First record of *Tamarixia dahlsteni* Zuparko (Hymenoptera, Eulophidae), a parasitoid of *Trioza eugeniae* Froggatt (Hemiptera, Triozidae) and current status of *Tamarixia* species in Mexico

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4

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19

20 **Abstract**

21 Parasitic wasps of the genus *Tamarixia* represent important biological control agents of

22 members of the Psylloidea group and are host specific; therefore, they can be used to control

23 insect pests. In this study we report, for the first time, the appearance of the parasitoid

24 *Tamarixia dahlsteni* in Mexico and its mitochondrial barcode region of the cytochrome

25 oxidase I gene (COI). We also reviewed the species diversity of the genus *Tamarixia* in

26 Mexico.

27

28 Key words: Biological control, parasitoids, first report

29

Introduction

31 Biological control agents represent a sustainable pest management option. They help to
32 maintain pest populations under accepted levels and in many cases, they are found as natural
33 enemies of their prey or hosts (Wang et al. 2019; Al-Ani et al. 2020). Parasitic wasps from
34 the Eulophidae family represent a very important group of biological control agents as they
35 have a wide range of insect hosts with different degrees of specialization. The genus
36 *Tamarixia,* Mercet represents one example of parasitoids with host specificity (Urbaneja-
37 Bernat et al. 2019). Their lifecycle is mainly as ectoparasitoids, but in some cases
38 endoparasitism has been reported (Noyes 2022). Species of the genus *Tamarixia* parasite
39 free-living and gall-forming species of Psylloidea. In fact, it was proposed that they reached
40 this host restriction through a specialization on their host (LaSalle 1994). Moreover, the
41 *Tamarixia* species have also been reported parasitizing aphids and species from the Triozidae
42 family (Zuparko et al. 2011). According to the Universal Chalcidoidea database, the genus
43 *Tamarixia* comprises 52 species to date, which are worldwide-distributed (Noyes 2022).

44

45 In Mexico, it is possible to find some native and other exotic species of *Tamarixia*; together
46 they total five species of this genus: *Tamarixia aguacatensis* Yefremova (Yefremova et al.
47 2014), *Tamarixia leucaenae* Boucek (McClay 1990), *Tamarixia radiata* (Waterston)
48 (González-Hernández et al. 2009), *Tamarixia schina* Zuparko (Zuparko et al. 2011), and
49 *Tamarixia triozae* (Burks) (Lomeli-Flores and Bueno 2002). Some of these species have
50 already been part of integrated pest management strategies with remarkable results. For
51 instance, *T. triozae,* the parasitoid of the potato psyllid *Bactericera cockerelli,* was introduced
52 to New Zealand for the biological control of the psyllids that vectors the bacteria *Candidatus*
53 Liberibacter *solanacearum* (CLso) (Workman and Whiteman 2009). This bacterium has been
54 linked to different diseases in plants of the Solanaceae family (Munyaneza et al. 2007). In
55 Mexico, *T. triozae* was found naturally in tomato crops and according to parasitism
56 evaluations, the percentage of parasitism reached by *T. triozae* on *B. cockerelli* has been up
57 to eighty percent when insecticides are not used to control the psyllid populations (Lomeli-
58 Flores and Bueno 2002). Other examples of *Tamarixia* species used for biological control

59   are *T. schina* Zuparko, which was introduced in California for the control of *Calophya schini*
60   Tuthill (Psyllidae: Calophyidae), *T. dahlsteni* Zuparko, which was introduced for the control
61   of *Trioza eugeniae* Froggatt (Hemiptera: Triozidae) (Zuparko et al. 2011) and *T. radiata*.
62   The latter is native to Pakistan (Chen and Stansly 2014), but some colonies were introduced
63   in countries such as Taiwan, the United States and France (Guadeloupe), to control
64   populations of the psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), vector of the
65   bacteria *Candidatus* Liberibacter *asiaticus* (Chien et al. 1989; Michaud 2002; De León and
66   Sétamou 2010). In Texas, a reduction of more than ninety percent of *D. citri* populations has
67   been observed in regions where *T. radiata* was released (Flores and Ciomperlik 2017). In
68   Mexico, the parasitoid was reported as an accidentally introduced species (De León and
69   Sétamou 2010).

70

71   Currently, DNA barcodes are important tools for species identification with potential for bio-
72   surveillance programs in agriculture (Ashfaq and Hebert 2016). DNA barcodes have been
73   useful to identify important arthropod pests even at immature stages (Ashfaq and Hebert
74   2016). The common barcoding method used for animal identification is based on the
75   sequencing of a part of the mitochondrial gene citocromo oxidasa subunit I (COI). COI had
76   served to create universal and public databases of sequences of all known animal species like
77   the Barcode of Life Data System (BOLD), which also includes agriculturally important insect
78   sequences (Hebert et al. 2003). In this regard, here we report the occurrence of *T. dahlsteni*
79   Zuparko, in Mexico for the first time, and contribute with the first mitochondrial cytochrome
80   oxidase subunit one (COI) sequences for this species. We also review the current diversity
81   status of the genus *Tamarixia* in Mexico.

82

83   **Methods**
84   Biological samples
85   We obtained parasitoids emerged from nymphs of *Trioza eugeniae* feeding on *Syzygium*
86   *paniculatum*, collected in an urban area from Zapopan, Jalisco, Mexico [Colonia Las
87   Palomas, Tesistan (20.7890 −103.4831) and Club Deportivo UdeG (20.7793 −103.6075)].

88   The nymphs were taken to the laboratory (HR 70%, T 25 ± 2°C) and were placed into Petri

89   dishes until the parasitoids emerged. The parasitoids were placed in 96% ethanol for their

90   morphological and molecular determination.

91

92   Morphological determination

93   According to Zuparko et al. (2011), the psyllid *Trioza eugeniae* is parasitized by *Tamarixia*

94   *dahlsteni*; therefore, we employed the original morphological description of such species to

95   identify the newly emerged parasitoids. Voucher specimens of the recovered parasitoids were

96   deposited in the Beneficial Insects Collection of the Universidad Autonoma de Nuevo Leon

97   (CIBE–UANL). All individuals followed the same diagnosis and given the low number of

98   specimens recovered, only one female was photographed with a scanning electronic

99   microscope (JEOL JSM–6510LV) in order to illustrate its diagnostic characteristics.

100

101   Barcoding determination

102   Genomic DNA was nondestructively isolated according to the protocol described by Giantsis

103   et al. (2016). We extracted it from three individual specimens that corresponded to the *T.*

104   *dahlsteni*, two specimens of *T. triozae* and two of *T. schina*. Polymerase chain reaction (PCR)

105   was carried out to amplify the DNA barcode region of the cytochrome oxidase subunit I

106   (COI) using the LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198

107   (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') primers (Folmer et al. 1994). PCRs

108   were performed in a 20 µl reaction volume: 2 µl of DNA, 2 µl of 10x Qiagen PCR buffer

109   containing 15 mM MgCl2, 0.9 µl of each primer (10 um), 0.6 µl of dNTPs (25 mM each),

110   0.2 µl of (5 U/µl) Taq DNA Polymerase (Qiagen, Hilden, Germany), and 13.4 µl of H2O.

111   PCR conditions were as follows: 94°C for 3 min, followed by 40 cycles of 94 °C for 30 s, 52

112   °C for 1 min, 72 °C for 1 min with a final extension at 72 °C for 10 min. All PCR products

113   were electrophoresed through an agarose gel (1%) and sequenced in both directions in an

114   Applied Biosystems model 3500 automated sequencer in Lanbama–Ipicyt (San Luis Potosi,

115   Mexico).

116

117   Phylogenetic analysis

118   We employed the resulting COI sequences to reconstruct the phylogenetic relations of the
119   emerged parasitoids. For this objective, we included COI sequences of all available species
120   of the genus *Tamarixia*, which were downloaded from the BOLD Systems database and the
121   Genbank. All sequences were aligned in Mesquite 3.70 (Maddison and Maddison 2021) with
122   the program MUSCLE v3 (Edgar 2004). Later, the alignment was used for the phylogenetic
123   analysis inferred with the Maximum Likelihood method in the online server IQ-tree version
124   1.6.12 (Trifinopoulos et al. 2016), and the model GTR+F+I+G4 which was inferred with the
125   function Model Finder (Kalyaanamoorthy et al. 2017). Branch support was obtained with the
126   ultrafast bootstrap approximation (Hoang et al. 2018) with 10000 replicates. Sequences
127   generated in this study were deposited in the Genbank database.

128

129   **Results**

130   Morphological and barcoding determination
131   From the collected material for this study, seven females and seven males of the genus
132   *Tamarixia* emerged. They followed the diagnosis of *T. dahlsteni*: the entire ventral surface
133   of the gaster was yellow, and in the dorsal part the yellow color extended to, or went to
134   slightly beyond the apex of the second tergite (Fig. 1). The specimens had a paraspicular
135   carina posteriorly bifurcated located medial to the propodeal spiracle (Figs 2–3). Regarding
136   the barcoding determination, the sequences generated in this study represent the first
137   barcoding evidence for the species *T. dahlsteni* (Genbank accession ON491415, ON491416,
138   ON491417) and *T. schina* (Genbank accession ON548243, ON684328).

139

140   Phylogenetic analysis
141   Our sequences alignment contained 893 bp, and included sequences of the species *Tamarixia*
142   *drukyulensis* Yefremova and Yegorenkova, *Tamarixia dryi* Waterston, *Tamarixia pronomus*
143   Walker, *Tamarixia pubescens* Nees, *T. radiata*, *T. triozae* and *Tamarixia upis* Walker,
144   obtained from public databases and those of *T. dahlsteni* and *T. schina* generated in this study.
145   In the phylogenetic reconstruction, sequences of each species were clustered in individual
146   subclades with high support (ultrabootstrap values >95) (Fig. 4). Therefore, the barcoding

147   region appears to be useful for the molecular identification of the *Tamarixia* species included
148   in this study. Our analysis did not resolve interspecific relations due to the low bootstrap
149   support for interior branches. On the other hand, the presence of highly supported
150   (ultrabootstrap values >95) intraspecific subclades within the species *T. drukyulensis* and *T.*
151   *dryi* suggests intraspecific genetic structure.

152
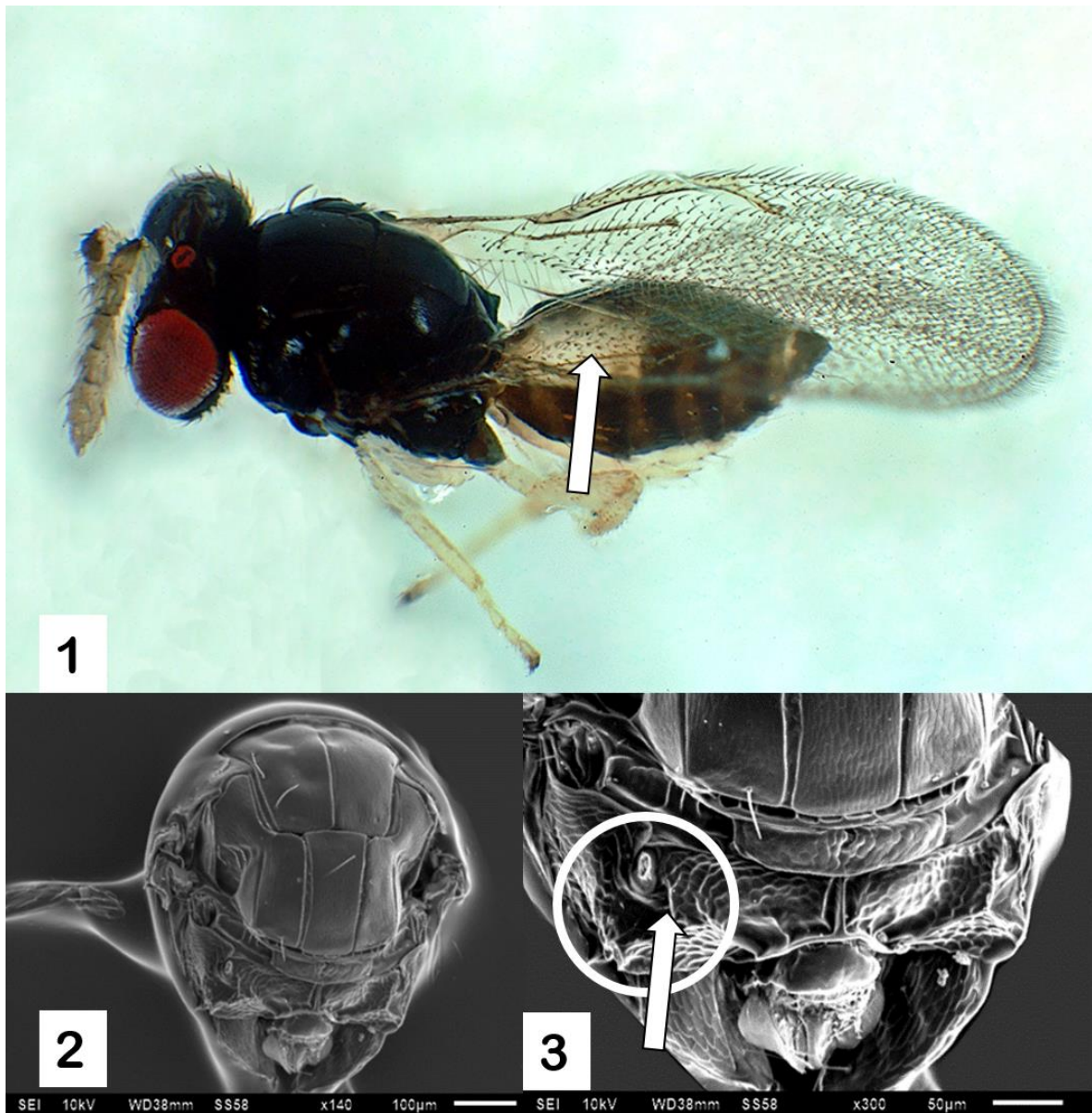
153   **Figures 1–3.** 1) *Tamarixia dahlsteni* gaster (dorsal view) the yellow color extended to the
154   apex of the second tergite. 2) Mesosoma (dorsal view). 3) Paraspicular carina. Scale bars:
155   100 μm (2); 50 μm (3).

156

157 **Figure 4.** Phylogenetic tree showing the genetic relations between species of the genus

158 *Tamarixia*. The species *Aprostocetus cerricola*, was included as an outgroup. Branch support

159 ≥ 95 is shown above branches; samples in bold represent sequences generated in this study,

160 while samples with an asterisk (*) were downloaded from the BOLD systems database. The

161 rest of the samples were downloaded from the Genbank. BOLD systems specimen records

162 and Genbank accession numbers are shown before species name.

163

164 **Discussion**

165 According to literature, of the 52 existent species of *Tamarixia*, seven are reported from the

166 Nearctic and Neotropical regions (Noyes 2022). Mexico is located in both regions and

167 following this study, the number of *Tamarixia* species was updated to six species. In addition,

168 our phylogenetic analysis suggested the COI barcoding region to be a useful molecular

169 marker for the distinction of *Tamarixia* species. Moreover, the phylogeny obtained suggested

170 intraspecific genetic structure for some of the species, which unveils the necessity of robust

171 and wider phylogenetic analysis at the genus level.

172

173   The host for *T. dahlsteni* is the Eugenia psyllid *Trioza eugeniae*. Both species were found in
174   Australia associated with the ornamental tree *Syzygium paniculatum* and in 1988 the psyllid
175   was found for the first time in California, USA. The damage caused by the psyllid on the *S.*
176   *paniculatum* trees prompted a search for its natural enemies in Australia. As a result, the wasp
177   *T. dahlsteni* was identified as the primary parasitoid of *T. eugeniae*, and was later imported
178   to the USA to control eugenia psyllid populations (Dahlsten et al. 1993). Notwithstanding,
179   when the parasitoid was imported to the USA, its identity was unknown (it was identified
180   only as *Tamarixia* sp.) and it took more than 20 years to describe it as a new species (Dahlsten
181   et al. 1993; Zuparko et al. 2011). A similar case is the one of *Tamarixia schina*. The wasp
182   was reported as a natural enemy of the exotic psyllid *Calophya rubra* (Blanchard) which
183   feeds on *Schinus molle* trees (Alvarez-Zagoya and Cibrián-Tovar, 1999). It was originally
184   described as an unknown species of *Tamarixia* until 2011 (Zuparko et al. 2011), so the list
185   of unnamed parasitoids of the genus *Tamarixia* might actually be longer. For instance,
186   according to Zuparko et al. (2011), another new putative species was reported in Florida,
187   USA, parasitizing psyllid species which have potential distribution in Mexico. However,
188   until now, that putative new species remains unnamed.

189

190   In research, names of species are essential to assure we are working with the same model of
191   study to obtain comparable results (Pante et al. 2015), and in agriculture they are also required
192   for biosecurity and quarantine concerns (Lyal et al. 2008). Notwithstanding, in some cases
193   species identification is not an easy task and deep taxonomic studies are needed. For instance,
194   in 2019 the misidentification of the eugenia psyllid *T. eugeniae* was uncovered by Taylor and
195   Martoni (2020), who indicated that the true name of the species should be *Trioza adventicia*
196   Tuthill. The authors mentioned that the two exotic species resemble each other, and only a
197   detailed study based on a series of morphological characters and DNA barcoding supported
198   the separation and validity of both species.

199

200   As already mentioned, psyllids are main hosts for the *Tamarixia* species and because of their
201   possible broad dietary tolerance some species might migrate and disperse to new geographic

202 regions (Percy et al. 2012). This might also promote the introduction and dispersion of exotic

203 or new species of parasitoids in countries like Mexico. For example, *T. schina* apparently

204 migrated to Mexico from California (Yefremova et al. 2014). According to the study by Percy

205 et al. (2012), in the same region (California, USA), different species of psyllids attacked by

206 some unidentified *Tamarixia* species exist, hosted by plants with potential distribution in

207 Mexico. Moreover, in this country more than 114 species of psyllids (Méndez-Tobar 2015)

208 exist, which may also represent possible hosts for exotic *Tamarixia* species. Therefore, the

209 diversity of this group of parasitoids might be currently underestimated in Mexico.

210

211 In agriculture, species identification protocols based on DNA represent powerful tools for

212 the success of early detection programs, or monitoring of species (Lyal et al. 2008; Boykin

213 et al. 2012; Poland and Rassati 2019). However, for some groups of insects, the lack of

214 reference barcodes, errors in databases, scarcity of voucher specimens and presence of

215 cryptic species represent strong limitations. As an example, recently a new *Tamarixia* species

216 (*T. aguacatensis*) was described based on morphological characters, but because their

217 sampling seems to be seasonally restricted (Yefremova et al. 2014), the generation barcodes

218 for further studies on their biology and phylogeny represents a challenge.

219

220 **Conclusion**

221 Besides reporting the presence of the *Tamarixia dahlsteni* species in Mexico, we also

222 provided barcodes that may be employed as a reference for further monitoring programs or

223 studies about this economically important group of wasps. Moreover, our phylogenetic

224 analysis suggests the need for a deeper and wider taxonomic revision of the genus.

225

226 **Credit author statement**

227 Conceptualization, MLRA and KIPC; Data curation, MLRA and KIPC; Investigation,

228 ARR, SGDR, IPRS; Methodology, MLRA, KIPC, ARR, SGDR, MAGG, IPRS, PZR;

229   Validation, MLRA, KIPC, MAGG, PZR; Writing–original draft, MLRA and KIPC;

230   Writing–review and editing, MLRA and KIPC.

231

235   Competing interests

236   The authors have declared that no competing interests exist.

237

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353 **Figures 1–3.** 1) *Tamarixia dahlsteni* gaster (dorsal view) the yellow color extended to the

354 apex of the second tergite. 2) Mesosoma (dorsal view). 3) Paraspicular carina.

355 **Figure 4.** Phylogenetic tree showing the genetic relations between species of the genus

356 *Tamarixia*. The species *Aprostocetus cerricola* was included as an outgroup. Branch support

357 ≥ 95 is shown above branches; samples in bold represent sequences generated in this study,

358 while samples with an asterisk (*) were downloaded from the BOLD systems database. The

359 rest of the samples were downloaded from the Genbank. BOLD systems specimen records

360 and Genbank accession numbers are shown before species name.