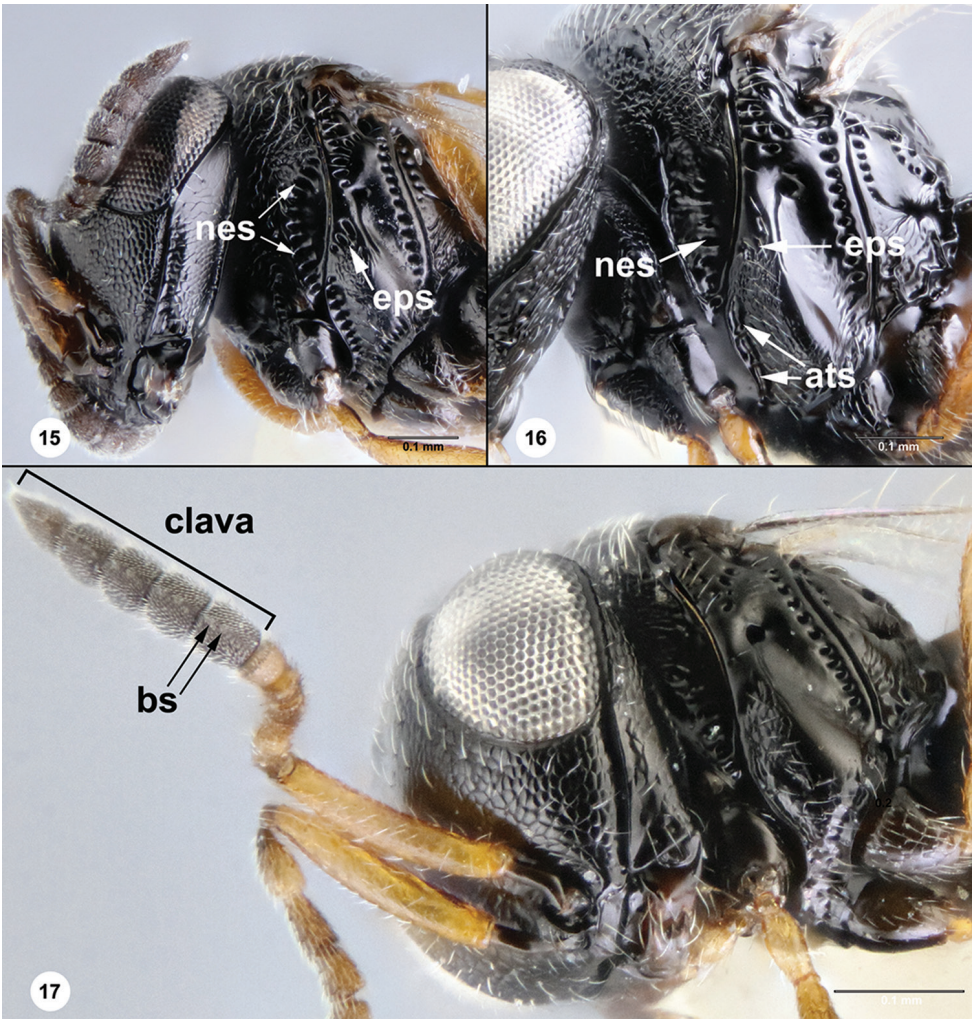


Figures 13–14. *Trissolcus basalis*. **13** male (DPI_FSCA 00009879), habitus, dorsolateral view **14** female (DPI_FSCA 00009651), habitus, dorsolateral view.

We here present a link to an online multichoice key to Nearctic *Trissolcus* that includes *T. hyalinipennis* and newly documented variation in *T. basalis*: http://idtools.org:8080/key_server/player.jsp?keyId=62.

Molecular identification

Over the past decade, the integration of molecular barcoding and traditional taxonomic methods has facilitated accurate identification and counting of insect species (Hebert et al. 2016). While the barcoding itself is not sufficient for robust phylogenetic tree generation in complicated taxonomic groups such as Nearctic and Palearctic *Trissolcus* (Bon, unpub-



Figures 15–17. 15 *Trissolcus erugatus*, female (USNMENT01197263), head and mesosoma, ventrolateral view **16** *Trissolcus basalis*, female (DPI_FSCA 00009651), head and mesosoma, ventrolateral view **17** *Trissolcus basalis*, female (DPI_FSCA 00009880), head and mesosoma, ventrolateral view. ats: postacetabular sulcus; bs: basiconic sensillum; eps: episternal foveae; nes: netrion sulcus.

lished data), it can provide an insightful means for disentangling species complexes and for preliminary species assignment. This is precisely the situation we have in the present study, where, after our first morphological examination, the initial male specimen collected in Riverside could be assigned to *T. hyalinipennis* or *T. erugatus*. When this study was initiated, Genbank and Barcode of Life Data Systems (BOLD) public nucleotide databases lacked reference barcode sequences of *T. hyalinipennis* and *T. erugatus*, although a multilocus DNA based sequence phylogeny of expertly-identified *Trissolcus* specimens associated with pentatomids, including *T. hyalinipennis* and *T. erugatus* is in progress (Talamas

Table 3. Barcode mean pairwise genetic distances(± STD) between this study's specimen and expertly identified *T. hyalinipennis* and *T. erugatus* (under the diagonal), and within taxa (along the diagonal).

	This study's specimen (*n*=1)	***T. hyalinipennis* (*n*= 6)**	***T. erugatus* (*n*= 3)**
This study's specimen	*NIA*	99[1]	87
T. hyalinipennis (*n*= 6)	0.005 ± 0.002	0.002 ± 0.001	n/c
T. erugatus (*n*= 3)	0.145 ± 0.017	0.143 ± 0.016	0.006 ± 0.002

n: number of sequences.

[1]: Blast identity score (in%) with all E values= 0 (above the diagonale)

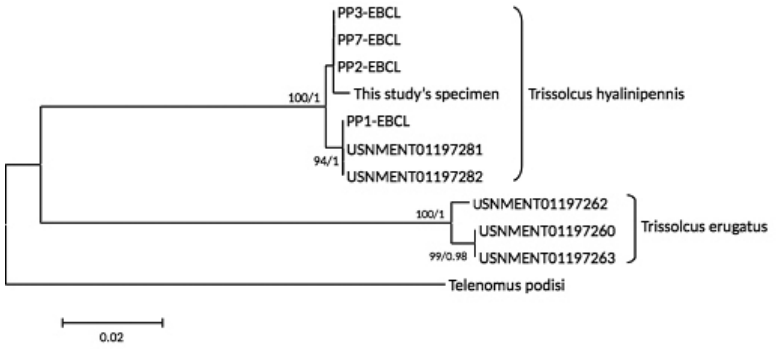


Figure 18. Rooted phylogram depicting the relationships between all specimens analyzed based on the barcode dataset alignment. Bootstrap percentages (BP) ≥ 75 and Posterior Probabilities (PP)≥ 0.90 are indicated at nodes. Each line represents a sequenced specimen with reference to its collection identifier.

and Bon, unpublished). We used the unpublished barcodes obtained for these two taxa to build a custom barcode database for comparison with the DNA barcode of the recovered specimen (Tab. 3). All of these unpublished barcodes were obtained as described hereafter. We also retrieved one barcode of *Telenomus podisi* (Accession number KR870964) from Genbank to be used as an outgroup for the phylogeny.

A barcode sequence of 661 base pairs (bp) was obtained from the specimen, and aligned with the nine barcode sequences of *T. hyalinipennis* and *T. erugatus* of our custom database (Tab. 3). The final alignment of 10 sequences consisted of 605 characters. To the best of our knowledge, these sequences constitute the first published barcode sequences for both *T. hyalinipennis* and *T. erugatus*. The Blast search of our query sequence against our custom database showed that the best identity score (99%) was obtained with all *T. hyalinipennis* whereas it was only 87% with all *T. erugatus* sequences (Tab. 3). Pairwise distance values within and among analyzed species are shown in Tab. 3. The genetic distances between the specimen from this study and all *T. hyalinipennis* specimens, which averaged 0.005 (± 0.002), were much lower than the mean pairwise distance observed between the specimen from this study and all of the *T. erugatus* specimens (0.145±0.017). The evolutionary model selected by jModelTest was the Tamura 3-parameter T92. ML topology obtained from the barcode dataset was

identical to the one produced from the Bayesian analysis, and the phylogenetic tree resulting from the ML analysis is presented in Fig. 18. We found that the distinctiveness of the two *Trissolcus* species always had high node supports, and that the present specimen was clearly nested within *T. hyalinipennis*. Taken as a whole, the molecular results confirm the morphological identification.

Discussion

The molecular genetic analyses clearly indicated that the sole male specimen collected in Riverside is indistinguishable from *T. hyalinipennis* from Pakistan, and thus appears to be an introduction to the USA. Our assertion that *T. hyalinipennis* is established in California is supported by the recovery of female specimens in Burbank, approximately 65 miles away. The discovery of *T. hyalinipennis* in southern California marks the third time in five years that an alien scelionid parasitoid of pentatomoid eggs has been found in the USA while under evaluation as a classical biological control agent (Gardner et al. 2013, Talamas et al. 2015b). These accidentally introduced parasitoids were discovered only because specialists were searching for the presence of parasitoids of their stink bug targets and working in conjunction with taxonomists. In the case of *T. hyalinipennis*, the fortuitous recovery of this species by the BioSCAN survey serves as a testament to the impact that citizen science programs can have in detecting species of agricultural and economic significance.

It is likely that accidental introductions of parasitoids are constant occurrences, but establishment does not become possible until their host is sufficiently available. In addition to these biocontrol candidates, the phenomenon of "tramp" species has recently been documented in other platygastroid wasps, including both scelionids and platygastrids (Masner et al. 2009, Popovici et al. 2018). We suspect that the number of cosmopolitan species is much higher than currently documented, primarily because recognition of them requires a world perspective for taxa that are often speciose and not well studied.

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