Chartocerus sp. (Hymenoptera: Signiphoridae) and Pachyneuron crassiculme (Hymenoptera: Pteromalidae) are Obligate Hyperparasitoids of Diaphorencyrtus aligarhensis (Hymenoptera: Encyrtidae) and Possibly Tamarixia radiata (Hymenoptera: Eulophidae)

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CHARTOCERUS SP. (HYMENOPTERA: SIGNIPHORIDAE) AND PACHYNEURON CRASSICULME (HYMENOPTERA: PTEROMALIDAE) ARE OBLIGATE HYPERPARASITOIDS OF DIAPHORENCYRTUS ALIGARHENSIS (HYMENOPTERA:ENCYRTIDAE) AND POSSIBLY TAMARIXIA RADIATA (HYMENOPTERA: EULOPHIDAE)

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ABSTRACT

Two species of suspected hyperparasitoids, Chartocerus sp. and Pachyneuron crassiculme, emerged from parasitized Diaphorina citri nymphs collected in Punjab Pakistan over 15-22 Apr 2013. Exposure tests conducted in quarantine on D. citri nymphs parasitized by Tamarixia radiata and Diaphorencyrtus aligarhensis, as well as unparasitized D. citri nymphs, confirmed that Chartocerus sp. and P. crassiculme are hyperparasitoids. Both Chartocerus sp. and P. crassiculme successfully reproduced on D. aligarhensis, with one instance of P. crassiculme reproducing on T. radiata. There was no emergence from unparasitized D. citri.

Key Words: choice test, no-choice test, quarantine

MATERIALS AND METHODS

Parasitized D. citri host material returned from Punjab Pakistan to quarantine at UCR (15-22 Apr 2013) yielded previously collected T. radiata and D. aligarhensis, along with several species of known (Marietta leopardina Motschulsky [Hymenoptera: Aphelinidae], Aprostocetus (Aprostocetus) sp. [Hymenoptera: Eulophidae] [Hoddle et al. 2013]) or suspected (Chartocerus sp. [Hymenoptera: Signiphoridae], Pachyneuron crassiculme Waterston [Hymenoptera: Pteromalidae] and Psyllaphycus diaphorinae [Hymenoptera: Encyrtidae]) hyperparasitoids.

To confirm that Chartocerus sp. (Fig. 1A. male, B. female) and P. crassiculme (Fig. 2A. male, B. female) are not primary parasitoids of D. citri, exposure trials using 10 sets of 4-7 Chartocerus sp. and 10 pairs of 1 male and 1 female P. crassiculme that emerged from material collected in Pakistan...
were rotated through each of 4 treatment types between 26 Apr and 24 May, 2013 in quarantine at UCR. It was not possible to reliably sex live *Chartocerus* sp., so this species was exposed in groups (assumed to contain at least 1 female each) unless a pair was otherwise observed mating. Exposure treatments consisted of: (A) nymphs parasitized by *T. radiata* (*n* = 8 replicates of 5-10 parasitized nymphs for *Chartocerus* sp. and 9 replicates of 5 parasitized nymphs for *P. crassiculme*), 5-9 days post-exposure to *T. radiata*; (B) nymphs parasitized by *D. aligarhensis* (*n* = 8 replicates of 5-10 parasitized nymphs for *Chartocerus* sp. and 10 replicates of 5 for *P. crassiculme*), 10-14 days post-exposure to *D. aligarhensis*; (C) unparasitized third to fourth instar *D. citri* nymphs (*n* = 9 replicates of 5-10 unparasitized nymphs for *Chartocerus* sp. and 10 replicates of 5 nymphs for *P. crassiculme*); and (D) each of the 3 previously listed host types (A, B, and C) presented simulta-

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**Fig. 1.** *Chartocerus* sp. male (A) and female (B). This figure is shown in color in the supplementary document in *Florida Entomologist* 97(2) (2014) online at http://purl.fcla.edu/fcla/entomologist/browse.

**Fig. 2.** *Pachyneuron crassiculme* male (A) and female (B). This figure is shown in color in the supplementary document in *Florida Entomologist* 97(2) (2014) online at http://purl.fcla.edu/fcla/entomologist/browse.
neously in a choice cage (n = 9 replicates of 5-10 of each host type for *Chartocerus* sp. and 9 replicates of 5 of each host type for *P. crassiculme*).

Each replicate was comprised of host material for each treatment type exposed to a group of potential hyperparasitoids for 24 h each. Hosts were exposed sequentially in a different order for each replicate to prevent bias due to presentation order. Emergence rates of *T. radiata* (n = 5 parasitized nymphs on each of 10 cuttings) and *D. aligarhensis* (n = 5 parasitized nymphs on each of 10 cuttings) determined baseline mortality for primary parasites in the absence of hyperparasitoids. Unparasitized *D. citri* nymphs (n = 5 fourth instar nymphs on each of 10 plants) provided data on nymph mortality in the absence of hyperparasitoids. Mummies of *T. radiata* and *D. aligarhensis* used in exposure experiments were sourced from colonies maintained in quarantine at UCR.

*Diaphorina citri* nymphs parasitized by either *T. radiata* or *D. aligarhensis* for no-choice treatments were presented on small *Citrus volkameriana* cuttings. *Citrus volkameriana* seedlings grown in 114 mL Cone-tainers™ (SC7 Stubby, 3.8 cm diameter, Stew and Sons Inc., Oregon) and infested with *D. citri* nymphs were used to expose unparasitized *D. citri* nymphs to *Chartocerus* sp. and *P. crassiculme*. Clear plastic vials (Thornton Plastic Co. 148 mL capacity, Salt Lake City, Utah) with three 12 mm diam ventilation holes covered with ultra-fine organza were inverted and placed over the top of the plant and fitted into the corresponding vial lid, which had a hole cut in the center to allow it to be fitted around the cone (Irvin et al. 2009).

Choice treatments were set up in 15 cm × 15.3 cm × 15.3 cm (h × w × d) clear plastic boxes (S&W Plastics, Riverside, California) with a 30 cm sleeve sewn from no-see-um netting (Skeeta Mosquito & Other Insect Protection Products, Bradenton, Florida). Unparasitized *D. citri* nymphs in Containers and *T. radiata- and D. aligarhensis-parasitized nymphs on *C. volkameriana* cuttings in water were placed in the cage without ventilated vials on top to allow free access to all 3 host types simultaneously. After 24 h, each host type was enclosed with an inverted ventilated vial to contain all insects that emerged from each host type. All experiments were conducted in quarantine at UCR’s Insectary and Quarantine facility, at 27 °C, 50% RH, and 14:10 h L:D. Replicates were observed daily after initial exposure, and total numbers of each emerged species were recorded per treatment.

### RESULTS

No-choice treatments resulted in *Chartocerus* sp. reproducing successfully only on *D. aligarhensis* (Table 1). Mean emergence time for *Char-

### Table 1. Emergence and Mortality Rates for *Chartocerus* sp. Exposed to Unparasitized Third and Fourth Instar *D. citri* Nymphs, and Nymphs Parasitized by *T. radiata*, and *D. aligarhensis* in No-Choice and Choice Treatments.

<table>
<thead>
<tr>
<th>Host</th>
<th>Total No. Exposed</th>
<th>% Host Emergence</th>
<th>% Host Parasitism</th>
<th>% Host Dead</th>
<th>% Host Missing</th>
<th>% Host Emergence, Choice</th>
<th>% Host Parasitism, Choice</th>
<th>% Host Dead, Choice</th>
<th>% Host Missing, Choice</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. citri</em></td>
<td>65</td>
<td>72.31%</td>
<td>0.00%</td>
<td>9.23%</td>
<td>18.46%</td>
<td>8.154%</td>
<td>26.87%</td>
<td>5.97%</td>
<td>14.61%</td>
</tr>
<tr>
<td><em>T. radiata</em></td>
<td>67</td>
<td>67.16%</td>
<td>0.00%</td>
<td>26.87%</td>
<td>5.97%</td>
<td>66.19%</td>
<td>25.81%</td>
<td>29.29%</td>
<td>29.29%</td>
</tr>
<tr>
<td><em>D. aligarhensis</em></td>
<td>60</td>
<td>33.33%</td>
<td>46.67%</td>
<td>16.67%</td>
<td>33.33%</td>
<td>53.85%</td>
<td>29.29%</td>
<td>29.29%</td>
<td>29.29%</td>
</tr>
</tbody>
</table>
tocerus sp. offspring from *D. aligarhensis* was 18.36 days ± 2.34 (SE). *Pachyneuron crassiculme* produced progeny on *D. aligarhensis* and *T. radiata* in no-choice treatments, though parasitism was much higher on *D. aligarhensis* (Table 2). Mean emergence times for males and females were 12.83 days ± 2.48 (SE) and 11.33 days ± 2.05 (SE), respectively. *Pachyneuron crassiculme* had a single male emerge from *T. radiata* after 11 days. Emergence rates for control treatments of *T. radiata*, *D. aligarhensis*, and *D. citri* were 84%, 88%, and 88%, respectively (Table 3). *Chartocerus* sp. and *P. crassiculme* failed to reproduce on unparasitized *D. citri* nymphs.

Immature *D. aligarhensis* exposed to *Chartocerus* sp. in no-choice tests experienced 47% parasitism, 17% died from undetermined causes, 3% were unaccounted for, and 33% emerged as adult *D. aligarhensis*. In 20% of trials (i.e., 2 of 10 replicates) *Chartocerus* sp. exhibited superparasitism, with 11 adults emerging from 9 *D. aligarhensis* mummies in 1 replicate, and 6 adults emerging from 3 mummies in the second. In no-choice tests, immature *T. radiata* exposed to *Chartocerus* sp. exhibited 0% parasitism, 27% of mummies died from unknown causes, 6% disappeared, and 67% emerged as adult *T. radiata*.

In no-choice tests where *P. crassiculme* was exposed to immature *D. aligarhensis*, 28% of hosts were parasitized by *P. crassiculme*, 19% died from unknown causes, and 53% emerged as adult *D. aligarhensis*. On *T. radiata*, *P. crassiculme* successfully parasitized only 2% of host material (i.e., one host), 40% died from unknown causes, 7% were unaccounted for, and 51% emerged as adult *T. radiata*. Unknown mortality may be attributable to superparasitism, host feeding, or a combination of both by *P. crassiculme*.

There was no successful parasitism of any host in choice tests for either *Chartocerus* sp. or *P. crassiculme*. However, elevated mortality rates were observed for *T. radiata* (26% when exposed to *Chartocerus* sp.; 28% for *P. crassiculme*) and *D. aligarhensis* (29%; 13%). In comparison, control mortality for *T. radiata* and *D. aligarhensis* were < 13% in the absence of these hyperparasitoids. When viewed collectively, data from exposure trials demonstrate that *Chartocerus* sp. and *P. crassiculme* are obligate hyperparasitoids within the *D. citri*-*Tamarixia-Diaphorencyrtus* system. Immediately following the conclusion of trials, all *Chartocerus* sp. and *P. crassiculme* material was killed in quarantine and preserved in 95% ethanol. Voucher specimens were deposited in the Entomology Museum at UCR (Table 4).

Assuming *Chartocerus* sp. and *P. crassiculme* preferentially parasitize *D. aligarhensis* as these exposure trial data suggest, the frequency of *Chartocerus* sp. and *P. crassiculme* emergence in quarantine from material collected from Punjab Pakistan in April 2013 was significant in compar-

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**Table 2: Emergence and Mortality Rates for *Pachyneuron crassiculme* Exposed to Unparasitized Third and Fourth Instar *D. citri* Nymphs, and Nymphs Parasitized by *Chartocerus* sp. and *D. aligarhensis* in No-Choice and Choice Treatments.**

<table>
<thead>
<tr>
<th>Host</th>
<th>No. Exposed</th>
<th>% Host</th>
<th>% Host Emergence</th>
<th>% Host Parasitism</th>
<th>% Host Dead</th>
<th>% Host Missing</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. citri</em></td>
<td>50</td>
<td>68.00%</td>
<td>51.11%</td>
<td>2.22%</td>
<td>40.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td><em>T. radiata</em></td>
<td>45</td>
<td>73.33%</td>
<td>71.74%</td>
<td>13.33%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td><em>D. aligarhensis</em></td>
<td>53</td>
<td>52.83%</td>
<td>28.30%</td>
<td>28.30%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
</tbody>
</table>

1. Percentage of *D. citri* adults that emerged from unparasitized nymphs.
2. Percentage of *T. radiata* adults that emerged from unparasitized nymphs.
3. Percentage of *D. aligarhensis* adults that emerged from unparasitized hosts.
4. Percentage of *P. crassiculme* adults that successfully emerged from parasitized hosts.
5. Percentage of hosts found dead.
6. Percentage of hosts unaccounted for at time of data collection.
ison to *D. aligarhensis* emergence rates. *Chartocerus* sp. (237 individuals reared), *P. crassiculme* (181), and *D. aligarhensis* (743) represented 20%, 16%, and 64% of material reared, respectively, within this complex. A total of 292 *T. radiata* were reared from April 2013 collections. Exposure trials suggest that the lower numbers of *T. radiata* obtained from Pakistan in April 2013 were not likely due to hyperparasitism.

**ACKNOWLEDGMENTS**

The authors would like to thank Roger Burks (UCR) for assistance with specimen mounting, photography, and identification. Serguei Triapitsyn (UCR) helped with specimen identification and deposition of voucher specimens. Anamaria Dal Molin (TAMU) identified *Chartocerus* sp. Funding for this project was provided, in part, by funds issued by the California Department of Food and Agriculture’s Specialty Crops Program and the California Citrus Research Board.

**REFERENCES CITED**


HODDLE, C. D., HODDLE, M. S., AND TRIAPITSYN, S. V. 2013 *Marietta leopardina* (Hymenoptera: Aphelinidae) and *Aprostocetus* (Aprostocetus) sp. (Hymenoptera: Eulophidae) are obligate hyperparasitoids of *Tamarixia radiata* (Eulophidae) and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae). Florida Entomol. 96: 643-646.


**Table 3. Emergence Rates of Unparasitized Third and Fourth Instar *D. citri* Nymphs and Nymphs Parasitized by *T. radiata* and *D. aligarhensis* in Control Treatments Not Exposed to Hyperparasitoids.**

<table>
<thead>
<tr>
<th>Host</th>
<th>Total No. Exposed</th>
<th>No. Adults Emerged</th>
<th>No. Dead Hosts 1</th>
<th>No. Missing Hosts 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. citri</em></td>
<td>50</td>
<td>44 1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>T. radiata</em></td>
<td>50</td>
<td>42 2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td><em>D. aligarhensis</em></td>
<td>52</td>
<td>46 3</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

1Total number of *D. citri* adults that matured from unparasitized nymphs.
2Total number of *T. radiata* adults that emerged from parasitized nymphs.
3Total number of *D. aligarhensis* adults that emerged from parasitized nymphs.

**Table 4. Specimen Accession Numbers for All Species Used in Exposure Trials and Deposited in the Entomology Museum at the University of California Riverside.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. citri</em></td>
<td>UCRC_ENT00334428</td>
</tr>
<tr>
<td><em>T. radiata</em></td>
<td>UCRC_ENT00334402-334418</td>
</tr>
<tr>
<td><em>D. aligarhensis</em></td>
<td>UCRC_ENT00334426-334427</td>
</tr>
<tr>
<td><em>Chartocerus</em> sp.</td>
<td>UCRC_ENT00417173-00417182</td>
</tr>
<tr>
<td><em>P. crassiculme</em></td>
<td>UCRC_ENT00417183-00417187</td>
</tr>
</tbody>
</table>

1Multiple individuals of Pakistani *D. citri* preserved in a single vial of 95% ethanol.
2Point-mounted individuals