An integrated approach reveals cryptic species of *Trissolcus* Ashmead (Hymenoptera, Scelionidae), egg parasitoids of Pentatomidae (Hemiptera)

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Abstract

Accurate identification of parasitoids is crucial for biological control of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål). A recent work by Talamas et al. (2017) revised the Palearctic fauna of *Trissolcus* Ashmead, egg-parasitoids of stink bugs, and treated numerous species as junior synonyms of *T. semistriatus* (Nees von Esenbeck). In the present paper, we provide a detailed taxonomic history and treatment of *T. semistriatus* and the species treated as its synonyms by Talamas et al. (2017) based on examination of primary types, molecular analyses and mating experiments. *Trissolcus semistriatus*, *T. belenus* (Walker), *T. colemani* (Crawford), and *T. manteroi* (Kieffer) are here recognized as valid and a key to species is provided. The identification tools provided here will facilitate the use of *Trissolcus* wasps as biological control agents and as the subject of ecological studies.

Keywords

Biological control, taxonomy, brown marmorated stink bug
Introduction

Taxonomy of the genus *Trissolcus* Ashmead has received renewed attention in recent years (Talamas et al. 2015, 2017), largely because accurate identification of these wasps is needed to use them as biological control agents against the invasive brown marmorated stink bug (*Halyomorpha halys* (Stål)) in Europe and North America. Morphological similarity, sharing of hosts by various species of *Trissolcus*, and the historical complications presented in Talamas et al. (2017) and Buffington et al. (2018) are some of the challenges faced by taxonomists working with this group.
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The revision of Palearctic *Trissolcus* (Talamas et al. 2017) provided keys to species, complete redescriptions, illustrations, and the utilization of new morphological characters. Many new synonymies were presented, including *T. grandis* (Thomson), *T. artus* Kozlov & Lê, *T. colemani* (Crawford), *T. djadetsko* (Rjachovskij), *T. manteroi* (Kieffer), *T. nigripedius* (Nakagawa), *T. pentatomae* (Rondani) and *T. pseudoturesis* (Rjachovskij) as junior synonyms of *T. semistriatus* (Nees von Esenbeck).

In support of studies on the egg-parasitoid complex of European Pentatomoida, a survey of egg masses was conducted and previously collected specimens were also examined. Using the key to species provided by Talamas et al. (2017), *Trissolcus* specimens that emerged from *Aelia rostrata* Boheman, *Arma custos* (F.), *Carpocoris* spp., *Eurygaster maura* (L.), *Graphosoma lineatum* (L.), *Palomena prasina* (L.) collected between 1996 and 2017 in Piedmont (NW Italy) were identified as *T. semistriatus*. However, some consistent morphological differences were detected among the specimens, which instigated closer examination using multiple methods. The focus of this paper is the morphological and molecular analysis of species synonymized under *T. semistriatus* by Talamas et al. (2017), and the integration of mating tests, when possible, to confirm species delimitation.

**Taxonomic history of *T. semistriatus* and related species**

**Species described by Walker**

*Telenomus belenus* was described by Walker (1836), then transferred by Kieffer (1912) to *Aphanurus* Kieffer, then transferred to *Microphanurus* Kieffer (Kieffer 1926). Walker (1838) described *Telenomus arminon* but did not provide distinctive characters by which it could be identified or separated from *Telenomus belenus*. Kieffer (1912) transferred *Te. arminon* to *Allophanurus* Kieffer and provided a redescription. Kieffer did not mention if his treatment was based on type material, and we consider it unlikely that it was. Lectotypes for *Te. belenus* and *Te. arminon* were designated by Fergusson (1984, 1983), respectively, from material housed in the National Museum of Ireland, Dublin. Despite their antiquity, and thus priority, these species received no further taxonomic treatment.

*Trissolcus semistriatus* vs. *T. grandis*

In taxonomic literature, the distinction between *T. semistriatus* and *T. grandis* has long been questioned. Mayr (1879) and Nixon (1939) ascertained *T. semistriatus* to be a highly variable species. Masner (1959) wrote “On base of the check of type of *Asolcus grandis* (Thomson), the latter species was synonymized with *semistriatus*”. However, the meaning of this sentence is unclear because we have not found in the literature previous synonymy of *T. grandis* under *T. semistriatus*, and it is not clear that Masner

sought to synonymize them for the first time. In this paper, Masner addressed characters considered to distinguish *T. grandis* and *T. semistriatus* (rugosity of the frons, leg color, longitudinal sculpture on the posterior mesoscutum, body length) based on the comparison of ~500 reared specimens and stated that these characters were variable within *T. semistriatus*. Viktorov (1967) considered *T. grandis* to be conspecific with *T. semistriatus*, but he did not formally treat it as a junior synonym. Subsequent authors considered *T. semistriatus* and *T. grandis* as different species, but without clearly defining the boundaries between them. Delucchi (1961) provided the first reliable character to distinguish *T. semistriatus* from *T. grandis*: the external surface of the hind femur is almost totally covered by setation in *T. grandis* (Figure 1), and he coupled this character with the color of the tibiae: reddish yellow in *T. semistriatus*, dark or black in *T. grandis*. Most authors continued to distinguish *T. semistriatus* and *T. grandis* by tibial color and ignored setation of the hind femora. This color-based distinction was employed in numerous previous and following papers (Delucchi 1961; Javahery 1968; Kozlov 1968, 1978; Safavi 1968; Voegelé 1969; Fabritius 1972; Kozlov and Lê 1977; Kozlov and Kononova 1983), and no substantial change was indicated in keys to species by Kononova (1995, 2014, 2015). Talamas et al. (2017) did not use tibial setation to differentiate between these species, but listed a new character, the form of the mesocutal humeral sulcus, and mentioned setation of the first laterotergite, which was first presented as a character for species of *Trissolcus* by Johnson (1987). Although Talamas et al. (2017) treated these characters as variable within *T. semistriatus*, analysis of these characters in light of molecular and mating experiments has allowed us to use them for species delimitation.

In a study on larval stages, Voegelé (1964) provided information about pigmentation of the membrane secreted by the larvae of different *Trissolcus* species reared in eggs of *Eurygaster austriaca* (L.). He distinguished *T. semistriatus* from *T. grandis* by the width of the pigmented band close to the margin of host egg operculum (see fig. 4 in Voegelé 1964). In his key to species, Safavi (1968) coupled color of the hind tibia (instead of mid tibia), and width of the pigmented band in larval membrane shown by Voegelé (1964), also adding different length ratios of the first two flagellomeres in males.

*Trissolcus artus* was distinguished by Kozlov and Kononova (1983) and Kononova (1995) from *T. grandis* (black tibiae) by its reddish-yellow tibiae, and from *T. semistriatus* by having a more elongate clava and infuscation in the fore wing. This last feature is used in the key by Kononova (2014, 2015) to distinguish *T. artus* from both *T. grandis* and *T. semistriatus*.

*Trissolcus manteroi*

*Trissolcus manteroi* was described by Kieffer (1909) as having the postmarginal vein (pm) slightly longer than the stigmal vein (st). In Kozlov and Kononova (1983), Koçak and Kilinciğer (2003) and Kononova (2014, 2015), *T. manteroi* was distinguished by its postmarginal vein 1.3× as long as the stigmal vein, compared to 1.8× in *Trissolcus rufiventris* (Mayr), and 2× in *T. grandis* (=*T. belenus*) and *T. semistriatus*. Kononova
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Figure 1. Illustrations published by Delucchi (1961) where differences in the bare area of the external side of hind femora of *Asolcus semistriatus* (Fig III, I) and *A. grandis* (Fig. III, H) are shown.
(2014, 2015) also distinguished *T. manteroi* by the sculpture of T2, in which short longitudinal rugae are arranged medially and do not extend to the posterior half of the tergite, contrasting with longitudinal rugae throughout the anterior two thirds of T2 in *T. belenus* and *T. semistriatus*.

**Trissolcus colemani**

Crawford (1912) described *Telenomus colemani* from specimens that emerged from an egg mass of *Dolycoris indicus* Stål, collected in India. Masner and Muesebeck (1968) transferred this species into *Trissolcus* and no other information was recorded until its treatment as a junior synonym of *T. semistriatus* in Talamas et al. (2017).

**Trissolcus pseudoturesis and T. djadetsbko**

The original description of *Microphanurus (=Trissolcus) pseudoturesis* Rjachovskij (Rjachovskij 1959) distinguished this species from *M. djadetsbko* Rjachovskij and *M. semistriatus* by tibial color: completely yellow in *M. pseudoturesis*; reddish or yellow in *M. djadetsbko*; almost black in *M. semistriatus*. Viktorov (1964) distinguished *Asolcus (=Trissolcus) djadetsbko* and *A. rufiventris* by the lack of longitudinal striae on the posterior margin of the mesoscutum in contrast to their presence in *A. pseudoturesis* and *A. semistriatus*. Viktorov (1967) then modified his concept, considering the color of the hind tibia as a valid character to distinguish *T. djadetsbko* from *T. semistriatus* and the color of femora to distinguish *T. djadetsbko* from *T. pseudoturesis*. The keys to species by Kozlov (1968) and Fabritius (1972) distinguished *T. djadetsbko* from *T. grandis*, *T. pseudoturesis* and *T. semistriatus* by the absence of longitudinal striation on the posterior mesoscutum and an absence of transverse striation on the frons, and *T. pseudoturesis* from *T. grandis* and *T. semistriatus* by color of the femora. Kozlov and Kononova (1983) separated *T. djadetsbko* from both *T. grandis* and *T. semistriatus* by the absence of longitudinal striation on the posterior mesoscutum. Safavi (1968) and Voegelé (1969) separated *T. djadetsbko* and *T. pseudoturesis* by their “ochraceous” femora from *T. semistriatus* and *T. grandis* (black femora), and separated *T. djadetsbko* from *T. pseudoturesis* by longitudinal striae on the posterior margin of mesoscutum (vs. striate throughout) and the presence of parapsidal furrows. Koçak and Kilinçer (2003) distinguished *T. djadetsbko* by its femora being reddish-yellow in contrast with dark brown or black femora in *T. semistriatus* and *T. grandis*, and separated *T. djadetsbko* from *T. pseudoturesis* by sculpture on mesoscutum as in Voegelé (1969). Petrov (2013) again distinguished *T. djadetsbko* on the basis of the mesoscutum without longitudinal wrinkles, contrasting with the clear longitudinal wrinkles of *T. grandis*, *T. pseudoturesis* and *T. semistriatus*, and he separated *T. pseudoturesis* from *T. grandis* and *T. semistriatus* by the color of femora. Kononova (2014, 2015) differentiated *T. djadetsbko* by its yellow legs and mesoscutum without longitudinal rugae posteriorly from *T. semistriatus*.
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and *T. grandis* having all femora black and mesoscutum with longitudinal rugae posteriorly, and *T. pseudoturesis* from *T. grandis* and *T. semistriatus* as in Kozlov (1968). *Trissolcus djadetshko* and *T. pseudoturesis* were treated as junior synonyms of *T. semistriatus* in Talamas et al. (2017).

**Trissolcus waloffae, T. nixomartini and T. silwoodensis**

Javahery (1968) described and keyed *T. waloffae* (Javahery) using leg color (predominantly brownish to reddish-yellow) and weakly indicated parapsidal furrows to separate it from *T. grandis*, *T. semistriatus*, *T. nixomartini* and *T. silwoodensis*, which he considered to have black femora in both sexes and be without parapsidal furrows. Characters provided to distinguish each of the last four species from each other were black vs. brown front tibiae, presence of infuscation of wings, color of wing venation, ratio between first flagellar segment and pedicel of male, sculpture of the head, distance between lateral ocelli and compound eye, and ‘weakly concave’ vs. ‘somewhat concave’ head. *Trissolcus silwoodensis* and *T. nixomartini* were previously treated as synonyms of *T. grandis* by Kozlov and Lê (1977).

**Trissolcus crypticus**

During a program for classical biological control of *Nezara viridula* L. in Australia, several ‘strains’ of different geographical populations of *Trissolcus basalis* (Wollaston) were introduced, starting in the 1930s (Clarke 1990). Of the strains introduced in subsequent years to the interior of Australia, one population imported from Pakistan (1961) was not able to efficiently control *N. viridula* (Clarke 1990). Clarke (1993) demonstrated that this ‘strain’ was indeed a different species, which he described as *Trissolcus crypticus* Clarke. Comparing *T. crypticus* with *T. basalis*, he considered the complete netrion sulcus (figure 1 in Clarke 1993) as the main diagnostic character for *T. crypticus*. Clarke analyzed specimens of *Trissolcus rungsi* (Voegelé) labelled by Voegelé and deposited in NHMUK and concluded that they were not the same species as *T. crypticus*, but did not present characters to support his hypothesis (Clarke 1993).

**Material and methods**

**Collections**

Primary types

Due to the challenge of historic confusion regarding species close to *T. semistriatus*, we treat only species for which the primary types were directly examined, or the diagnostic characters are clearly visible in photographs.
Images of the primary types of *Telenomus colemani* Crawford, *Microphanurus djadetskoi* Rjachovskij, *Trissolcus grandis* Thomson, *Telenomus Manteroi* Kieffer, *Microphanurus pseudoturesis* Rjachovskij and *Telas semistriatus* Nees von Esenbeck were made available via Specimage (specimage.osu.edu) by Talamas et al. (2017). Images of the lectotype of *Telenomus nigripes* Thomson, syntypes of *Telenomus ovulorum* Thomson, and additional images of the lectotype of *Telenomus grandis* were provided by Dr Hege Vårdal (Naturhistoriska Riksmuseet, Stockholm, Sweden).

Institutional acronyms

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<thead>
<tr>
<th>Acronym</th>
<th>Institution</th>
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<tr>
<td>CNCI</td>
<td>Canadian National Collection of Insects – Ottawa, Canada;</td>
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<tr>
<td>DISAFA</td>
<td>Dipartimento di Scienze Agrarie, Forestali e Alimentari, University of Torino – Torino, Italy;</td>
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<tr>
<td>EIHU</td>
<td>Hokkaido University Museum, Entomology – Sapporo, Japan;</td>
</tr>
<tr>
<td>HMIM</td>
<td>Hayk Mirzayans Insect Museum, Plant Pests and Diseases Research Institute – Tehran, Iran;</td>
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<tr>
<td>NHMUK, BMNH</td>
<td>The Natural History Museum – London, United Kingdom;</td>
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<td>NHMW</td>
<td>Naturhistorisches Museum Wien – Wien, Austria;</td>
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<td>NMID</td>
<td>National Museum of Ireland – Dublin, Ireland;</td>
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<tr>
<td>MSNG, MCSN</td>
<td>Museo Civico di Storia Naturale “Giacomo Doria” – Genoa, Italy;</td>
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<tr>
<td>MZUF</td>
<td>Museo di Storia Naturale di Firenze, Sezione di Zoologia “La Specola”, Università degli Studi di Firenze – Florence, Italy;</td>
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<tr>
<td>NHRS</td>
<td>Naturhistoriska Riksmuseet, Entomology – Stockholm, Sweden;</td>
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<tr>
<td>UCRC</td>
<td>University of California, Riverside – CA, USA;</td>
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<tr>
<td>UNIPA</td>
<td>Dipartimento di Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo – Palermo, Italy;</td>
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<tr>
<td>USNM</td>
<td>National Museum of Natural History, Smithsonian Institution – Washington, DC, USA;</td>
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<tr>
<td>ZIN</td>
<td>Zoological Museum, Academy of Sciences – St. Petersburg, Russia.</td>
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Geographical distribution and host association

The identification tools of previous literature are not reliable for identifying the species that we treat here. Hence, the geographical distribution and host associations presented in Material Examined sections derive only from specimens examined as part of this study.

Cybertaxonomy

Specimens used in this study were assigned collecting unit identifiers (CUIDs) and their associated collection and host association data were deposited in Hymenoptera
Online (hol.osu.edu). In addition to the abbreviated Material examined sections, a DarwinCore archive is provided for each species (Suppl. material: S2–S5). These files contain the totality of specimens for which data is deposited in Hymenoptera Online, including specimens for which updated identification has not yet occurred, which can be assessed by the dates of determination. Taxonomic synopses, descriptions, and material examined sections were generated in the online, matrix-based program vSysLab (vsyslab.osu.edu) with a matrix based on that of Talamas et al. (2017).

Photography

A Leitz Großfeld-Stereomikroskop TS with magnification up to 160×, a Stereomicroscope Wild M3B with oculars 15×, and a spot light Leica CLS 150× were used for biometric diagnosis. A semi-transparent light shield was used to reduce glare and to diffuse the light. The lectotypes of *T. belenus* and *T. arminon* were photographed with a Macroscopic Solutions Macropod MicroKit with individual slices rendered in Helicon Focus 6. All other images were produced using a Leitz Dialux 20 EB compound microscope with a Leica DFC 290 Camera with LED spot light or dome light based on different points of view after techniques summarized in Buffington et al. (2005), Kerr et al. (2008) and Buffington and Gates (2008). LEICA APPLICATION SUITE V 3.7.0 software was used to manage image acquisition and ZERENE STACKER was used for merging of the image series into a single in-focus image.

Morphology

Terminology for surface sculpture follows the glossary by Harris (1979), Mikó et al. (2007), Yoder et al. (2010) and Talamas et al. (2017). Measurements of the head, mesosoma, metasoma, total body, and wing venation follow Masner (1980) and Tortorici et al. (2016). In the wing ratio expressed as st:pm:mg, the stigmal vein is treated as the benchmark unit (=1). Morphological terms largely follow Mikó et al. (2007) and were matched to concepts in the Hymenoptera Anatomy Ontology (Yoder et al. 2010) using the text analyzer function and a table of these terms and URI links is provided in Suppl. material: S1.

Additional abbreviations and terminology used in this paper: HL: head length; HW: head width; HH: head height, from vertex to distal end of clypeus; FCI: frontal cephalic index (HW/HH); LCI: lateral cephalic index (HH/HL); OOL:POL:LOL: ocular distance ratio, OOL as the benchmark unit (=1); IOS: interorbital space (Mikó et al. 2010); claval formula: the sequence of sensilla, from the apical antennomere (A11) to the last functional clavomere (Bin 1981), i.e. the last antennomere bearing one or two multiporous gustatory sensilla, as defined by Isidoro et al. (1996); compound eye height and width: measured when eye longitudinal axis is parallel to the focal plane.
Insect collecting and rearing

A host colony of *E. maura* used for rearing *Trissolcus* was established from adults collected on wheat in Piedmont (NW Italy) and maintained in cages under laboratory conditions (climatized chambers at 24 ± 1 °C, 65 ± 5% RH, L:D = 16:8). All eggs laid in the cages were collected and frozen at -20 °C. Because of the short egg-laying period of *E. maura*, freezing the eggs allowed the eggs to be used for a much longer time.

To obtain *Trissolcus* specimens, egg masses of *E. maura* and *P. prasina* were collected in the field in Piedmont (NW Italy) in the spring and summer of 2017. The field-collected egg masses were reared and checked daily. *Trissolcus* specimens that emerged from field-collected egg masses were allowed to mate. Some females were isolated in small plastic boxes (64.5 × 40.9 × 16 mm), fed with water and honey, and provided with *E. maura* frozen egg masses to produce progeny for use in subsequent tests.

For interbreeding experiments, specimens were isolated immediately following emergence to prevent mating, and females and males were maintained singly in plastic boxes as described above. When the parasitoids reach the early pupal stage inside the eggs, their red eyes are clearly visible through the transparent operculum of the host egg. Following observation of this feature (Figure 2), the eggs were checked at a frequency of 4–5 times per day to ensure that they were isolated prior to mating.

Some of the progeny from isolated, mated females were selected for preservation, identification and molecular analysis. The remaining progeny were used in breeding experiments.

Molecular analyses

Molecular analyses were performed to confirm morphological identification and characterize the species. Genomic DNA was extracted from the metasoma of specimens from rearing experiments and pinned collection specimens according to Kaartinen et al. (2010), but doubling the proteinase K dose (5 μl of 20 mg ml⁻¹ proteinase K). The barcode region of the cytochrome oxidase I (COI) gene was amplified using universal PCR primers for insects LCO1490 (5’-GGT CAA CAA ATC ATA AAG ATA TTG G-3’) and HCO2198 (5’-TAA ACT TCA GGG TGA CCA AAA AAT CA-3’) (Folmer et al. 1994). The PCR was performed in a 50 μl reaction volume: 2 μl of DNA, 37.9 μl molecular grade water, 5 μl 10× Qiagen PCR buffer, 3 μl dNTPs (25 mM each), 1.5 μl MgCl₂, 0.2 μl of each primer (0.3 μM each), 0.2 μl Taq DNA Polymerase (Qiagen, Hilden, Germany). Thermocycling conditions were optimized to shorten reaction times and included initial denaturation at 94 °C for 300 s, followed by 35 cycles of 94 °C for 30 s, annealing at 52 °C for 45 s and extension at 72 °C for 60 s; then further 600 s at 72 °C for final extension. PCR products were purified using a commercially available kit (QIAquick PCR Purification Kit, Qiagen GmbH, Hilden, Germany) following the manufacturer’s instructions, and sequenced by a commercial service (Genethron S.r.l., Rome, Italy). The sequences were compared with the GenBank database and each other using the Basic Local Alignment Search Tool (http://www.ncbi.nlm.
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nih.gov/BLASTn). All sequences were aligned using ClustalW with default settings as implemented in Mega X. The pairwise nucleotide sequence distances among and within taxa were estimated using the Kimura 2-parameter model (K2P) of substitution (Kimura 1980) using Mega X (Kumar et al. 2018). The sequences generated from this study are deposited in the GenBank database. All residual DNA is archived at DISAFA.

**Figure 2.** Pupal stage of *Trissolcus* sp. in *Halyomorpha halys* eggs, clearly indicated by the presence of eyes and ocelli, which are visible through the semi-transparent host egg.

**Mating tests and reproductive isolation between *T. belenus* and *T. semistriatus***

For mating experiments, 1–2-day old virgin females and males were used. Four combinations for mating tests were done: *T. semistriatus* (♀) × *T. belenus* (♂); *T. belenus* (♀) × *T. semistriatus* (♂); *T. semistriatus* (♀) × *T. semistriatus* (♂); *T. belenus* (♀) × *T. belenus* (♂). The total number of interbreeding tests was 24: four replicates for each intraspecific mating combination and eight replicates for each interspecific mating combination. Each pair of wasps was observed at the stereomicroscope until the end of copulation or for 10 minutes if copulation did not occur. The pair then remained together in isolation for 24 hours. After the mating test, an egg mass of *E. maura* was provided to each female wasp for 24 hours of exposure. The egg masses were then moved to other plastic boxes until offspring emergence. Each mating test was considered successful when emerged offspring included females, because in all known *Trissolcus* species, only mated females can produce female offspring. We compared the percentage of mating success among the four combinations and the significance of the results was assessed with a chi-square test.
Results

Morphological analysis

The easiest task regarded the distinction of _T. manteroi_ from _T. semistriatus_, _T. belenus_ and _T. colemani_. _Trissolcus manteroi_ clearly has a shorter postmarginal vein, only slightly longer than the stigmal vein; A7 has only one papillary sensillum instead of two in the other three species; and _T. manteroi_ has no episternal foveae. The holotype of _T. manteroi_ is thus morphologically very close to _T. rufiventris_, from which it can be differentiated by the length of the postmarginal vein.

The distinction of _T. belenus_ and _T. colemani_ from _T. semistriatus_ is more nuanced and required an integrative approach to determine which morphological characters were congruent with the biological and molecular data. The results of this in-depth analysis demonstrate that some of the characters that Talamas et al. (2017) treated as intraspecifically variable have diagnostic power.

The presence or absence of setation on the external face of the hind femur, described in the key and figure III (I) (H) in Delucchi (1961), is a reliable character to distinguish _T. grandis_ from _T. semistriatus_. However, in the lectotype of _T. grandis_ and neotype of _T. semistriatus_ this character is opposite to what was stated by Delucchi (1961). Furthermore, the holotype of _T. colemani_ has the external surface of hind femur setose, as in the lectotype of _T. grandis_. The association proposed in Delucchi (1961): ‘external face of hind femora uncovered by hair’ – ‘reddish yellow tibiae’ is the typical combination for _T. colemani_, while Delucchi (1961) proposed it for _T. semistriatus_, and ‘external face of hind femora covered by hair’ – ‘dark or black tibiae’ is the typical combination for _T. semistriatus_. We conclude that this interpretation is contrary to what is found in type material.

Synonymy in _T. belenus_

In the analysis of original descriptions and images of lectotype of _T. arminon_ and _T. grandis_, no remarkable characters were recognized to distinguish them from _T. belenus_, which we therefore consider it to be their senior synonym. In the analysis of type material of _T. silwoodensis_ and _T. nixomartini_, previously considered junior synonym of _T. grandis_ (Kozlov & Lê, 1977), we confirmed the findings of previous authors, and thus treat these species as junior synonyms of _T. belenus_. Mayr (1879) considered _Telenomus ovulorum_ Thomson to be a junior synonym of _Telenomus semistriatus_ Nees von Esenbeck, but through analysis of the photographs of type material of _T. ovulorum_ Thomson, we recognized the character states of _T. belenus_, and therefore treat _T. ovulorum_ as a junior synonym of _T. belenus_.

Synonymy in _T. colemani_

One paratype of _T. djadetsbko_ and three syntypes of _T. pseudoturesis_ were analyzed via photographs and compared with the original description and photographs of the holo-
type of *T. colemani*. The character states of the two first species matched perfectly with those of the latter, leading us to treat *T. colemani* as the senior synonym of *T. djadetshko* and *T. pseudoturesis*. We conclude that the characters of *T. crypticus* match those in the holotype of *T. colemani* based on examination of *T. crypticus* paratypes collected in Pakistan and its original description (see figs 1, 3, 5 in Clarke 1993). We thus treat *T. crypticus* as a junior synonym of *T. colemani*.

Clarke (1993) also reported that “Examination of material of *T. rungsi* labelled by Voegelé (deposited in NHMUK) shows that this species is not the same of *T. crypticus*” but he did not provide any distinguishing characters between the two species. Contrary to what was reported by Clarke (1993), in our analysis of the material deposited at NHMUK, 37 specimens labelled as “*Asolcus rungsi* Voegelé” were identified as *T. colemani* and four specimens labelled as “rungsi 1965 Voegele” were identified as *T. basalis*, while other 25 with the same last cumulative label were identified as *T. colemani*. This confirms our interpretation of the description and analysis of figures regarding *A. rungsi* and demonstrates confusion of species in the Moroccan rearing efforts at École Nationale d’Agriculture in Meknès.

The original description of *Asolcus rungsi* mentioned the presence of short traces of notauli (fig. 1, c. in Voegelé 1965); these traces are visible in all specimens *T. colemani* (Figure 23). However, because the location of the holotype of *A. rungsi* is not known, we were unable to examine it and at this time do not treat this species name as a synonym. The morphological analyses of the holotype and paratypes of *T. waloffae* showed the conspecificity of this species with *T. colemani*.

### Molecular analysis

Barcode sequences were obtained from 17 *Trissolcus* specimens (Table 1). The Blast search showed that the sequences of *T. semistriatus* from Italy and from Iran had a 98% sequence identity with the GenBank sequence from *Trissolcus nigripedius* (accession no. AB971830). The sequences from the two specimens of *T. colemani* showed a 98% identity with a GenBank sequence with a Platygastridae sp. (accession no. KY839581), while the sequences from the specimens of *T. manteroi*, *T. belenus* and *T. rufiventris* showed a lower similarity with GenBank sequences. The final alignment consisted of 548 characters. Pairwise distance values within and among analyzed species are shown in Table 2. The genetic distances between the specimens identified as of the same species (which averaged between 0.000 ± 0.000 and 0.005 ± 0.002), were much lower than the mean pairwise distances observed between the specimens identified as of different species (from 0.105 ± 0.001 to 0.149 ± 0.000).

### Mating tests

Specimen pairs tested for intraspecific combination mated within ten minutes; pairs tested for interspecific combination did not mate within the 10-minute observation period.
**Figure 3.** Sex ratio of emerged specimens. Combinations: $T. belenus (\varnothing \times \mathcal{O})$, $n = 4$; $T. semistriatus (\varnothing \times \mathcal{O})$, $n = 4$; $T. belenus (\varnothing) \times T. semistriatus (\mathcal{O})$, $n = 8$; $T. semistriatus (\varnothing) \times T. belenus (\mathcal{O})$, $n = 8$. Bars indicate standard deviation.

**Table 2.** Barcode mean pairwise genetic distances (± SE) between $T. manteroi$, $T. semistriatus$, $T. belenus$, $T. colemani$ and $T. rufiventris$ (under the diagonal), and within taxa (along the diagonal). $n = $ number of sequences.

<table>
<thead>
<tr>
<th>Species</th>
<th>$T. manteroi$ (n = 2)</th>
<th>$T. semistriatus$ (n = 6)</th>
<th>$T. belenus$ (n = 6)</th>
<th>$T. colemani$ (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T. manteroi$ (n = 2)</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T. semistriatus$ (n = 6)</td>
<td>0.139 ± 0.000</td>
<td>0.005 ± 0.002</td>
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<td></td>
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<tr>
<td>$T. belenus$ (n = 6)</td>
<td>0.139 ± 0.000</td>
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<td>0.000</td>
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<tr>
<td>$T. colemani$ (n = 2)</td>
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<td>0.105 ± 0.001</td>
<td>0.107 ± 0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>$T. rufiventris$ (n = 1)</td>
<td>0.144 ± 0.000</td>
<td>0.141 ± 0.001</td>
<td>0.149 ± 0.000</td>
<td>0.133 ± 0.000</td>
</tr>
</tbody>
</table>

**Table 1.** Specimen information and GenBank Accession Number for the sequences generated by this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Country</th>
<th>Year of collection</th>
<th>GenBank accession number</th>
<th>Collecting unit identifier</th>
</tr>
</thead>
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<tr>
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<td>ITALY</td>
<td>2010</td>
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<td>ITALY</td>
<td>2017</td>
<td>MK906048</td>
<td>DISAFA-draw1465-HYM-0233</td>
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<td>MK906049</td>
<td>USNMENT01223088</td>
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<tr>
<td><em>Trissolcus manteroi</em></td>
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<td>IRAN</td>
<td>2017</td>
<td>MN603796</td>
<td>DISAFA-draw1465-HYM-0238</td>
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<tr>
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<td>DISAFA-draw1465-HYM-0240</td>
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<td>ITALY</td>
<td>2017</td>
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<td>DISAFA-draw1465-HYM-0019</td>
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<td>USNMENT01223455</td>
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<tr>
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<td>♀</td>
<td>IRAN</td>
<td>2017</td>
<td>MN603807</td>
<td>UNIPA-HYM-S01347</td>
</tr>
</tbody>
</table>

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All females used for the two intraspecific combinations successfully produced female offspring (Figure 3); as expected the sex ratio was similar, in *T. belenus* (♀ × ♂) combination 31 females and 5 males emerged, and in *T. semistriatus* (♀ × ♂) 21 females and 4 males emerged. Females used for the two interspecific combinations produced only male offspring, 78 males in the *T. belenus* (♀ × *T. semistriatus* ♂) combination, and 65 males in the *T. semistriatus* (♀ × *T. belenus* ♂) combination. A total of 3 females failed to reproduce, producing no offspring in either the intraspecific or interspecific combinations.

Key to *Trissolcus* of the Palearctic region (females)

Modified couplets for the Key to *Trissolcus* of the Palearctic region (females) in Talamas et al. (2017)

29 Ventral mesopleuron distinctly bulging; mesocoxa oriented parallel to long axis of body; dorsal frons with sculpture effaced, sometimes entirely smooth and shining; A7 with two papillary (basiconic) sensilla (figures 128–132 in Talamas et al. 2017).......................................................... *Trissolcus perepelovi* (Kozlov)

– Ventral mesopleuron not distinctly bulging; mesocoxa oriented at an angle of ~45° relative to long axis of body (Figure 6); dorsal frons evenly and densely covered in microsculpture; A7 with one papillary (basiconic) sensillum (Figure 10)........................................................................................................ 29A

29A Postmarginal vein in fore wing about twice as long as stigmal vein (Figure 14); metasoma yellow to dark brown, typically reddish-brown.................

.......................................................... *Trissolcus rufiventris* (Mayr)

– Postmarginal vein only slightly longer than stigmal vein (Figure 13); metasoma dark brown to black (Figure 18)....................... *Trissolcus manteroi* (Kieffer)

32 Lateral mesoscutum with mesoscutal humeral sulcus present as a smooth furrow (Figure 25)........................................................................ 32A

– Lateral mesoscutum with mesoscutal humeral sulcus comprised of distinct foveae (Figures 20–23).................................................. 32B

32A Lateral pronotum with netrion sulcus incomplete dorsally, netrion often poorly defined; medial part of occipital carina rounded in dorsal view........

.......................................................... *Trissolcus basalis* (Wollaston)

– Lateral pronotum with netrion sulcus complete dorsally (Figures 5, 50, 53, 55, 60), netrion distinct; medial part of occipital carina angled (Figure 36), vertex of angle with short carina directed toward median ocellus ..........

.......................................................... *Trissolcus semistriatus* (Nees von Esenbeck)

32B Laterotergite 1 with line of 3 setae (Figures 30, 45).................................

.......................................................... *Trissolcus belenus* (Walker)

– Laterotergite 1 without setae (Figure 32).... *Trissolcus colemani* (Crawford)

A matrix of the diagnostic characters used in this key is provided in Suppl. material: S6.
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Figures 8–11. Basiconic sensilla, indicated by arrows, in the ventral surface of female antennal clava:
8 *Trissolcus belenus* [DISAFA-draw1465-HYM-0009] 9 *T. colemani* [DISAFA-draw1466-HYM-0484]
10 *T. manteroi* [DISAFA-draw1465-HYM-0430] 11 *T. semistriatus* [DISAFA-draw1465-HYM-0227].


*Trissolcus belenus* (Walker)
https://bioguid.osu.edu/xbiod_concepts/3190
Figures 4, 8, 12, 16, 20, 21, 26, 30, 33, 37, 41, 45–51.

_Telenomus Belenus_ Walker, 1836: 352 (original description).
_Telenomus arminon_ Walker, 1838: 457 (original description).
_Telenomus Nigrita_ Thomson, 1860: 172 (original description, synonymized by Kozlov (1968)); Kozlov 1968: 214 (junior synonym of *Trissolcus grandis* (Thomson)).
_Telenomus frontalis_ Thomson, 1860: 170 (original description, synonymized by Kozlov (1968)); Kozlov 1968: 214 (junior synonym of *Trissolcus grandis* (Thomson)).
_Telenomus grandis_ Thomson, 1860: 169 (original description).
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Telennomus ovulorum Thomson, 1860: 171 (original description, synonymized by Mayr (1879)); Mayr 1879: 704 (junior synonym of *Telennomus semistriatus* (Nees von Esenbeck)).

Telennomus ovulorum Thomson: Mayr 1879: 704. Junior synonym of *Telennomus semistriatus* (Nees von Esenbeck)

*Telennomus nigritus* Thomson: Dalla Torre 1898: 517 (emendation).

*Telennomus pentatomae* (Rondani): Dalla Torre, 1898: 518 (generic transfer).


*Allophanurus arminon* (Walker): Kieffer, 1926: 23 (description, keyed).


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Figures 37–44. 37–40 Head; malar area and gena  
37 *Trissolcus belenus* [DISAFA-draw1465-HYM-0009]  
38 *T. colemani* [DISAFA-draw1466-HYM-0484]  
39 *T. manteroi* [DISAFA-draw1465-HYM-0430]  
40 *T. semistriatus* [DISAFA-draw1465-HYM-0227]  
41–44 Head in frontal view  
41 *Trissolcus belenus* [DISAFA-draw1465-HYM-0009]  
42 *T. colemani* [DISAFA-draw1466-HYM-0484]  
43 *T. manteroi* [DISAFA-draw1465-HYM-0430]  
44 *T. semistriatus* [DISAFA-draw1465-HYM-0227].
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**Asolcus grandis** (Thomson): Masner, 1959: 376. (diagnosis, variation); Delucchi 1961: 44, 60 (description, keyed); Voegelé 1964: 28 (keyed); Javahery 1968: 419 (keyed); Voegelé 1969: 150 (keyed).

**Trissolcus grandis** (Thomson) syn. nov.: Viktorov, 1967: 91 (generic transfer, keyed); Safavi 1968: 416 (keyed); Kozlov 1968: 200, 214 (description, lectotype designation, synonymy, keyed); Fabritius 1972: 32, 35. (keyed; host catalogue; distribution); Viggiani and Mineo 1974: 156, 160, 161 (description, keyed); Kozlov and Lê 1977: 512 (synonymy, keyed); Kozlov 1978: 636 (description); Kozlov 1981: 187 (keyed); Kozlov and Kononova 1983: 110 (description); Johnson 1992: 629 (cataloged, type information); Kononova 1995: 96 (keyed); Doganlar 2001: 112 (description); Koçak and Kiliçer 2003: 302, 307 (keyed, description); Fabritius and Popovici 2007: 158 (host informations, distribution); Buhl & O’Connor, 2010: 154 (distribution); Ali 2011: 10 (keyed); Ghahari et al. 2011: 596 (host association, listed); Guz et al. 2013: 87 (description, phylogenetic relationships); Petrov 2013: 326 (keyed); Kononova 2014: 1424 (keyed); Kononova 2015: 262 (keyed); Talamas et al. 2017: 129, 135 (junior synonym of *Trissolcus semistriatus* (Nees von Esenbeck), type information).


**Trissolcus pentatomae** (Rondani) syn. nov.: Bin, 1974: 463 (generic transfer, lectotype designation); Johnson 1992: 634 (cataloged, type information); Talamas et al. 2017: 130, 135 (junior synonym of *Trissolcus semistriatus* (Nees von Esenbeck), type information).

**Trissolcus belenus** (Walker): Fergusson, 1978: 120 (generic transfer); Fergusson 1984: 230 (lectotype designation); Johnson 1992: 623 (cataloged, type information); Kononova 2014: 1426 (possibly in *Telanomus*); Kononova 2015: 264 (possibly in *Telanomus*).

**Trissolcus nigripes** (Thomson) syn. nov.: Fergusson, 1978: 120 (generic transfer).

**Trissolcus nixomartini** (Javahery) syn. nov.: Fergusson, 1978: 120 (generic transfer); Fergusson 1984: 230 (type information).

**Trissolcus silwoodensis** (Javahery) syn. nov.: Fergusson, 1978: 120 (generic transfer); Fergusson 1984: 230 (type information).

**Trissolcus arminon** (Walker) syn. nov.: Fergusson 1983: 208 (generic transfer, description, lectotype designation); Fergusson 1984: 230 (type information); Johnson 1992: 622 (cataloged, type information).

**Trissolcus ovulorum** (Thomson) comb. nov., syn. nov.

**Diagnosis.** The presence of setae on the first laterotergite (Figures 30, 45) allows *T. belenus* to be easily diagnosed, as only two other Palearctic species share this character: *T. saakowi* and *T. mitsukurii* (Ashmead). Both of these species have distinct notauli,
which are absent in *T. belenus* (Figures 20, 22). Additionally, the hyperoccipital carina is entirely absent in *T. belenus* (Figures 33, 47, 49), whereas it is complete in *T. saakowi* and present posterior to the lateral ocellus in *T. mitsukurii*.

**Description.** Body length: 1.03–1.1 mm, median = 1.06 mm, SD = 0.02, n = 20. Body color: head, mesosoma, and metasoma black.


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Metasoma. Width of metasoma: about equal to width of mesosoma. Longitudinal striae on T1 posterior to basal costae: pair of longitudinal submedial carinae separate a lateral smooth area from an internal area where striate sculpture starts with basal grooves. Number of sublateral setae (on one side): 1. Setation of laterotergite 1: present. Length of striation on T2: extending two-thirds the length of the tergite. Setation of T2: present in a transverse line and along lateral margin. Setation of laterotergite 2: present.

Host associations. Pentatomidae: Aelia rostrata; Arma custos; Carpocoris sp.; Dolycoris sp.; Graphosoma italicum Müller; Palomena prasina; Picromerus bidens (L.); Piezodorus sp.; sentinel frozen eggs of Halyomorpha halys. Scutelleridae: Eurygaster integriceps Puton; Eurygaster matura.

Link to distribution map. [https://hol.osu.edu/map-large.html?id=3190]

Figures 45–48. Holotype of *Trissolcus belenus* [NMINH_2018_11_49]: 45 mesoscutal humeral sulcus and laterotergite 1 in lateral view 46 body in lateral view 47 body in antero-lateral view 48 body in dorsal view.
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Morocco: 24 female, 1 pin with multiple specimens, OSUC 17729 (BMNH); USNMENT01223131 (UNIPA). Portugal: 6 females, 1 male, 1 pin with multiple specimens, USNMENT00916191, 00916210–00916213, 00916217 (BMNH). Russia: 4 females, 2 males, 2 pins with multiple specimens, OSUC 17796–17797 (BMNH).

Figures 49–51. Holotype of *Trissolcus arminon* [NMINH_2018_11_46]: 49 body in dorsal view 50 head and mesosoma in antero-lateral view 51 body in postero-lateral view.

**Trissolcus colemani** (Crawford)
https://bioguid.osu.edu/xbiod_concepts/3203
Figures 5, 9, 17, 22, 23, 27, 31, 34, 38, 42, 52–55

**Telenomus colemani** Crawford, 1912: 2 (original description).

**Microphanurus djadetshko** Ryakhovskii, 1959: 84, 87 (original description, keyed).


**Asolcus nigribasalis** Voegelé, 1962: 155 (original description); Voegelé 1964: 28 (keyed); Voegelé 1965: 96, 108 (variation, diagnosis, keyed); Voegelé 1969: 151 (junior synonym of *Asolcus djadetsbko* (Ryakhovskii)).

**Asolcus djadetsbko** (Ryakhovskii): Viktorov, 1964: 1015, 1021 (description, generic transfer, removed from synonymy with *Telenomus scutellaris* Thomson, keyed); Voegelé 1969: 151 (synonymy, keyed, spelling error).

**Asolcus pseudoturesis** (Ryakhovskii): Viktorov, 1964: 1013, 1021 (description, generic transfer, synonymy, keyed); Voegelé 1969: 151 (synonymy, keyed).

**Asolcus bennisi** Voegelé, 1964: 119 (original description); Voegelé 1965: 96, 108 (variation, diagnosis, keyed); Voegelé 1969: 151 (junior synonym of *Asolcus pseudoturesis* (Ryakhovskii)).

**Trissolcus djadetshko** (Ryakhovskii) syn. nov.: Viktorov, 1967: 91 (generic transfer, keyed); Safavi 1968: 415 (keyed); Kozlov 1968: 200 (keyed); Fabritius 1972: 31 (keyed); Kozlov and Lê 1977: 512 (keyed); Kozlov 1978: 636 (description); Kozlov 1981: 187 (keyed); Kozlov and Kononova 1983: 115 (description); Johnson 1992: 626 (cataloged, type information); Kononova 1995: 96 (keyed); Koçak and Kilinçer 2000: 171 (description, diagnosis, new distribution record for Turkey); Koçak and Kilinçer 2003: 303, 313 (keyed, description); Fabritius and Popovici 2007: 159 (checklist, host information, distribution); Ghahari et al. 2011: 595 (listed); Petrov 2013: 326 (keyed); Kononova 2014: 1425 (keyed); Kononova 2015: 263 (keyed); Tálamas et al. 2017: 129 (junior synonym of *Trissolcus semistriatus* (Nees von Esenbeck)).

**Trissolcus pseudoturesis** (Ryakhovskii) syn. nov.: Viktorov, 1967: 91 (generic transfer, keyed); Safavi 1968: 415 (keyed); Kozlov 1968: 200 (keyed); Fabritius 1972: 31 (keyed); Kozlov and Lê 1977: 512 (keyed); Kozlov 1978: 636 (description); Kozlov and Kononova 1983: 114 (description); Johnson 1992: 635 (cataloged, type
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information); Kononova 1995: 96 (keyed); Koçak and Kilincer 2003: 302, 310 (keyed, description); Ghahari et al. 2011: 596 (listed); Petrov 2013: 326 (keyed); Kononova 2014: 1425 (keyed); Kononova 2015: 263 (keyed).

Trissolcus colemani (Crawford): Masner and Musebeck 1968: 72 (type information, generic transfer); Johnson 1992: 625 (cataloged, type information).

Asolcus waloffae (Javahery, 1968: 419 (original description, keyed).


Trissolcus waloffae (Javahery) syn. nov.: Kozlov and Lê 1977: 516 (keyed, generic transfer); Kozlov 1978: 637 (description); Fergusson 1984: 231 (type information); Johnson 1992: 640 (cataloged, type information); Kononova 2014: 1425 (keyed); Kononova 2015: 263 (keyed).

Trissolcus crypticus Clarke syn. nov., 1993: 524 (original description); Ghahari et al. 2011: 595 (new distribution record for Iran, host association, listed); Kononova 2014: 1426 (status unknown (not examined)); Kononova 2015: 264 (status unknown (not examined)).

Diagnosis. Trissolcus colemani is identified by a combination of characters more than by the presence of a distinct feature. The foveate mesoscutal humeral sulcus (Figures 21, 23) separates it from all the species treated here with the exception of T. belenus (Figures 20, 22). Trissolcus colemani and T. belenus are very similar in general appearance and these two species can be separated most reliably by setation of laterotergite 1: present in T. belenus (Figures 30, 45) and absent in T. colemani (Figure 31, 53). The anteroventral extension of the metapleuron reaches the mesocoxa in lateral view in both T. belenus and T. colemani and exhibits difference in the shape of its apex between these species. In T. colemani, the anteroventral extension of the metapleuron is very slender (Figure 5) compared to T. belenus, in which it is thicker, and the apex is rounded (Figure 4).

Description. Body length: 0.96–1.10 mm, m = 1.01 mm, SD = 0.03, n = 22. Body color: head, mesosoma, and metasoma black.


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**Figures 52–55.** Holotype of *Trissolcus colemani* [USNMENT00989063]: 52 body in dorsal view 53 body in lateral view 54 head in frontal view 55 head in ventral view and mesosoma in lateral view.
Metasoma. Width of metasoma: about equal to width of mesosoma. Longitudinal striae on T1 posterior to basal costae: pair of longitudinal submedial carinae separate a lateral smooth area from an internal area where striate sculpture starts with basal grooves. Number of sublateral setae (on one side): 1. Setation of laterotergite 1: absent. Length of striation on T2: extending two-thirds the length of the tergite. Setation of T2: present in a transverse line and along lateral margin. Setation of laterotergite 2: present.

Host associations. Pentatomidae: Dolycoris indicus (Type host); Aelia acuminata L.; Aelia sp.; Brachynema germarii (Kolenati); Dolycoris sp.; Graphosoma semipunctatum (F); Graphosoma sp. Scutelleridae: Eurygaster integriceps; Eurygaster maura.

Link to distribution map. [https://hol.osu.edu/map-large.html?id=3203]


Trissolcus manteroi (Kieffer)
https://bioguid.osu.edu/xbiod_concepts/3260
Figures 6, 10, 13, 18, 24, 28, 35, 39, 43, 56–58

Telenomus Manteroi Kieffer, 1909: 268 (original description).
Aphanurus Manteroi (Kieffer): Kieffer 1912: 84 (description, generic transfer).
Microphanurus manteroi (Kieffer): Kieffer 1926: 91, 102. (description, generic transfer, keyed); Boldaruyev 1969: 163, 170 (description, keyed)

**Diagnosis.** Trissolcus manteroi and *T. rufiventris* are the only two species of Palearctic *Trissolcus* in which females exhibit a 1-2-2-2-1 claval formula (Figure 10). These two can be separated from each other by the length of the postmarginal vein in the fore wing: slightly longer than the stigmal vein in *T. manteroi* (Figure 13) and about twice as long as the stigmal vein in *T. rufiventris* (Figure 14). These two species can also be separated from each other by the form of the mesopleural epicoxal sulcus, which is comprised of cells in *T. manteroi* and is a smooth furrow in *T. rufiventris*.

**Description.** Female body length: 0.99–1.09 mm, m = 1.04 mm, SD = 0.02, n = 16. Body color: head, mesosoma, and metasoma black.


Figures 56–58. Holotype of *Trissolcus manteroi* [MCSN 0013]: 56 body in dorsal view 57 head in antero-lateral view 58 body in lateral view.
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**Metasoma.** Width of metasoma: about equal to width of mesosoma. Longitudinal striae on T1 posterior to basal costae: pair of longitudinal submedial carinae separate a lateral smooth area from an internal area where striate sculpture starts with basal grooves. Number of sublateral setae (on one side): 1. Setation of laterotergite 1: absent. Length of striation on T2: extending one-third the length of the tergite. Setation of T2: present in a transverse line and along lateral margin. Setation of laterotergite 2: present.

**Host associations.** Pentatomidae: *Carpocoris* sp. (Type host); *Aelia rostrata* Bohemian; *Dolycoris* sp.

**Link to distribution map.** [https://hol.osu.edu/map-large.html?id=13225](https://hol.osu.edu/map-large.html?id=13225)


*Trissolcus semistriatus* (Nees von Esenbeck)
[https://bioguid.osu.edu/xbiod_concepts/3305](https://bioguid.osu.edu/xbiod_concepts/3305)

Figures 7, 11, 15, 19, 25, 29, 32, 36, 40, 44, 59, 60.

**Teleas semistriatus** Nees von Esenbeck, 1834: 290 (original description); Ratzeburg 1852: 182 (description); Johnson 1992: 519 (cataloged).

**Telenomus semistriatus** (Nees von Esenbeck): Thomson, 1860: 171 (description, generic transfer); Mayr 1879: 699, 701, 704 (description, synonymy, keyed).

**Asolcus nigripedius** Nakagawa, 1900: 17 (original description); Watanabe 1951: 21, 25 (description, type information, keyed); Watanabe 1954: 22 (keyed).

**Aphanurus Semistriatus** (Nees von Esenbeck): Kieffer, 1912: 74 (description, generic transfer).

**Microphanurus semistriatus** (Nees von Esenbeck): Kieffer, 1926: 91, 97 (description, generic transfer, keyed); Nixon 1939: 131, 134 (description, keyed); Meier 1940: 80 (description, keyed); Ryakhovskii 1959: 84 (keyed).

**Microphanurus alexceevi** Meier, 1949: 114 (original description, not seen: reference from Kozlov (1963), synonymized with *Asolcus semistriatus* (Nees von Esenbeck))
by Kozlov (1963)); Ryakhovskii 1959: 83 (keyed); Kozlov 1963: 295 (junior synonym of *Asolcus semistriatus* (Nees von Esenbeck)).


*Trissolcus artus* Kozlov and Lê 1977: 512, 519 (original description, keyed); Kozlov 1978: 636 (description); Kozlov and Kononova 1983: 112 (description); Johnson 1992: 622 (cataloged, type information); Kononova 1995: 96 (keyed); Kononova 2014: 1424 (keyed); Kononova 2015: 262 (keyed); Talamas et al. 2017: 128, 135 (junior synonym of *Trissolcus semistriatus* (Nees von Esenbeck), type information).

**Diagnosis.** *Trissolcus semistriatus* is most similar to *T. belenus* and *T. colemani*, with which it overlaps in distribution and host range. It can be separated from both by the mesoscutal humeral sulcus present as a smooth furrow (Figure 25) and the short anteroventral extension of the mesopleuron, which does not extend to the base mesocoxa (Figure 7). Additionally, the angular form of the occipital carina in dorsal view, with a short carina extending toward the median ocellus, is found only in this species (Figure 36). The anteroventral area of the hind femur that is covered by setae (Figure 29) is
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useful when separating *T. semistriatus* from *T. belenus* (Figure 26), *T. colemani* (Figure 27) and *T. manteroi* (Figure 28).

**Description.** Body length: 1.07–1.11 mm, median = 1.08 mm, SD = 0.01, n = 20. Body color: head, mesosoma, and metasoma black.


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**Metasoma.** Width of metasoma: about equal to width of mesosoma. Longitudinal striae on T1 posterior to basal costae: pair of longitudinal submedial carinae separate a lateral smooth area from an internal area where striate sculpture starts with basal grooves. Number of sublateral setae (on one side): 1. Setation of laterotergite 1: absent. Length of striation on T2: extending two-thirds the length of the tergite. Setation of T2: present in a transverse line and along lateral margin. Setation of laterotergite 2: present.

**Host associations.** Pentatomidae: *Aelia rostrata*; *Brachynema germarii* (Kolenati); *Carpocoris* sp.; *Dolycoris baccarum* (L.); *Graphosoma semipunctatum*; *Rhaphigaster* sp.; Scutelleridae: *Eurygaster maura*.

**Link to distribution map.** [https://hol.osu.edu/map-large.html?id=3305](https://hol.osu.edu/map-large.html?id=3305)


**Discussion**

More than 180 years have passed between the original descriptions of *T. semistriatus* and *T. belenus* and the development of identification tools that can reliably distinguish them. This can be viewed as a glacial rate of progress, but also as an indication that modern methods can resolve long-standing taxonomic challenges. The taxonomy of *Trissolcus* illustrates that the examination of primary types and detailed comparison of specimens across a broad geographical range is necessary to advance the field, and that further refinement may be required even when these practices are implemented. Talaumas et al. (2017) significantly advanced the taxonomy of Palearctic *Trissolcus* but additional analysis was needed to distill diagnostic characters from those that were treated as intraspecifically variable. Specifically, setation on the first laterotergite, the form of the mesoscutal humeral sulcus, and the length of the anteroventral extension of the
metapleuron were treated as variable within *T. semistriatus*. Although the utility of these characters for separating *T. belenus* and *T. colemani* was not recognized, Talamas et al. (2017) did bring attention to them, as they had not yet been used in the taxonomy of Palearctic *Trissolcus*. Setation of the hind femora, not mentioned by Talamas et al. (2017), represents a case in which a diagnostic character was previously recognized, but incorrectly associated with a taxonomic name (Delucchi 1961), and is now treated as useful for identifying *T. belenus*. *Trissolcus manteroi* is a different matter, in which reexamination of the type specimen was needed for its diagnostic characters (wing venation, claval formula, absence of episternal foveae) to be correctly characterized. These features place *T. manteroi* closer to *T. rufiventris* than to *T. semistriatus*, *T. belenus* or *T. colemani*.

The trail of photographic evidence provided by Talamas et al. (2017) enabled junior synonyms of *T. semistriatus* to be rapidly redistributed among *T. belenus* and *T. colemani* once the characters that delimit these species were identified, as well as the resurrection of *T. manteroi*. Given that producing a natural classification is an iterative process, explicit presentation of data that underlies taxonomic decisions accelerates further refinement. This is perhaps the only means by which the various quagmires of inadequate species descriptions in Platygastroidea can be transformed into a useful classification.

The need for reliable identification can be clearly seen in examples where quality taxonomy was absent. In the early part of the 20th century, *Trissolcus* specimens identified as *T. semistriatus* or *T. grandis* were reared and released in Russia and Iran as classical biological control agents against *Eurygaster* (Alexandrov 1947; Saakov 1903; Vaezi 1950; Vassiliev 1913; Zomorrodi 1959). Some of these authors did not indicate how they identified the species, and in any case, the characters that reliably separate these species were not established. It is only by retroactively identifying voucher specimens, if they exist, that the results of these efforts can be interpreted in a meaningful way.

The presence of *H. halys* in Europe has created a similar situation, with the same species involved in studies of its biological control. The refined species concepts presented here are thus of immediate relevance, given that *T. belenus* was recorded from frozen sentinel eggs of *H. halys* in Europe, and was previously identified as *T. semistriatus*.

Finally, it should be noted that independent testing of species concepts, ideally using multiple methods, is the best means by which they can be verified or improved. This study employed such an approach, using morphology, mating studies and molecular analysis to resolve four species from the concept of *T. semistriatus* provided in Talamas et al. (2017). In a manner conforming with this perspective, our results have been confirmed by a concomitant study by Talamas et al. (2019), in which a phylogeny of *Trissolcus* based on five molecular markers retrieved *T. belenus*, *T. colemani* and *T. semistriatus* as distinct entities.

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**Supplementary material 1**

**URI table of HAO morphological terms**

Authors: Francesco Tortorici, Elijah J. Talamas, Silvia T. Moraglio, Marco G. Pansa, Maryam Asadi-Farfar, Luciana Tavella, Virgilio Caleca

Data type: species data

Explanation note: This table lists the morphological terms used in this publication and their associated concepts in the Hymenoptera Anatomy Ontology.

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**Supplementary material 2**

*Trissolcus belenus* occurrence data
Authors: Francesco Tortorici, Elijah J. Talamas, Silvia T. Moraglio, Marco G. Pansa, Maryam Asadi-Farfar, Luciana Tavella, Virgilio Caleca
Data type: species data
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**Supplementary material 3**

*Trissolcus colemani* occurrence data
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Supplementary material 4

Trissolcus manteroi occurrence data
Authors: Francesco Tortorici, Elijah J. Talamas, Silvia T. Moraglio, Marco G. Pansa, Maryam Asadi-Farfar, Luciana Tavella, Virgilio Caleca
Data type: species data
Explanation note: DarwinCore archive of occurrence records for *T. manteroi.*
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Link: https://doi.org/10.3897/jhr.73.39052.suppl4

Supplementary material 5

Trissolcus semistriatus occurrence data
Authors: Francesco Tortorici, Elijah J. Talamas, Silvia T. Moraglio, Marco G. Pansa, Maryam Asadi-Farfar, Luciana Tavella, Virgilio Caleca
Data type: species data
Explanation note: DarwinCore archive of occurrence records for *Trissolcus semistriatus.*
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Supplementary material 6

Matrix of diagnostic characters
Authors: Francesco Tortorici, Elijah J. Talamas, Silvia T. Moraglio, Marco G. Pansa, Maryam Asadi-Farfar, Luciana Tavella, Virgilio Caleca
Data type: species data
Explanation note: This table provides a matrix of diagnostic characters to separate Palearctic species that are morphologically close to *Trissolcus semistriatus.*
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