



A remarkable new species of *Trissolcus* Ashmead (Hymenoptera, Scelionidae), parasitoid of eggs of *Phloea subquadrata* (Hemiptera, Phloeidae) in Brazil

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Academic editor: Miles Zhang | Received 11 December 2024 | Accepted 18 February 2025 | Published 31 March 2025

<https://zoobank.org/9A1723A0-9212-4F93-B9F8-598D4923BFFF>

Citation: Costa VA, Johnson N, Salomão A, Vasconcellos-Neto J, Wengrat A, Schoeninger K (2025) A remarkable new species of *Trissolcus* Ashmead (Hymenoptera, Scelionidae), parasitoid of eggs of *Phloea subquadrata* (Hemiptera, Phloeidae) in Brazil. Journal of Hymenoptera Research 98: 405–417. <https://doi.org/10.3897/jhr.98.144215>

Abstract

Trissolcus adrianae Costa & Johnson, **sp. nov.** is described. It is an egg parasitoid of the neotropical bark bug *Phloea subquadrata* Spinola (Hemiptera, Phloeidae) on *Plinia cauliflora* (Mart.) Kausel (Myrtaceae). *Trissolcus adrianae* belongs to the *flavipes* species group and can easily be distinguished from other *flavipes* group members by its noticeably depressed habitus; head oblong in anterior view; bidentate mandibles; frontal depression delimited dorsally by carina; orbital furrow sculptured, constricted through most of its length by lateral expansion of frons; notauli absent; and the length of T3–T6 subequal to the length of T2. This is the first species of Scelionidae associated with the family Phloeidae (Hemiptera: Pentatomoidae) and the first report of parasitism of Phloeidae eggs as well.

Keywords

Atlantic forest, natural enemy, Platygastroidea, taxonomy, Telenominae

Introduction

Trissolcus Ashmead (Hymenoptera, Scelionidae) is a cosmopolitan genus comprising ca. 150 described species (Johnson 1992). *Trissolcus* species are egg parasitoids of Pentatomoida (Hemiptera, Heteroptera). The genus is divided into five species groups (Kozlov and Kononova 1983). The *flavipes* group is particularly abundant in the Neotropical Region (Johnson 1984a, 1987) and is characterized by the presence of a hyperoccipital carina; frons with large setigerous punctures at least laterally; frons strongly bulging between antennal insertions and inner orbits; orbital furrow usually strongly expanded ventrally; antenna with radicle concolorous or lighter than the scape; gena rounded from malar sulcus to occipital carina, with no carina extending from base of mandibles dorsad; notauli well-developed; and sublateral setae on T1 usually absent (Johnson 1984a). In addition, Talamas et al. (2015) recently added that the clypeus in this group has 4 or fewer setae.

The fauna of *Trissolcus* in the Northern Hemisphere has been receiving great attention in recent years, due mainly to the introduction of three economically important Pentatomoida species into new areas (Talamas et al. 2015, 2017, 2019; Tortorici et al. 2019). However, there are few papers dealing with *Trissolcus* species of the Neotropics, and the *flavipes* group has not been treated since Johnson (1987).

Salomão and Vasconcellos-Neto (2010), while studying the population structure and dynamics of the Neotropical bark bug *Phloea subquadrata* Spinola (Hemiptera, Phloeidae) on *Plinia cauliflora* (Mart.) Kausel (Myrtales, Myrtaceae) in southeast Brazil, reared an undescribed and peculiar species of *Trissolcus* parasitizing its eggs. This paper deals with the description of this scelionid, increasing our understanding of morphological and biological diversity within Neotropical *Trissolcus*.

Methods

Specimen collection

Parasitoid specimens were reared from *P. subquadrata* eggs collected on the trunk of *Pl. cauliflora* trees in a fruit garden located in the Reserva Ecológica da Serra do Japi ($23^{\circ}14'04''S$, $46^{\circ}55'28''W$, 900 m.a.s.l.), in Jundiaí, São Paulo state, south-eastern Brazil. After initial storage in 70% ethanol, the specimens were dried and glue-mounted on paper points.

Imaging

Examinations and photographs were made with a Leica M165C stereomicroscope, equipped with a Leica DFC 420 digital camera and a dome for light dispersion (Kerr et al. 2008). Image stacks were combined using Leica Application Suite v3.8 to obtain final images with extended focus. Scanning electron micrographs were taken of a series of uncoated paratype specimens with a Quanta 250 scanning electron microscope under low vacuum. The final images were edited in Adobe Illustrator and Photoshop v. 2025.

Morphology

Terminology for surface sculpture follows Harris (1979). Morphological terms largely follow Masner (1980), with some terms from Johnson (1984a) (hyperocapital carina, orbital furrow), Johnson (1984b) (episternal foveae), Mikó et al. (2007) (mesopleural carina, mesopleural epicoxal sulcus, metasomal depression of propodeum, paracoxal sulcus, preocellar pit, postacetabular patch, postacetabular sulcus, speculum), Vilhelmsen et al. (2010) (mesopleural pit), Yoder et al. (2010) (antecostal sulcus) and Talamas et al. (2015) (axillar crescent). The claval formula follows Bin (1981), from the apical antennomere to the last antennomere bearing one or two papillary sensilla ventrally. The only abbreviation used is T1, T2, T3, ..., for tergites. The species description format follows Talamas et al. (2015) and Tortorici et al. (2019).

All morphological terms were matched to concepts in the Hymenoptera Anatomy Ontology (<http://portal.hymao.org/projects/32/public/ontology>) by use of the text analyzer function (Suppl. material 1). The color distribution map was generated using the web software SimpleMappr (Shorthouse 2010).

Collection acronyms

Collection acronyms used in this paper are: **MZUSP**, Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil; **OSUC**, C.A. Triplehorn Insect Collection, Columbus, USA.

DNA extraction, amplification and sequencing of the COI gene fragment

Total genomic DNA was extracted by destructively sampling a single male or female as outlined in Wengrat et al. (2021).

The mitochondrial cytochrome c oxidase subunit I (COI) fragment was amplified by Polymerase Chain Reaction (PCR) using the primers SCEL (Gariepy et al. 2014) and HCO-2198 (Folmer et al. 1994). The PCRs and thermocycling conditions were carried out following Gariepy et al. (2014). The amplicons were observed under ultraviolet light, after electrophoresis on 1.5% agarose gel stained with SYBR Safe (Life Technologies). The PCR purification was performed using 1 μ L (20 U μ L $^{-1}$) of Exonuclease I (Thermo Fisher Scientific™) and 2 μ L (1 U μ L $^{-1}$) of thermostable alkaline phosphatase FastAP™ (Thermo Fisher Scientific™) per 10 μ L of the final PCR product. The purification conditions were 37 °C for 30 min, followed by 80 °C for 15 min. The bidirectional Sanger sequencing was performed at the Agricultural Biotechnology Laboratory (CEBTEC), Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ), Piracicaba, SP, Brazil.

The sequence was checked, edited, and aligned to produce the consensus sequence using Geneious Prime 2022.1 (<https://www.geneious.com>). The sequence was submitted to the National Center for Biotechnology Information (NCBI) GenBank (www.ncbi.nlm.nih.gov).

Molecular analyses

Sequences of the mitochondrial gene COI from five genera belonging to subfamily Telenominae (Scelionidae) were obtained from GenBank, previously deposited by Chen et al. (2021) (Table 1) and added with our sequence from the new species *Trissolcus adrianae* sp. nov. The choice of taxa focused on *Trissolcus* species and related genera members of Telenominae, following the results proposed by Chen et al. (2021).

Matrix visualization, manipulation, and alignment were performed using the software MEGA X (Kumar et al. 2018). The protein coding gene COI was aligned using MUSCLE (Edgar 2004) with default gap opening, extension, and substitution costs as implemented in MEGA X. All codons were translated to functional amino acids using the invertebrate mitochondrial code. Maximum likelihood analysis was conducted on the molecular data using RAXML-HPC2 v. 8.2.10 on XSEDE (Stamatakis 2014), using the GTRGAMMA evolutionary model, through the CIPRES Science Gateway (Miller et al. 2010). Branch support was measured using 1000 bootstrap replicates. Interspecific distance among specimens was calculated using the p-distance in MEGA X. All resulting trees were visualized in FigTree 1.4.4, and the outgroup, *Paratelenomus saccharalis* (Dodd, 1914), was assigned; the final tree figure was generated using Adobe Illustrator v. 2025.

Results

Based on morphological, molecular and biological data we describe both sexes of *T. adrianae*, a new species that parasitizes eggs of *Phloea subquadrata* (Hemiptera, Phloeidae). The COI sequence was obtained for the new species at 586 bp. The BLAST search revealed that the sequence of *T. adrianae* does not have any similar ($\geq 85\%$) or closely related species/accessions in GenBank and BOLD (*Barcode of Life Data System*), since the genetic distance between the deposited sequences is 15%.

Table 1. Specimens of *Trissolcus* and some related genera of subfamily Telenominae (Scelionidae), with the respective GenBank accession numbers.

TAXON	COI GENBANK ACCESSION NUMBERS
<i>Baeoneurella</i> sp.	KC778481
<i>Paratelenomus saccharalis</i> (Dodd, 1914)	KC778441
<i>Psix tunetanus</i> (Mineo & Szabó, 1979)	KC778435
<i>Psix</i> sp.	MF583552
<i>Phanuromyia</i> sp.	KC778444
<i>Telenomus podisi</i> Ashmead, 1893	MF583566
<i>Telenomus grenadensis</i> Ashmead, 1895	KC778462
<i>Trissolcus adrianae</i> Costa & Johnson, sp. nov.	OQ720992
<i>Trissolcus</i> sp. 1	KC778492
<i>Trissolcus</i> sp. 2	MF583576
<i>Trissolcus hullensis</i> (Harrington, 1900)	KC778491
<i>Trissolcus strabus</i> Johnson, 1984	KC778486
<i>Trissolcus thyantae</i> Ashmead, 1893	KC778487
<i>Trissolcus urichi</i> Crawford, 1913	KC778485

Our previous molecular analyses (Fig. 13) focused on the placement and relationship between *T. adrianae* and the related genera of Telenominae, since its morphology is very distinct (see Remarks below). *Trissolcus adrianae* is placed as sister group of *Trissolcus urichi* + *Trissolcus* sp. 1 and, with a interspecific distance of 14% and 16%, respectively. This cladogram (Fig. 13) illustrates the limited usefulness of using only the COI fragment to infer relationships. For example, a more expanded analysis using morphological data and 4 genes - 18S, 28S, COI, and wingless places *Phanuromyia* sp. as the sister group to the remaining *Trissolcus*, *Baeoneurella*, and *Telenomus* as proposed by Chen et al. (2021) (but not *Parateleomus* and *Psix*). Furthermore, *T. urichi* and *Trissolcus* sp. 1 are not closely related since they belong to different species groups, *flavipes* group and *basalis* group, respectively (see Chen et al. 2021).

In our analyses, *T. adrianae* demonstrated a very high genetic divergence in relation to the other genera of Telenominae (see Suppl. material 2).

Taxonomy

Trissolcus adrianae Costa & Johnson, sp. nov.

<https://zoobank.org/6939EE1A-E68E-4543-A7D1-E7CFB6B427B8>

Figs 1–12

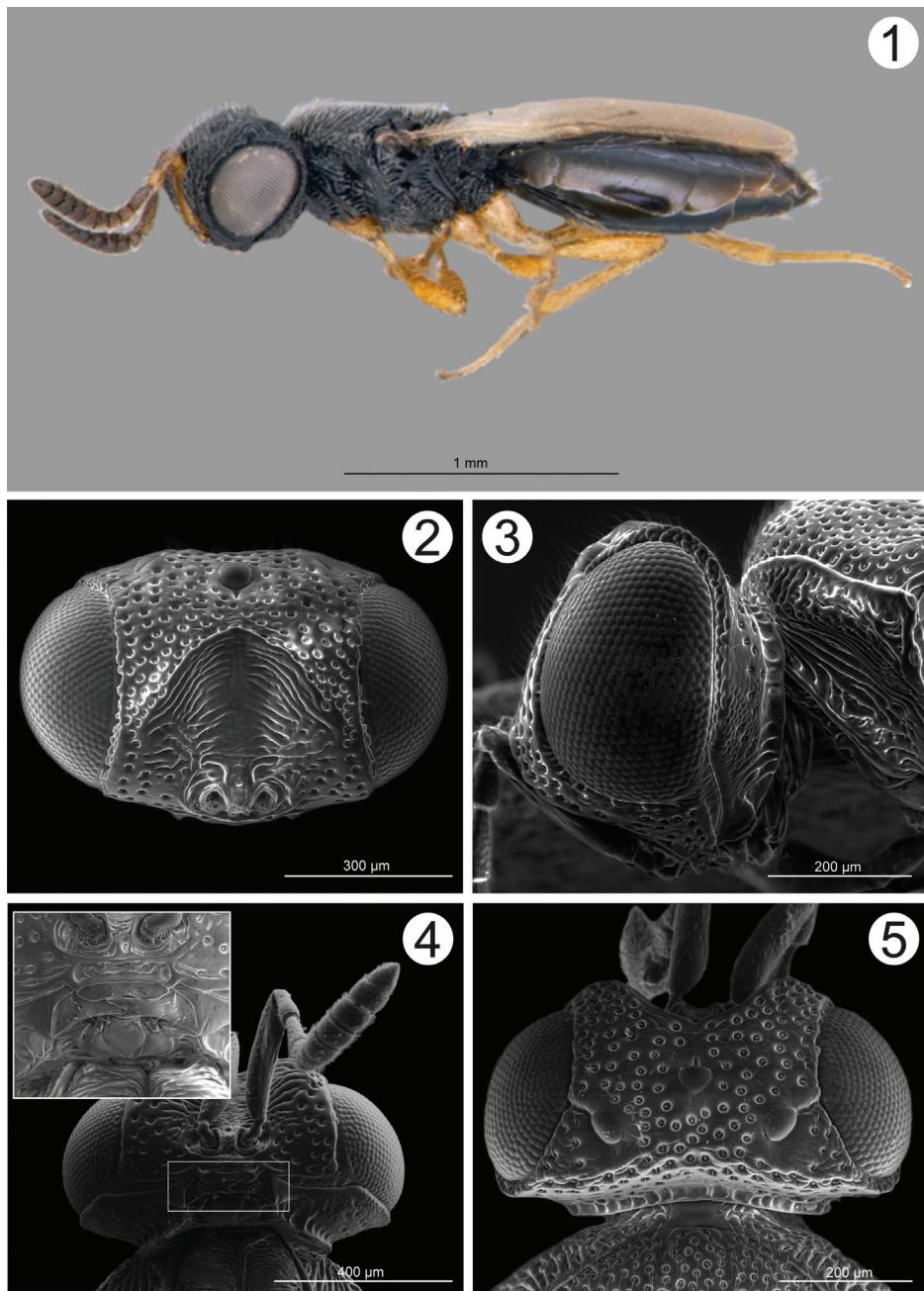
GenBank accession number. [OQ720992](#).

Diagnosis. *Trissolcus adrianae* Costa & Johnson, sp. nov. is a distinctive species which is easily distinguished from other members of the *flavipes* group by its noticeably depressed habitus; head oblong in anterior view; mandible with only two teeth; frontal depression delimited dorsally by carina; orbital furrow sculptured, constricted through most of its length by lateral expansion of frons; notauli absent; and length of T2 subequal to length of T3–T6.

Description (female). Body length: 2.18–2.44 mm (n = 12). Habitus: distinctly depressed dorsoventrally (Fig. 1).

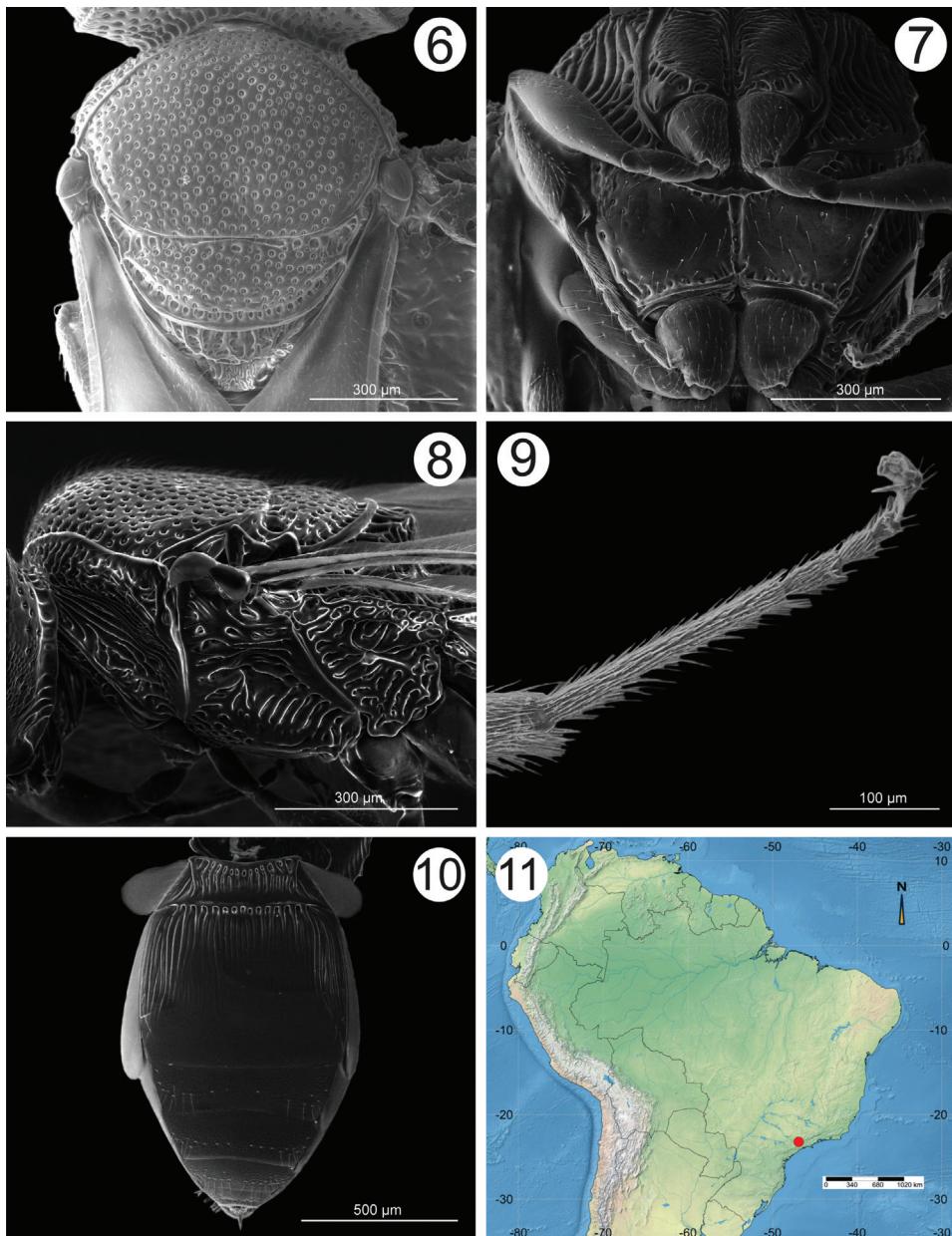
Color. Body: head, mesosoma, and metasoma black. Color of radicle: yellow to dark brown. Color of A1–A6: A1, A2 yellow, otherwise brown. Color of A7–A11: brown. Coxae: brownish yellow. Legs beyond coxae: brownish yellow.

Head. Claval formula: 1-2-2-2-2. Shape of head in anterior view: oblong (Fig. 2), distinctly bulging between antennal insertion and inner orbits. Shape of head in lateral view: strongly protruding anteriorly (Fig. 3). Number of mandibular teeth: two (Fig. 4). Number of clypeal setae: 4. Facial striae: absent. Shape of gena in lateral view: receding posteriorly. Genal carina: absent. Macrosculpture of lateral frons: absent. Microsculpture of lateral frons: punctate, with large, well-defined setigerous punctures irregularly distributed as on vertex, interspaces smooth. Macrosculpture inside antennal scrobe: transversely rugulose. Frontal depression: delimited dorsally by carina (Fig. 2). Orbital furrow: present, constricted through most of its length by lateral expansion of frons (Figs 2, 4). Hyperoccipital carina: weakly developed (Fig. 5). Vertex: angulate, particularly laterally. Preocellar pit: present.



Figures 1–5. *Trissolcus adrianae* Costa & Johnson, sp. nov., female **1** lateral habitus, paratype (OSUC 782300) **2** head, frontal view **3** head, lateral view **4** head, ventral view **5** head, dorsal view.

Mesosoma. Epomia: absent. Netrion sulcus: complete but obscured by coarse pro-natal sculpture. Mesoscutal suprakumeral sulcus: not distinguishable from surrounding surface sculpture. Mesoscutal humeral sulcus: present as a thin groove. Pattern of



Figures 6–11. *Trissolcus adrianae* Costa & Johnson, sp. nov., female **6** mesosoma, dorsal view **7** mesosoma, ventral view **8** mesosoma, lateral view **9** metabasitarsus ventral view **10** metasoma dorsal view **11** distributional map.

mesoscutal microsculpture: absent between punctures. Macrosculpture of mesoscutum: punctate, with large, well-defined setigerous punctures irregularly distributed as on vertex. Area bounded by axillar crescent: smooth. Parapsidal signum: absent. Notaulus: absent (Fig. 6). Median mesoscutal line: absent. Median mesoscutal sulcus: absent. Sculpture of

mesoscutellum: punctate, with large, well-defined setigerous punctures irregularly distributed as on vertex, interspaces smooth. Postacetabular sulcus: comprised of cells (Fig. 7). Episternal fovea: present. Shape of episternal foveae: elongate with rounded edges. Number of episternal foveae: 3–5. Course of episternal foveae ventrally: abutting cells of postacetabular sulcus. Course of episternal foveae dorsally: extending dorsally to mesopleural pit. Sculpture of postacetabular patch: densely punctate. Sculpture of anterior mesepisternum: smooth with punctures. Mesopleural epicoxal sulcus: comprised of cells anteriorly. Mesopleural carina: weakly indicated ventrally. Speculum: transversely striate. Paracoxal sulcus in ventral half of metapleuron: indicated by a line of distinct foveae. Length of intercoxal space: exceeding length of mesocoxae. Anteroventral extension of metapleuron: short and truncated. Line of pits along metapleural carina: present. Setation of metapleuron: absent (Fig. 8). Metapostnotum: invaginated at edges of metascutellum and separating metanotum from propodeum. Metasomal depression of propodeum: with striae radiating from propodeal foramen. Length of postmarginal vein: about $1.8 \times$ as long as stigmal vein. Length of metabasitarsus: longer than combined length of metatarsomeres 2–5 (Fig. 9).

Metasoma. Sublateral setae on T1: absent. Setation of laterotergite 1: absent. Sculpture of T2 posterior to antecostal sulcus: distinctly striate in basal half, striae longer laterally, smooth in apical half. Length of T3–T6: subequal to length of T2 (Fig. 10).

Male. Body length: 1.82–2.11 mm (n = 5). Color of antenna: scape and flagellum yellow (Fig. 12).

Etymology. This species is named in honor of Adriana Trevizoli Salomão, the collector. The epithet is treated as a noun in the genitive case.

Distribution. Brazil (São Paulo state) (Fig. 11).

Host. The eggs of *Phloea subquadrata* Spinola, 1837 (Hemiptera, Phloeidae).

Material examined. Holotype. BRAZIL • ♀; São Paulo, Jundiaí; 23°14'04"S, 46°55'28"W, 900 m.a.s.l.; 02.xi.2011; Ex *Phloea subquadrata* eggs on *Plinia cauliflora*; A.T. Salomão; MZUSP 133071.



Figure 12. *Trissolcus adrianae* Costa & Johnson, sp. nov., male antennae, lateral view.

Paratypes. BRAZIL • 7♀♂, same data as for holotype; 5♀, 1♂ MZUSP 133072–133077, 1♀ OSUC 782300.

Remarks. In the key to the New World species of the *flavipes* group (Johnson 1987), *T. adrianae* keys to couplet 6, as the scutellum has no median carina. The key of Johnson (1987) is modified to accommodate *T. adrianae*:

6a Scutellum punctate *T. adrianae* Costa & Johnson, sp. nov.
 – Scutellum without distinct punctuation..... 6

Another species of the group with a more elongate and depressed habitus is *T. decumbens* Johnson. However, besides the differences mentioned above in diagnosis, the two species would be distinguished immediately in the first couplet of Johnson (1987) key for the species of the group: while a distinct median longitudinal carina is present in the mesoscutellum of *T. decumbens*, such a carina is absent in *T. adrianae*. There are other differences; in *T. adrianae* the antennae of the female are almost entirely brown, mesonotum nearly flat, with posterior two thirds of mesoscutum and mesoscutellum in the same plane, mesonotum punctate, with well-defined setigerous punctures irregularly distributed and with interspaces smooth, and the coxae yellow. In *T. decumbens* the female antennae are abruptly bicolored, with A1–A7 yellow and A7–A11 dark brown, mesonotum convex, the mesoscutum with coriaceous to reticulate microsculpture throughout, and the coxae dark.

Trissolcus adrianae is the first species of Scelionidae associated with the family Phloeidae (Hemiptera: Pentatomoidea) and the first report of parasitism of Phloeidae

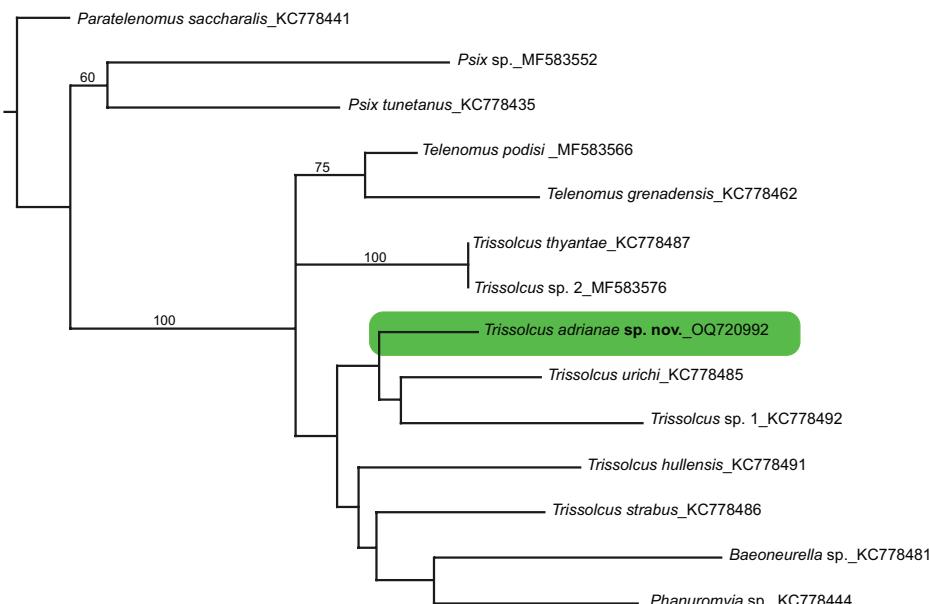


Figure 13. Maximum likelihood phylogeny elucidating placement of *Trissolcus adrianae* Costa & Johnson, sp. nov., based on the mitochondrial gene COI fragment. Support values based on 1000 bootstrap replicates.

eggs as well. Phloeidae is a small family that contains only 2 genera and 3 species endemic to the Neotropical region (Bernardes et al. 2005; López and Schwertner 2024). *Phloea subquadrata* eggs are laid in clusters on tree bark, forming an egg mass which is completely covered by the guarding female's flattened body (Magalhães 1909). Similar guarding behavior in the pentatomid *Antiteuchus tripterus* (Fabricius) has been associated with defense against egg parasitism by the telenomines *Trissolcus bodkini* Crawford and *Phanuropsis semiflaviventris* Girault (Eberhard 1975). The remarkably flattened body of *T. adrianae* may be an adaptation so that the female wasp can have access to the eggs. Another possibility is that *T. adrianae* may be phoretic on the adult of its host. The flattened body and elongate hind basitarsi are features found in other phoretic species of *Trissolcus* as well as the mantid egg parasitoids of the genus *Mantibaria* Kirby (Hymenoptera: Scelionidae).

Acknowledgements

To Zachary Lahey (United States Department of Agriculture, Agricultural Research Service, U.S. Vegetable Laboratory, Charleston, South Carolina, USA) for the critical review and valuable suggestions on the preliminary version of the manuscript; to Zachary Lahey again, to Rupam Debnath (M-Block, Zoological Survey of India, Kolkata, India) and Yu-anmeng Miles Zhang (Institute of Ecology and Evolution, University of Edinburgh, Edinburgh, UK) for reviewing and improving the manuscript; to Luciana B. R. Fernandes (Universidade Federal de São Carlos, SP, Brazil), for the SEM images; to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant numbers 2017/50334-3 and 2018/18965-6); to the INCT - Hympar (Instituto Nacional de Ciência e Tecnologia dos Hymenoptera Parasitoides, CNPq process 465562/2014-0). KS thanks the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant 2020/16051-7).

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Supplementary material 1

URI table of HAO morphological terms

Author: Valmir A. Costa

Data type: xlsx

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

Genetic divergence analyses

Author: Karine Schoeninger

Data type: xls

Explanation note: This table lists the genetic divergence data between *Trissolcus adrianae* sp. nov. and other taxa (other species of *Trissolcus* and related genera).

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