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***Marietta leopardina* (Hymenoptera: Aphelinidae) and  
*Aprostocetus* (*Aprostocetus*) sp. (Hymenoptera: Eulophidae) are  
Obligate Hyperparasitoids of *Tamarixia radiata* (Eulophidae) and  
*Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae)**

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**MARIETTA LEOPARDINA (HYMENOPTERA: APHELINIDAE) AND APROSTOCETUS (APROSTOCETUS) SP. (HYMENOPTERA: EULOPHIDAE) ARE OBLIGATE HYPERPARASITOIDS OF TAMARIXIA RADIATA (EULOPHIDAE) AND DIAPHORENCYRTUS ALIGARHENESIS (HYMENOPTERA: ENCYRTIDAE)**

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Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), was first discovered in California, USA in 2008. Establishment of *D. citri* in southern California is viewed as a serious threat to California's multibillion dollar citrus industry because of its capacity to spread a bacterium responsible for a lethal disease of citrus, huanglongbing (HLB) (Anonymous 2010). This disease was discovered in California in March 2012 (Leavitt 2012). Part of the California response to the ACP invasion has been the initiation of a classical biological control program with host specific parasitoids. The search for natural enemies has focused exclusively on the Pakistan Punjab because of a good climate match to major citrus production areas in California and the assumption that the Punjab is part of the evolutionary area of origin for this pest (Hussain & Nath 1927; Beattie et al. 2008; Hoddle 2012a). To date, 1 parasitoid has passed mandatory host specificity testing and been released in California, this being *Tamarixia radiata* (Waterston) (Hymenoptera: Encyrtidae) (Hoddle 2012b).

Collections of ACP nymphs from citrus growing areas around Faisalabad, Punjab, Pakistan,

over the period from 24 May to 1 Jun 2012 by co-operators from the University of Agriculture Faisalabad and by CDH and MSH over 2-5 Jun 2012 resulted in the emergence of 3 primary parasitoids of ACP nymphs: *T. radiata*, *Diaphorencyrtus aligarhensis* (Shafee, Alam and Agarwal) (Hymenoptera: Encyrtidae), and *Psyllaphycus diaphorinae* Hayat (Encyrtidae) (Triapitsyn et al. 2013). Additionally, 2 suspected hyperparasitoids, *Marietta leopardina* Motschulsky (Hymenoptera: Aphelinidae) (Fig. 1) and *Aprostocetus* (*Aprostocetus*) sp. (Hymenoptera: Eulophidae) (Fig. 2) emerged from parasitized ACP nymphs over 13-25 Jun 2012, in quarantine at the University of California, Riverside (UCR). The Pakistani *A.* (*Aprostocetus*) sp. does not key to any described species in the genus *Aprostocetus* Westwood from the Indian Subcontinent (Narendran 2007). In Taiwan, *M. leopardina* and *Tetrastichus* sp. were reared from field collected ACP suspected to be parasitized by *T. radiata* or *D. aligarhensis* [= *D. diaphorinae*] (Lin & Tao] (Chien et al. 1989). The *Tetrastichus* sp. most likely was an *A.* (*Aprostocetus*) sp., but we don't know if it was conspecific with *A.* (*Aprostocetus*) sp. from Pakistan. To demonstrate that

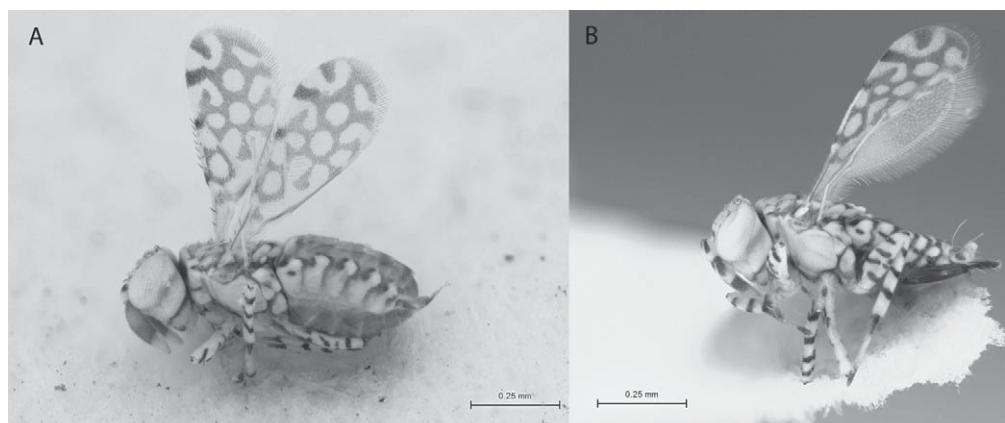


Fig. 1. *Marietta leopardina* male (A) and female (B). The color version of this figure is available online in Florida Entomologist 96(2) (2013) at <http://purl.fcla.edu/fcla/entomologist/browse>.



Fig. 2. Male *Aprostocetus (Aprostocetus) sp.* (all females were slide mounted for identification and were not photographed prior to mounting). The color version of this figure is available online in Florida Entomologist 96(2) (2013) at <http://purl.fcla.edu/fcla/entomologist/browse>.

*M. leopardina* and *A. (Aprostocetus) sp.* are not primary parasitoids of ACP, but are indeed hyperparasitoids of *T. radiata* and *D. aligarhensis* as hypothesized by Chien et al. (1989), exposure studies were conducted in quarantine at UCR.

Over the period 13-25 Jun 2012, eight pairs of male (Fig. 1A) and female (Fig. 1B) *M. leopardina* and 3 unmated individual females of *A. (Aprostocetus) sp.* reared from material collected in Pakistan were set up and exposed for 3-7 days to 1 of 3 treatments before being moved to a new treatment. Suspected hyperparasitoids were rotated across each of the 3 treatments at least once. Treatment (1) consisted of 10 unparasitized fifth instar ACP nymphs [ $n = 10$  replicates for *M. leopardina*, and 4 replicates for *A. (Aprostocetus) sp.*], (2) ACP parasitized by *T. radiata*, 6-9 days post-exposure to ovipositing *T. radiata* females [ $n = 6$  replicates of 4-20 parasitized ACP for *M. leopardina* and 3 replicates for *A. (Aprostocetus) sp.*], and (3) ACP parasitized by *D. aligarhensis* 12-16 days post-exposure to ovipositing *D. aligar-*

*hensis* females [ $n = 6$  replicates of 6-20 parasitized ACP for *M. leopardina* and 4 replicates for *A. (Aprostocetus) sp.*]. Emergence of *T. radiata* ( $n = 57$  parasitized ACP on 5 plants [range = 5-23 parasitized nymphs per plant] or *D. aligarhensis* ( $n = 90$  on 5 plants, range = 8-25) from ACP nymphs not exposed to either *M. leopardina* or *A. (Aprostocetus) sp.*, provided data on mortality rates for these primary parasitoids in the absence of hyperparasitoids. Similarly, 10 fifth instar ACP nymphs on each of 5 host plants not exposed to *M. leopardina* or *A. (Aprostocetus) sp.* provided a measure of ACP mortality in the absence of these hyperparasitoids.

Host plants used for experiments were *Murraya exotica* L. [formerly *M. paniculata* (L.) Jack] seedlings grown in Ray Leach Cone-tainers<sup>TM</sup> (SC7 Stubby, 3.8 cm diam and 114 mL capacity, Stuewe and Sons Inc., Oregon, USA). Clear plastic vials, 148 mL capacity (Thornton Plastic Co., Salt Lake City, Utah, USA), with three 12 mm diam holes (2 on opposite sides, 1 on the bottom) covered with ultra-fine organza mesh were inverted to fit on the vial lid that was firmly secured around the cone-tainer. This ventilated inverted vial enclosed the test plant infested with parasitized or unparasitized psyllids and confined *M. leopardina* or *A. (Aprostocetus) sp.* with the target of interest. Exposure experiments were conducted in quarantine at 27 °C, 50% RH and 14:10 h L:D. ACP nymphs parasitized by *T. radiata* or *D. aligarhensis* were sourced from colonies being maintained in quarantine at UCR. Following exposure to *M. leopardina* or *A. (Aprostocetus) sp.*, treatments were observed daily and the numbers of adult ACP, *T. radiata*, *D. aligarhensis*, *M. leopardina*, or *A. (Aprostocetus) sp.* that emerged were recorded by treatment.

*Marietta leopardina* successfully emerged only from ACP nymphs parasitized by *T. radiata* or *D. aligarhensis* (Table 1). The mean time to emergence for *M. leopardina* males and females from *T. radiata* was 24.73 days  $\pm$  0.97 (SE) and 26.67 days  $\pm$  1.55, respectively. The mean time to emergence for *M. leopardina* males and females from

TABLE 1. EMERGENCE RATES OF *MARIETTA LEOPARDINA* (ACCESSION NUMBERS: UCRC ENT 328025-328043 AND 329620) WHEN EXPOSED TO NON-PARASITIZED FIFTH INSTAR ASIAN CITRUS PSYLLID (ACP) NYMPHS; ACP NYMPHS PARASITIZED BY *TAMARIXIA RADIATA*, OR *DIAPHORENCYRTUS ALIGARHENSISS*.

Host	No. Exposed	No. Adults Emerged	No. <i>Marietta leopardina</i> Emerged		
			Males	Females	Total
ACP	100	74 <sup>1</sup>	0	0	0
<i>T. radiata</i>	60	0 <sup>2</sup>	11	9	20
<i>D. aligarhensis</i>	59	13 <sup>3</sup>	22	8	30

<sup>1</sup>Number of ACP adults that emerged from unparasitized nymphs.

<sup>2</sup>Number of adult *T. radiata* that emerged from parasitized ACP nymphs.

<sup>3</sup>Number of adult *D. aligarhensis* that emerged from parasitized ACP nymphs.

*D. aligarhensis* was 25.45 days  $\pm$  0.70 (SE) and 24.75 days  $\pm$  0.90, respectively. Male progeny resulted from attacks by unmated *A. (Aprostocetus)* sp. females on *T. radiata* and *D. aligarhensis*. No emergence was recorded from unparasitized ACP nymphs (Table 2). The mean time to emergence for *A. (Aprostocetus)* sp. males from *T. radiata* and *D. aligarhensis* was 27.50 days  $\pm$  0.50 (SE) and 21.62 days  $\pm$  1.17, respectively. Emergence rates for ACP, *T. radiata*, and *D. aligarhensis* not exposed to *M. leopardina* or *A. (Aprostocetus)* sp., were high, at 76%, 91%, and 96%, respectively (Table 3).

Under quarantine conditions, *Marietta leopardina* and *A. (Aprostocetus)* sp. were only able to produce progeny on ACP nymphs parasitized by *T. radiata* or *D. aligarhensis*. Exposure experiments demonstrated that *M. leopardina* and *A. (Aprostocetus)* sp. are almost certainly obligatory hyperparasitoids in the ACP-*Tamarixia-Diaphorencyrtus* system. Once this was determined, all *M. leopardina* and *A. (Aprostocetus)* sp. in quarantine were killed via freezing and then preserved in 95% ethanol.

When *M. leopardina* was exposed to immature *T. radiata*, 33% of potential hosts were successfully parasitized, while the remainder (67%) died from undetermined causes (control mortality for *T. radiata* not exposed to *M. leopardina* was 9%). It is unknown if this high mortality rate for *T. radiata* was due to host feeding or superparasitism, or a combination of both of these potential events. *Marietta leopardina* emerged from 51% of exposed *D. aligarhensis*, and 27% of potential hosts died from undetermined causes (control mortality for *D. aligarhensis* was 4%).

Mortality for *T. radiata* in the presence of *A. (Aprostocetus)* sp. was also 100%: 7% of exposed hosts died from parasitism, while 93% died from unknown causes. Again, either host feeding or superparasitism could be responsible for this high mortality. *Aprostocetus (Aprostocetus)* sp. parasitized 57% of *D. aligarhensis*, and 26% died from undetermined causes. As with *M. leopardina*,

< 20% of *D. aligarhensis* survived when exposed to foraging *A. (Aprostocetus)* sp. females.

The mortality rates recorded in quarantine from these exposure experiments are probably overestimating the negative impact *M. leopardina* and *A. (Aprostocetus)* sp. have on ACP parasitized by *T. radiata* or *D. aligarhensis*. Parasitism rates for *M. leopardina* and *A. (Aprostocetus)* sp. in citrus in the Pakistan Punjab were 6% and 1%, respectively, of parasitized ACP collected during 24 May-5 Jun 2012. Similar low rates of hyperparasitism by *M. leopardina* and *A. (Aprostocetus)* sp. (as *Tetrastichus* sp.) were observed on *T. radiata* and *D. aligarhensis* (as *D. diaphorinae*) in Taiwan by Chien et al. (1989). It is unlikely these hyperparasitoids play a significant and deleterious role in naturally-occurring biological control in Pakistani citrus, but field studies across multiple sites and yr would be needed to verify this.

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## SUMMARY

*Marietta leopardina* and *Aprostocetus (Aprostocetus)* sp. that emerged from *Diaphorina citri* nymphs collected from the Pakistani Punjab and exposed in quarantine to *D. citri* nymphs parasitized by *Tamarixia radiata* or *Diaphorencyrtus aligarhensis* successfully emerged from both of these primary parasitoids. *Marietta leopardina* and *A. (Aprostocetus)* sp. did not reproduce on unparasitized *D. citri* nymphs. These exposure experiments confirmed that *M. leopardina* and *A. (Aprostocetus)* sp. are obligatory hyperparasitoids of *T. radiata* and *D. aligarhensis* in the *Diaphorina-Tamarixia-Diaphorencyrtus* system.

Key Words: developmental times, foreign exploration, Pakistan, Punjab, quarantine

TABLE 2. EMERGENCE RATES OF *APROSTOCETUS (APROSTOCETUS)* SP. (ACCESSION NUMBERS: UCRC ENT 334419-334425) WHEN UNMATED FEMALES WERE EXPOSED TO NON-PARASITIZED FIFTH INSTAR ASIAN CITRUS PSYLLID (ACP) NYMPHS; ACP NYMPHS PARASITIZED BY *TAMARIXIA RADIATA*, OR *DIAPHORENCYRTUS ALIGARHENESIS*.

Host	No. Exposed	No. Adults Emerged	No. <i>A. (Aprostocetus)</i> sp. Emerged		
			Males	Females	Total
ACP	40	27 <sup>1</sup>	0	0	0
<i>T. radiata</i>	27	0 <sup>2</sup>	2	0	2
<i>D. aligarhensis</i>	23	4 <sup>3</sup>	13	0	13

<sup>1</sup>Number of ACP adults that emerged from unparasitized nymphs.

<sup>2</sup>Number of adult *T. radiata* that emerged from parasitized ACP nymphs.

<sup>3</sup>Number of adult *D. aligarhensis* that emerged from parasitized ACP nymphs.

TABLE 3. EMERGENCE RATES FOR UNPARASITIZED FIFTH INSTAR ASIAN CITRUS PSYLLID (ACP) NYMPHS, AND ACP PARASITIZED BY *TAMARIXIA RADIATA* OR *DIAPHORENCYRTUS ALIGARHENESIS* THAT WERE NOT EXPOSED TO *MARIETTA LEOPARDINA* OR *APROSTOCETUS* (*APROSTOCETUS*) SP. ACCESSION NUMBERS ARE PROVIDED FOR VOUCHER SPECIMENS MAINTAINED AT THE ENTOMOLOGY RESEARCH MUSEUM, UNIVERSITY OF CALIFORNIA, RIVERSIDE (UCRC).

Host	No. Set Up	No. Adults	Accession No.
ACP	50	38 <sup>1</sup>	UCRC ENT 334428 (vial of Pakistani ACP preserved in 95% ethanol)
<i>T. radiata</i>	57	52 <sup>2</sup>	UCRC ENT 334402-334418
<i>D. aligarhensis</i>	90	86 <sup>3</sup>	UCRC ENT 334426-334427

<sup>1</sup>Number of ACP adults that emerged from unparasitized nymphs.

<sup>2</sup>Number of adult *T. radiata* that emerged from parasitized ACP nymphs.

<sup>3</sup>Number of adult *D. aligarhensis* that emerged from parasitized ACP nymphs.

## RESUMEN

*Marietta leopardina* y *Aprostocetus* (*Aprostocetus*) sp. emergidos de ninfas de *Diaphorina citri* recolectadas en Punjab de Pakistán fueron expuestos en cuarentena sobre ninfas de *D. citri* parasitadas por *Tamarixia radiata* o *Diaphorencyrtus aligarhensis*, logrando emerge con éxito de estos dos parásitoides primarios. *Marietta leopardina* y *A. (Aprostocetus)* sp. no se reprodujeron en ninfas de *D. citri* no parasitadas. Estos experimentos de exposición confirmaron que *M. leopardina* y *A. (Aprostocetus)* sp. son hiperparásitoides obligatorios de *T. radiata* y *D. aligarhensis* en el sistema de *Diaphorina-Tamarixia-Diaphorencyrtus*.

Palabras Clave: tiempo de desarrollo, exploración extranjero, Pakistán, Punjab, cuarentena

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