

First report in Colombia and diagnosis of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), a parasitoid wasp of *Diaphorina citri* (Hemiptera: Liviidae)

Primer reporte en Colombia y diagnóstico de *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), una avispa parasitoide de *Diaphorina citri* (Hemiptera: Liviidae)

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ABSTRACT

Diaphorencyrtus aligarhensis (Hymenoptera: Encyrtidae) is herein reported for the first time from Colombia based on specimens collected in the municipality of Palmira, department of Valle del Cauca. Adult male and female wasps of this endoparasitoid are diagnosed based on published literature and character states taken from specimens collected in the present study. The adult parasitoids were extracted from parasitized nymphs (mummies) of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae). Information is provided on the differences in the morphology of parasitized nymphs of *D. citri* with exit holes made by the two main primary parasitoids, i.e., *Tamarixia radiata* (Hymenoptera: Eulophidae) and *D. aligarhensis*. Rates of parasitization on *D. citri* ranged from 1.5 to 24.2 % for *T. radiata* and 0.3 to 1.0 % for *D. aligarhensis*. With the present study, the presence of *D. aligarhensis* in Colombia becomes the second confirmed report of the species in South America, after Ecuador.

Keywords: Biological control, endoparasitoid, new record, Neotropical region, taxonomy.

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RESUMEN

Diaphorencyrtus aligarhensis (Hymenoptera: Encyrtidae) se reporta por primera vez en Colombia con base en especímenes recolectados en el municipio de Palmira, departamento del Valle del Cauca. Los machos y hembras adultos del endoparásitoide *D. aligarhensis* se diagnostican con base en la literatura publicada y los estados de carácter tomados de las muestras recolectadas en el presente estudio. Los parasitoides adultos se extrajeron de ninfas parasitadas (momias) del psílido asiático de los cítricos, *Diaphorina citri* (Hemiptera: Liviidae). También se proporciona información sobre las diferencias en la morfología de las ninfas parasitadas de *D. citri* con orificios de salida de los dos principales parasitoides primarios, i.e., *Tamarixia radiata* (Hymenoptera: Eulophidae) y *D. aligarhensis*. Las tasas de parasitismo en *D. citri* variaron entre 1,5 y 24,2 % para *T. radiata* y entre 0,3 y 1,0 % para *D. aligarhensis*. Con el presente estudio, la presencia de *D. aligarhensis* en Colombia se convierte en el segundo reporte confirmado de la especie en América del Sur después de Ecuador.

Palabras clave: Control biológico, endoparásitoide, nuevo registro, región neotropical, taxonomía.

INTRODUCTION

In Colombia, efforts to prevent the spread of Huanglongbing (HLB), one of the most devastating diseases of citrus worldwide, have been carried out through chemical control of the insect vector (*D. citri*) and the eradication of infected trees (Kondo *et al.* 2020). On the other hand, in areas free of HLB, since 2013 the Colombian Corporation for Agricultural Research – Agrosavia has been conducting a mass rearing and release program of the wasp *Tamarixia radiata* (Waterston, 1922) (Hymenoptera: Eulophidae), the main parasitoid of *D. citri*, for research purposes. The program also is aimed at maintaining populations of *D. citri* at low levels in house gardens, hedge trees, and shrubs of the family Rutaceae (e.g., *Citrus* spp., *Swinglea glutinosa* (Blanco) Merr. and *Murraya paniculata* (L.) Jack) in urban areas because these populations can act as reservoirs for the HLB causing bacteria (Kondo 2018, Kondo *et al.* 2020).

During a study carried out in a citrus orchard in Agrosavia, Palmira Research Station, in order to determine the rates of parasitization of *T. radiata*, a second parasitoid which was hitherto not detected in the area, was identified as *Diaphorencyrtus aligarhensis* (Shafee, Alam and Agarwal, 1975) (Hymenoptera: Encyrtidae). Until now in Colombia, there was a record of *Diaphorencyrtus* sp. from the department of Caldas (Arias-Ortega *et al.* 2016), however, this is the first record of *D. aligarhensis* in Colombia. The present paper provides information on i) rates of parasiti-

zation of *T. radiata* and *D. aligarhensis* on *D. citri* under field conditions; ii) differences between these two primary parasitoids of *D. citri*, based on the field morphology of the adult wasps and of the parasitized nymphs; and iii) diagnoses and photographs of the adult male and female of *D. aligarhensis*.

MATERIALS AND METHODS

Eight field surveys were carried out between February 2020 and February 2021 in order to monitor the rates of parasitization (natural and after mass release) of *D. citri* by the parasitoid *T. radiata* in a citrus orchard (03°30'04"N, 76°18'46"W, 1005 m altitude) in Agrosavia, Palmira Research Station, municipality of Palmira, department of Valle del Cauca, Colombia. The citrus orchard was planted with Tahiti lime, *Citrus latifolia* Tanaka, Frost Valencia orange, *Citrus × sinensis* (L.) Osbeck and mandarin orange, *Citrus reticulata* Blanco var. Arrayana, with a planting density of 450 trees spaced 3.5 m between plants and 5 m between rows for an area of 7927 m². For each survey, 20–40 young shoots infested with nymphs of *D. citri* were selected, marked with a colored tape, and monitored daily from day one until all nymphs were parasitized, consumed by predators, fallen from the host, died for various reasons, or reached adulthood. Each infested shoot had between six and 110 nymphs, with a mean infestation of 20–30 nymphs per shoot. Observations of each infested shoot lasted up to 13 days.

The surveys conducted in 2020 were aimed at determining the natural rate of parasitization of *D. citri* in the field prior to mass releases of *T. radiata*, and the survey in 2021 was carried out to determine the rate of parasitization of *D. citri* after a field release of adult parasitoids of *T. radiata*. Surveys between February and October 2020 were non-destructive and the impact of *T. radiata* was determined by field counting of the number of mummies (with and without exit holes). With the aid of a magnifying glass, mummies can be recognized easily in the field by their dark brown to reddish brown coloration (Fig. 1a). A non-destructive method was chosen to prevent the removal of parasitoids from the field, which could affect the populations of the parasitoid in the next generation and result in a lower rate of parasitization in the following survey.

Surveys carried out in December 2020, and January and February 2021 were destructive; psyllid-infested shoots and/or leaf buds were trimmed off the trees and put in Petri dishes for daily observations in the laboratory. In the laboratory, the number of parasitized nymphs of *D. citri* was determined based on the presence of eggs, larvae, and pupae of *T. radiata*, as well as nymphs of *D. citri* with a parasitoid emergence hole. Nymphs were individually checked with the help of a fine pencil brush and entomological forceps under a stereo microscope. The shoots with parasitized nymphs were placed individually in 16-ounce plastic tubes to observe the emergence of the parasitoids and confirm that *T. radiata* had parasitized the psyllids. The shoots were checked daily for an average period of seven to ten days, counting the adults of *T. radiata* that emerged each day. To reduce the dehydration of the citrus shoot, the basal part of the stem where the cut was made was covered with a piece of cotton moistened with distilled water and cotton mesh was fitted in the lid of the plastic tube to allow the passage of air. This destructive method was adapted from various studies (Pluke *et al.* 2008, Chong *et al.* 2010, Cortez *et al.* 2010, Branco and Postali 2012, Chávez *et al.* 2017) and was used in the last three surveys because other natural enemies, especially coccinellids, were consuming the nymphs of *D. citri* that were being evaluated, making it difficult to calculate the parasitization rate of the parasitoids.

In February 2021, 320 adults of *T. radiata* were released in the citrus orchard. The parasitoids were obtained from a mass rearing program established at Agrosavia, Palmira Research Center, and were packed in 60 cc plastic bottles

with a screw cap (each bottle contained 40 unsexed adults of *T. radiata*) for a total of eight bottles. One bottle of parasitoids was released at each of eight sites in the lot, following a diagonal x-shaped pattern covering as much area as possible within the orchard. The number of nymphs on each of the selected shoots was counted just prior to the field release of the parasitoids. Observations were carried out daily from day one until either the emergence of the parasitoids, the adult psyllids emerged, or the nymphs disappeared due to the reasons described above. The rate of parasitization was determined by using the following formula:

$$\text{Rate of parasitization (\%)} = \left[\frac{\text{number of parasitized nymphs}}{\text{total number of evaluated nymphs}} \right] \times 100$$

Most of the emerged parasitoids identified as *Diaphorencyrtus* sp. were preserved in 75 % ethyl alcohol. A number of the alcohol-preserved specimens were critical-point-dried and mounted following the techniques described by Noyes (1982). Some individuals were point-mounted without previous treatments and others were mounted on slides in Hoyer's mounting medium (distilled water 50 cc, gum Arabic 30 cc, chloral hydrate 200 cc and glycerin 20 cc) or Euparal. Except for the antennae, which are used to determine the distribution pattern of coloration, the specimens for slide-mounting were cleared by heating them for about 10 to 20 minutes in potassium hydroxide (10 % w/v) prior to mounting. The specimens were identified to genus and species level by using the keys of Noyes and Hayat (1984) and Hayat (2006) and the descriptions of Robinson (1960), Shafee *et al.* (1975) and Hayat (1981). Morphological terminology used for the parasitoids follows that of Gibson (1997).

Depositories

TAMUIC: Texas A&M University Insect Collection, USA.

CTNI: Colección Taxonómica Nacional de Insectos "Luis María Murillo", Corporación Colombiana de Investigación Agropecuaria, Agrosavia, Mosquera, Cundinamarca, Colombia. Voucher specimens in alcohol were deposited at CTNI. Voucher catalogue numbers are given in the material studied section.

RESULTS

In the first three surveys (February-June 2020), all nymphs of *D. citri* were parasitized by *T. radiata*, with the typical parasitoid exit hole located in the thoracic

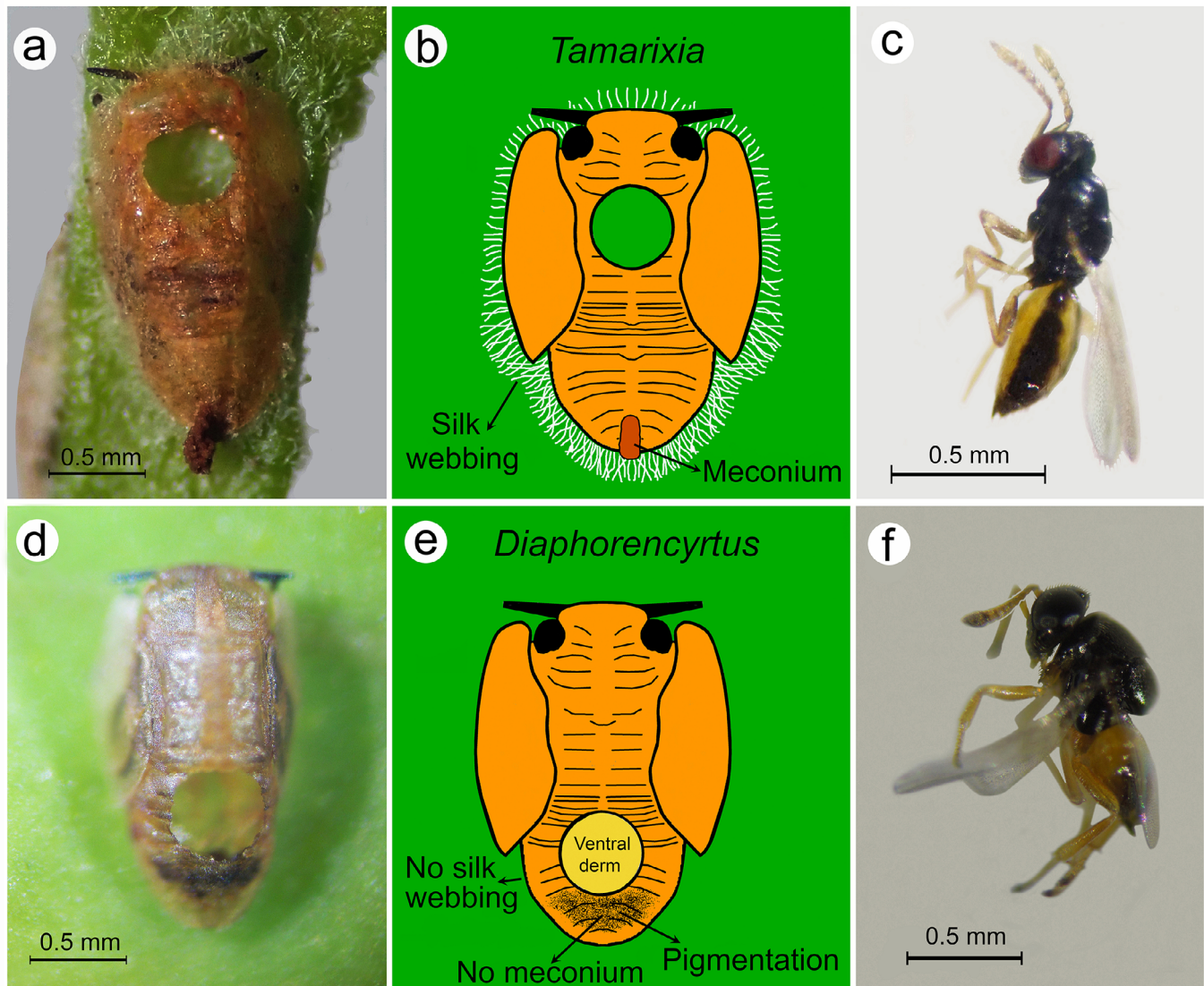


Figure 1. Morphological differences between *Tamarixia radiata* and *Diaphorencyrtus aligarhensis*. **a.** A close-up photograph of a mummy after emergence of *T. radiata*. **b.** Color illustration based on the photograph in **a.** **c.** Adult female of *T. radiata*. **d.** A close-up photograph of a mummy after emergence of *D. aligarhensis*. **e.** Color illustration based on the photograph in **d.** **f.** Adult female of *D. aligarhensis*. Illustrations and photographs: **a, b, e** by TK; **c, d, f** by KTA.

region. The rates of parasitization of *T. radiata* in these first surveys were as follows: 2.1 % (25 parasitoids from 1172 nymphs) in February (17/02/2020–28/02/2020), 1.5 % (18 parasitoids from 1188 nymphs) in March (02/03/2020–11/03/2020), and 3.0 % (21 parasitoids from 697 nymphs) in June (09/06/2020–21/06/2020). In the survey carried out in July (07/07/2020–22/07/2020), of the 1278 nymphs of *D. citri* surveyed, 103 (8.1 %) nymphs were parasitized by *T. radiata* (mummies with exit holes on the thorax). However, in this survey, 12 (0.9 %) mummified nymphs of *D. citri* had parasitoid exit holes in the abdominal area, very similar to that reported for *Diaphorencyrtus* sp., however, at that time, adults of the parasitoid were not recovered. In the following survey,

conducted in August 2020 (20/08/2020–28/08/2020), a total of 990 nymphs were surveyed, of which 240 (24.2 %) nymphs were parasitized by *T. radiata* and three (0.3 %) nymphs by a second parasitoid, tentatively identified as *Diaphorencyrtus* sp. based on the position of the parasitoid exit hole on the abdomen. Populations of *D. citri* in September (07/09/2020–25/09/2020) were very low (n = 62) and no evidence of parasitoids was detected. In the survey conducted in October 2020 (11/10/2020–28/10/2020), a total of 1746 nymphs were counted at the start of the survey, however, the manager of the citrus orchard mistakenly sprayed the trees with a mixture of pesticides [Confidor® (neocotinoid, active ingredient Imidacloprid) + Oberon® (acaricide, active ingredient

Spiromesifen), in addition to the application of Inex-A®, a penetrating surfactant adjuvant]. This chemical treatment decimated the population of *D. citri*, however, we were able to collect 32 psyllid mummies just prior to the chemical treatment, from which 11 adults of *T. radiata* and 14 adults of the tentatively identified *Diaphorencyrtus* sp. were recovered; eight mummies did not produce any parasitoids. Parasitization rates by parasitoids were not calculated for the October survey because the original population was killed by the pesticides. Due to the negative effect and persistence of the pesticide treatment, populations of *D. citri* were not detected during November–December 2020 and January 2021. In the survey carried out in February 2021 (04/02/2021–17/02/2021), the population of *D. citri* recovered in the citrus orchard and 320 unsexed adult parasitoids of *T. radiata* were released in the field and on this occasion a total of 2110 nymphs of *D. citri* were evaluated of which 187 (8.9 %) nymphs were parasitized by *T. radiata* and 21 (1.0 %) nymphs were parasitized by the probable *Diaphorencyrtus* sp. Specimens tentatively identified as *Diaphorencyrtus* sp. were sent to the second author (J.B.W.) who identified the parasitoid as *Diaphorencyrtus aligarhensis*. As a further check, images corresponding to Figs. 2a–f were sent to Prof. Mohammad Hayat, Aligarh Muslim University, India, who kindly compared them to paratypes of *D. aligarhensis* in his collection and confirmed our identification.

Taxonomy

Diaphorencyrtus aligarhensis (Shafee, Alam and Agarwal, 1975) (Hymenoptera: Encyrtidae)

Synonymy (taken from Noyes [c2019](#)): *Aphidencyrtus aligarhensis* Shafee, Alam and Agarwal, 1975; *Aphidencyrtus diaphorinae* Myartseva and Trjapitzin, 1978; *Aphidencyrtus sacchari* Kaul and Agarwal, 1986; *Diaphorencyrtus diaphorinae* (Lin and Tao, 1979); *Diaphorencyrtus diaphorinae* (Myartseva and Trjapitzin, 1978); *Psyllaephagus diaphorinae* Lin and Tao, 1979; *Syrphophagus aligarhensis* (Shafee, Alam and Agarwal, 1975).

Material studied. *Diaphorencyrtus aligarhensis*, ex *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Liviidae). Colombia, Valle del Cauca, Palmira, Agrosavia, Palmira Research Station, citrus orchard, 03°30'04"N, 76°18'46"W, 1000 m a.s.l., 12.viii.2020, coll. K. T. Arciniegas (voucher #6692-1: 3 female specimens) (CTNI); *Diaphorencyrtus aligarhensis*, ex *Diaphorina citri* Ku-

wayama (Hemiptera: Liviidae), Colombia, Valle del Cauca, Palmira, Agrosavia, Palmira Research Station, citrus orchard, 03°30'04"N, 76°18'46"W, 1000 m a.s.l., 12.xi.2020, coll. K. T. Arciniegas (voucher #755: 7 females and 1 male specimen) (TAMUIC), (voucher #6692-2: 4 females and 1 male specimen) (CTNI).

Field recognition

Parasitized nymphs (mummies): *Tamarixia radiata* vs *Diaphorencyrtus aligarhensis*

While studying the parasitization rate of *T. radiata* in the field using a non-destructive method, the authors first recognized the presence of a second parasitoid by the position of the exit hole of the adult parasitoids. In the field, the position of the exit hole on the mummies of *D. citri* can be used to distinguish nymphs parasitized by *D. aligarhensis* from those parasitized by *T. radiata*, i.e., the emerging adult of *T. radiata* exits dorsally through the thorax, whereas the adult of *D. aligarhensis* exists dorsally through the abdomen. There are also other differences induced by both parasitoids such as the shape of the mummies. The parasitized nymphs or mummies of *D. citri* hosting *T. radiata* and *D. aligarhensis* can be differentiated by the following combination of features [features of *D. aligarhensis* in square brackets]: 1) exit hole of the adult parasitoid usually found on the thorax (Hoy [2005](#)); the plant substrate visible inside the exit hole (Figs. 1a, b) [exit hole usually found on the abdomen; dried thin ventral derm of the parasitized nymph visible inside the exit hole (Figs. 1d, e)]; 2) mummies normally attached to the plant substrate by silk webbing that is visible around the margins of the mummy, mainly around the posterior abdomen (Figs. 1a, b) [mummies attached ventrally to the plant substrate by a sticky substance; without silk webbing around the mummy (Figs. 1d, e)]; 3) body shape similar to a non-parasitized nymph, rather flat, dark brown (Figs. 1a, b), with the ectoparasitoid larva found externally under the psyllid body [body shape different from non-parasitized nymphs, becoming cylindrical (Fig. 1d), the endoparasitoid larva found within the psyllid body]; and 4) parasitized nymph with a meconium on posterior part of body, its abdomen without pigmentation, usually of the same color as rest of body (Figs. 1a, b) [parasitized nymph without a meconium on posterior part of body; abdomen often with a pigmentation on posterior abdominal segments, clearly darker than rest of body (Figs. 1d, e)].

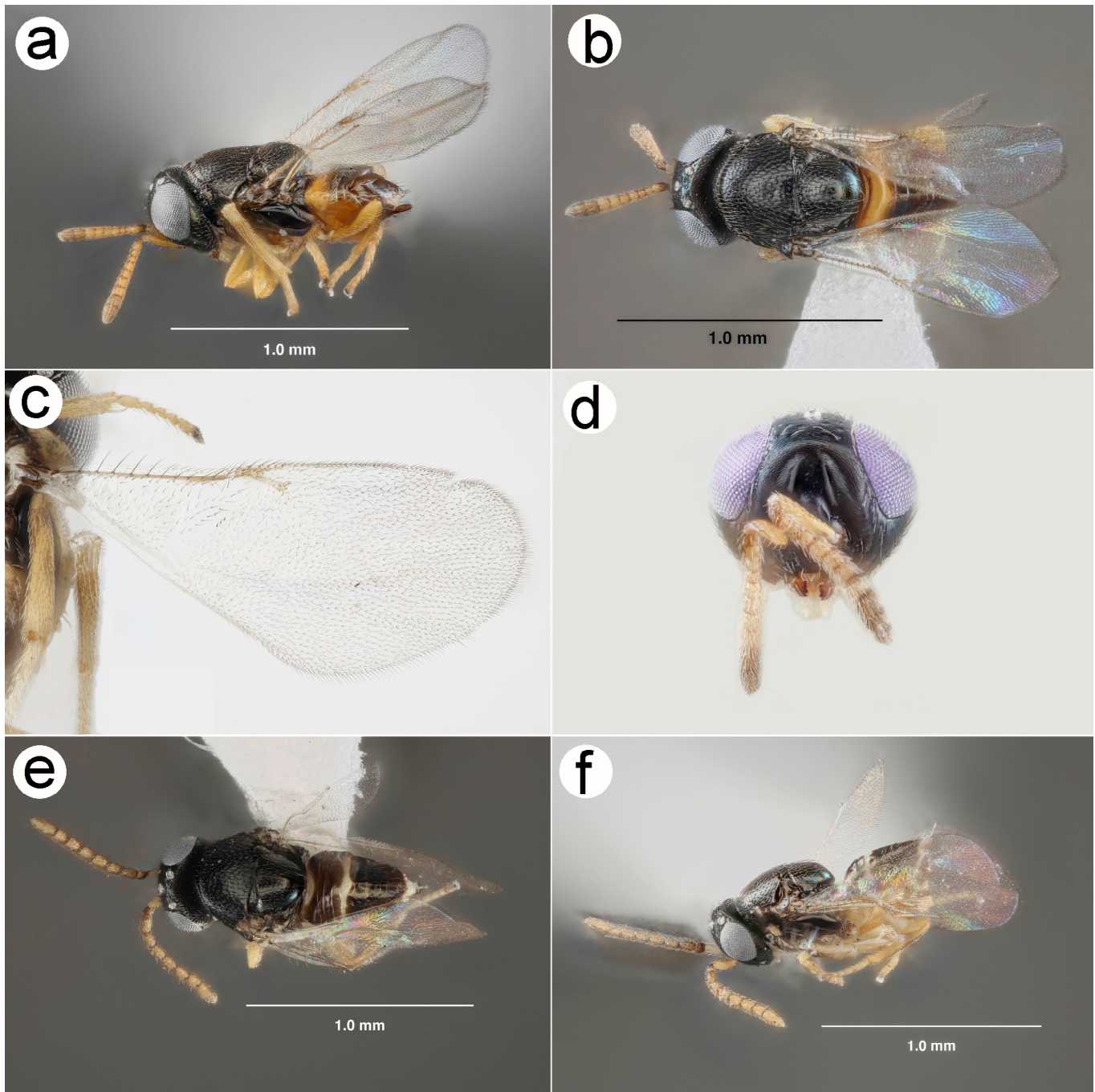


Figure 2. *Diaphorencyrtus aligarhensis*. **a.** Adult female, lateral habitus. **b.** Adult female dorsal habitus. **c.** Female fore wing. **d.** Female, face, and antenna. **e.** Adult male, dorsal habitus. **f.** Adult male, lateral habitus. Photos by JBW (a & b are TAMU x1756468, c & d are TAMU x1756467, e & f are TAMU x1756460).

The adults: *Tamarixia radiata* vs *Diaphorencyrtus aligarhensis*

Considering there are only two primary parasitoids of *D. citri*, adults of *T. radiata* (Fig. 1c) and *D. aligarhensis* (Fig. 1f) can be differentiated in the field by the following combination of features [features of *D. aligarhensis* in square

brackets]: 1) body elongate, 1 mm long, head black, with reddish eyes (Fig. 1c) [body somewhat stout, 1.5–1.8 mm long, head and eyes both black (Figs. 1f and 2a-f)]; 2) antennae and legs pale cream, pale white or pale yellow (Fig. 1c) (male antennae plumose) [antennae and legs yellow (Fig. 2a) (male antennae without a clava and covered in small hairs) (Fig. 2e, f)]; 3) metasoma dark laterally and

yellow dorsomedially (Fig. 1c) (yellow patch of color less pronounced and more anterior in male) [abdomen yellow, dark posteriorly (Figs. 1f and 2a, b)].

Note. Descriptions of nymphs of *D. citri* parasitized by *T. radiata* can also be found in Aubert and Quilici (1984), Chen and Stansly (2014), and Hoy (2005); information on nymphs of *D. citri* parasitized by *D. aligarhensis* are found in Rohrig (c2010, c2014) and Qing (1990). Character states of the adults of *T. radiata* were taken from Kondo et al. (2012) and Qing and Aubert (1990), and those of *D. aligarhensis* taken from Rohrig (c2014). These reports agree well with our observations. Additionally, some features were taken from the studied material, i.e., the dark coloration around posterior abdominal segments and the dried thin ventral derm of the parasitized nymph visible inside the exit hole in nymphs of *D. citri* parasitized by *D. aligarhensis*.

Diagnosis of the adults of *Diaphorencyrtus aligarhensis*

According to Qing and Aubert (1990), *D. aligarhensis* can be diagnosed by the following combination of features: female body 1.5–1.8 mm long, head and mesosoma black, basal four metasomal segments yellow, apical metasoma dark brown; antennae yellowish brown, funicle 6-segmented, funiculars wider than long, gradually larger towards clava; clava 3-segmented, subcylindrical, rounded apically; legs yellow, with 3–6 spines on apical parts of mid tibiae and 1st–3rd tarsi of middle leg. Male antennae yellowish, funicle 6-segmented, funiculars longer than wide, clava slender, 1-segmented; legs white-yellowish, basal abdomen yellowish brown. The studied material agrees well with the above diagnosis.

Taxonomic notes. *Diaphorencyrtus* can be recognized by a combination of features of the antennal segmentation and coloration (Figs. 2a–c) in which the clava is slightly darker than the funicle segments and apically rounded, the robust and convex mesosoma, the yellow or orange terga at the base of the metasoma (Figs. 2a, b), and the wing venation (Fig. 2c) with a pronounced postmarginal vein that is shorter than the long stigmal vein. The metapleuron is covered in conspicuous silvery setae (Fig. 2a) which serves to distinguish *Diaphorencyrtus* from the similar genus *Syrphophagus* (Hayat 2006). There are currently two valid species of *Diaphorencyrtus* (Noyes c2019). *Diaphorencyrtus harrisoni* (Robinson, 1960) can be distinguished from *D. aligarhensis* by having only a single yellow tergum at the base of the metasoma (at least two in *D. aligarhen-*

sis), the antennal scape which is dark brown in the basal half (all yellow in *D. aligarhensis*) and differences in leg coloration (see description in Robinson (1960)).

According to Prinsloo (1985), cleared, slide-mounted female specimens of *D. aligarhensis* from Reunion Island differ slightly from Indian specimens by having the antennal pedicel about as long as the basal two funicle segments together (a little longer in Indian specimens), with the head and thorax black (brownish-black to dark brown in Indian specimens), although he considered these differences as part of the morphological variation of the species.

DISCUSSION

Arias-Ortega et al. (2016) reported *Diaphorencyrtus* sp. from Colombia as a possible undescribed species, stating that unlike the features described by Mani (1989) and Rohrig (c2014), the postmarginal vein of the anterior wing is not slightly longer than the marginal vein, the female has setae on the antennae and the antennae of the male are highly pilose, which may indicate that it is a different species. However, we disagree with the character states listed by Arias-Ortega et al. (2016) as evidence for a different species because in the photograph they provide the postmarginal vein is slightly longer than the marginal vein as stated in the original description of Shafee et al. (1975). Concerning the pilosity of the antennae, Mani (1989) does not illustrate nor mention the pilosity for either male or female antennae, and Rohrig (c2014) described the female antennae as smooth and clubbed and the male antennae as slightly longer than those of the female, lacking a club and being covered with short hairs. Although Rohrig (c2014) describes the antennae of *D. aligarhensis* as being smooth, the photos of the adult female in his factsheet clearly shows small hairs covering the antennal segments. Thus, we consider that the report of *Diaphorencyrtus* sp. by Arias-Ortega et al. (2016) from the municipality of Manizales, department of Caldas, Colombia refers to *D. aligarhensis*.

In South America, *D. aligarhensis* has been recorded previously only from Ecuador where the parasitoid was probably accidentally introduced with its psyllid host, *D. citri* (Portalanza et al. 2017). In a checklist of natural enemies of *D. citri* of the world compiled by Kondo et al. (2015a), the authors erroneously listed Argentina as part of the distribution of *D. aligarhensis* citing a data sheet by García-Darderes (2009), however, this publication mere-

ly mentioned the species as a biological control agent. The distribution of *D. aligarhensis* in South America is now revised to include only Colombia and Ecuador. It is likely that *D. aligarhensis* is more widespread in Colombia and elsewhere in South America because *D. aligarhensis* is out-competed by *T. radiata* (see below for discussion) and thus not as easy to detect, and intraguild predation by coccinellids and other predators may generally keep their populations very low.

Diaphorencyrtus aligarhensis is native to the Oriental region, known from Afghanistan (CABI [c2021](#)), China (Yang *et al.* [2006](#)), India (Shafee *et al.* [1975](#)), Philippines and Vietnam (Aubert [1987](#)); and has been introduced deliberately to Reunion Island (Aubert and Quilici [1984](#)), Taiwan (Chien and Chu [1996](#)), South Africa (Prinsloo [1985](#)), and the U.S.A. (Hoy and Nguyen [2001](#), Michaud [2004](#), Vankosky and Hoddle [2016](#), Milosavljević *et al.* [2017](#)) for the control of citrus psyllids. Populations of *D. aligarhensis* from Taiwan, Vietnam (Hoy and Nguyen [2001](#), Hoy *et al.* [c2006](#)), mainland China (Rohrig *et al.* [2011](#), [2012](#)), and Pakistan (Hoddle [2012](#), Milosavljević *et al.* [2017](#)) were introduced to mainland U.S.A. However, populations of *D. aligarhensis* introduced to Florida from Taiwan and mainland China (Guangdong province) have failed to establish (Rohrig *et al.* [2012](#), Bistline-East *et al.* [2015](#)). Furthermore, populations of *D. aligarhensis* introduced to California from Pakistan have apparently also failed to establish (Milosavljević *et al.* [2021](#)). The parasitoid has been found also on the island of Oahu, Hawaii, where it was accidentally introduced (Matsunaga [2014](#)). Furthermore, there is a report of *Diaphorencyrtus* sp. from Mexico (Cortez *et al.* [2010](#)) as a parasitoid of *D. citri*.

Hussain and Nath ([1927](#)) reported nine parasitoid species attacking *D. citri* nymphs in Punjab province, Pakistan. However, Hoddle *et al.* ([2014](#)) confirmed that most species reported by Hussain and Nath ([1927](#)) are hyperparasitoids or parasitoids of other insect species and that there are only two primary parasitoids of *D. citri* in the province of Punjab, i.e., *T. radiata* and *D. aligarhensis*. Another study also reports these two primary parasitoids in the Asian Pacific region, and 13 secondary or tertiary parasitoids (Qing [1990](#)). Mummies from which hyperparasitoids emerge, generally have exit holes on the lateral side of the psyllid nymphs (Qin [1990](#)), thus they can be differentiated from mummies induced by primary parasitoids in which

the exit holes are found in the midline of the mummies (Qing [1990](#)).

Between 2014 and 2017, over 300 000 *D. aligarhensis* wasps were released in urban areas of Southern California, U.S.A. by the California Department of Food and Agriculture (CDFA) and the University of California, Riverside (Milosavljević *et al.* [2017](#)). More than 20 million parasitoids (*T. radiata* and *D. aligarhensis* combined) were mass-produced and released at >1 500 sites in southern California by the CDFA, however, only *T. radiata* spread rapidly and established, whereas *D. aligarhensis* apparently did not become established (Milosavljević *et al.* [2021](#)).

A study conducted under laboratory conditions indicated that when *D. aligarhensis* and *T. radiata* compete, *T. radiata* will have an advantage, suggesting that it may be difficult for *D. aligarhensis* to contribute significantly to the biological control of *D. citri* when both parasitoids are present (Vankosky and Hoddle [2019b](#)). When a nymph of *D. citri* is parasitized by *T. radiata* before or within five days following oviposition by *D. aligarhensis*, the emerging parasitoid will be an offspring of *T. radiata* (Rohrig *et al.* [2012](#)). Nevertheless, in Reunion Island, in the absence of hyperparasitoids, both parasitoids have been reported to be extremely efficient in controlling populations of *D. citri* (Aubert and Quilici [1984](#)).

In a study conducted in Punjab, Pakistan, on nymphs of *D. citri* on *Citrus reticulata* L. and *Citrus sinensis* Osbeck (Rutaceae), average rate of parasitization of 26 % by *T. radiata* and 17 % for *D. aligarhensis* have been reported (Khan *et al.* [2014](#)). During its lifetime, an adult female of *D. aligarhensis* can kill up to 280 nymphs of *D. citri* through a combination of host feeding and parasitization (Chien [1995](#)). On the other hand, an adult female of *T. radiata* can kill up to 500 nymphs of *D. citri* through a combination of host feeding and parasitization during its lifetime (Chien [1995](#)).

During the present study, we observed that the number of nymphs of *D. citri* usually started to decline from day one in the field due to predation and other undetermined factors. In some cases, all nymphs disappeared from a shoot from one day to the next, making it impossible to evaluate the rate of parasitization. In the Valle del Cauca region, apart from *T. radiata*, numerous other natural enemies of *D. citri* have been reported, including ladybird

beetles (Coleoptera: Coccinellidae), hover flies (Diptera: Syrphidae), assassin bugs (Hemiptera: Reduviidae), vespid wasps (Hymenoptera: Vespidae), lacewings (Neuroptera: Chrysopidae), ants (Hymenoptera: Formicidae), and a dragonfly (Odonata: Gomphidae), among others (Kondo et al. 2015a, b, 2018).

A study conducted in California, U.S.A., determined that *D. aligarhensis* shows a high preference for the Asian citrus psyllid, *D. citri*, but may parasitize the potato psyllid *Bactericera cockerelli* (Sulc., 1909), at low levels (< 14 %) (Bistline-East et al. 2015). *Diaphorina citri* and its two main parasitoids, *D. aligarhensis* and *T. radiata* have similar survival rates under different relative humidity and temperature regimes (McFarland and Hoy 2001). The developmental time from psyllid egg to the start of parasitoid emergence is 24 days for *T. radiata* and 28 days for *D. aligarhensis* (Skelley and Hoy 2004).

Diaphorencyrtus aligarhensis wasps oviposit on second-, third- and fourth-instar nymphs of *D. citri* and host feed on first- to fourth-instar nymphs (Skelley and Hoy 2004, Rohrig et al. 2011). On the other hand, *T. radiata* prefers to parasitize third-, fourth- and fifth-instar nymphs of *D. citri* (Sule et al. 2014). However, populations of *D. aligarhensis* and *T. radiata* from Pakistan, where both species exist in sympatry, shared a preference for fourth-instar nymphs of *D. citri* (Vankosky and Hoddle 2019a). The adult female of the ectoparasitoid *T. radiata* usually lays one egg, although rarely two on the ventral side of the nymph of *D. citri*, frequently in the division between the thorax and the abdomen, near the point of attachment of the posterior coxae, but only one adult parasitoid will emerge per nymph (Mann and Stelinski 2010, García-Córdoba et al. 2017). The adult female of the endoparasitoid *D. aligarhensis* also oviposits a single egg (rarely two eggs) into the host, usually through the psyllid's abdomen; and like *T. radiata* only one individual will complete development to adulthood when multiparasitism occurs (Rohrig et al. 2011).

The egg to adult development of Pakistani individuals of *D. aligarhensis* in California lasts from 18–19 days in the females and 20 days in the males at 25 °C (Milosavljević et al. 2019). The life cycle of Taiwanese and Chinese (mainland) individuals of *D. aligarhensis* from egg to adult lasts 16 to 18 days at 25 °C (Chien 1995, Rohrig et al. 2011). The developmental stages of *D. aligarhensis* include an embryonic stage (ca. 2 days), four larval instars (ca. 6 days),

a prepupal stage (ca. 1 day), and a pupal stage (ca. 7 days) (Rohrig 2014). After about 8 days post oviposition, the parasitized nymphs of *D. citri* die and harden, becoming a brown mummy (Rohrig 2014). There are no significant differences in the mean number of progeny produced by females of *D. aligarhensis* when given second-, third-, or fourth-instar hosts (Skelley and Hoy 2004).

The population of *D. aligarhensis* introduced to Florida from Taiwan are infected by the intracellular endosymbiont *Wolbachia* (Jeyaparakash and Hoy 2000, Meyer and Hoy 2007), which probably explains why they are thelytokous, comprised of only females (Chien 1995). The population in Colombia produces both males and females. Thus, it is possible that the population of *D. aligarhensis* found in Colombia may belong to the Pakistani stock introduced into California, U.S.A. Further molecular studies may help elucidate the introduction path of this parasitoid in Colombia.

In the Valle del Cauca region, Colombia, the two main parasitoids of *D. citri* coexist, whilst the populations of *D. aligarhensis* are much lower than those of *T. radiata*, as reported in other studies. Although parasitization rates by *D. aligarhensis* are low compared to *T. radiata*, its parasitization added to that of *T. radiata* and predation by the numerous predators in the environment makes it an important natural enemy in the control of the Asian citrus psyllid.

AUTHORS' CONTRIBUTIONS

JBW, TK, KTA, and YCP contributed to the writing of the manuscript, agree with manuscript results and conclusions, made critical revisions and approved the final version. TK prepared the original draft and the illustrations. JBW wrote the diagnosis and measured the specimens. KTA and YCP conducted the field work. Photographs were taken by JBW and KTA and TK. All authors reviewed and approved the final manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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