

BIOLOGY OF *TAMARIXIA RADIATA* (HYMENOPTERA: EULOPHIDAE),
PARASITOID OF THE CITRUS GREENING DISEASE VECTOR *DIAPHORINA*
CITRI (HEMIPTERA: PSYLLOIDEA): A MINI REVIEW

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ABSTRACT

Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) is an ectoparasitoid of the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama (Hemiptera: Psylloidea) a citrus pest and vector of huanglongbing (HLB) or citrus greening disease. First described from what is now Pakistan, the parasitoid has been introduced deliberately or inadvertently throughout Asia and the Americas wherever the psyllid now occurs. Interest in *T. radiata* for biological control of *D. citri* has grown in response to continued spread of ACP and HLB, and the evident searching and colonization capabilities of the parasitoid. Mass release is seen as a potential strategy to augment area wide management of *D. citri*, particularly where pesticides are not extensively used. Efficient mass rearing and eventual success of these programs will require the best possible information on biology of *T. radiata* including life history parameters, host relationships, sensory perception, environmental responses and genetics. Much early literature on *T. radiata* is in Chinese and therefore inaccessible to those not able to read the language. The present review covers the literature through 2014. The intent is to summarize what is known about biology of *T. radiata* to aid research efforts, with the objective of contributing to more effective biological control of this pest.

Key Words: life history, mass rearing, huanglongbing, biological control

RESUMEN

Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) es un ectoparasitoide del psílido asiático de los cítricos (PAC) *Diaphorina citri* Kuwayama (Hemiptera: Psylloidea) una plaga de los cítricos y vector de la enfermedad Huanglongbing (HLB) o enverdecimiento de los cítricos. Descrita por primera vez en lo que hoy es Pakistán, el parasitoide ha sido introducido de forma deliberada o inadvertidamente a través de Asia y las Américas donde el psílido actualmente ocurre. El interés por *T. radiata* para el control biológico de *D. citri* ha crecido en respuesta a la continua propagación de la PAC y la HLB, y las capacidades de búsqueda y colonización evidentes del parasitoide. La liberación masiva es visto como una estrategia potencial para aumentar el manejo de *D. citri* por una amplia área en particular cuando no se utilizan ampliamente pesticidas. La cría en masa eficiente y eventual éxito de estos programas requieren la mejor información posible sobre la biología de *T. radiata* incluyendo los parámetros del ciclo de vida, su relación con sus hospederos, la percepción sensorial, respuestas ambientales y su genética. Mucha de la literatura temprana sobre *T. radiata* está en chino y por lo tanto inaccesibles para los que no pueden leer el idioma. La presente revisión se refiere a la literatura a través de las perspectivas del 2014 para su futuro. La intención es resumir lo que se conoce acerca de la biología de *T. radiata* para ayudar a los esfuerzos de investigación, con el objetivo de contribuir al control biológico más eficaz de esta plaga.

Palabras Clave: historia de vida, cría en masa, huanglongbing, control biológico

Citrus greening or huanglongbing (HLB) is considered the world's most destructive citrus disease (Halbert & Manjunath 2004; Texeira et al. 2005; Bové 2006; Wang et al. 2006; Batool et al. 2007; Manjunath et al. 2008). The putative causative agents are three known vector-borne α -proteobacteria of which the most widespread and pervasive is '*Candidatus Liberibacter asiati-*

cus', vectored by the Asian citrus psyllid, *Diaphorina citri* (Bové 2006) (Hemiptera: Psylloidea). *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae, an ectoparasitoid) and *Diaphorocytus aligarhensis* (Shafee, Alam and Argarwal) (Hemiptera: Encyrtidae, an endoparasitoid) are the only known primary parasitoids of *D. citri* nymphs (Tang 1990). Both were first described

from the northern Indian subcontinent (Waterston 1922; Shafee et al. 1975).

Tamarixia radiata has been successfully introduced in Réunion (Aubert & Quilici 1984), Taiwan (Chien et al. 1989), Mauritius (Quilici 1986), Philippines (Gavarrá et al. 1990), Saudi Arabia (Aubert 1984), East Java, Indonesia (Nurhadi 1988), Guadalupe (Étienne et al. 2001), California (Hoddle 2012) and Florida (Skelley & Hoy 2004), where it has spread throughout the state (Qureshi et al. 2009). *T. radiata* appeared inadvertently in Brazil (Gomez-Torres et al. 2005; Torres et al. 2006), Argentina (Lizondo et al. 2007), Venezuela (Cermeli et al. 2007), Mexico (De León & Sétamou 2010), Puerto Rico (Pluke et al. 2008) and Texas (French et al. 2001). *Tamarixia radiata* has a high host specificity on *D. citri*, although *Bactericera cockerelli* Sulc was found parasitized at a low level (5%) (Hoddle & Pandey 2014).

Given the rapidity with which *T. radiata* has established and spread, it is an obvious choice for augmentative biological control of *D. citri*. Such efforts are underway in numerous states and countries in the Americas including São Paulo, Costa Rica, Jamaica, Mexico, Florida and California. Given the interest in mass rearing and

release of *T. radiata*, we felt it would be useful to review the literature on biology of this species, much of which is inaccessible to those unable to read Chinese.

Taxonomy and Identification

Tamarixia radiata was originally described as *Tetrastichus radiatus* by Waterston (1922) from specimens collected at Lyallpur, India (now Faisalabad Pakistan). *Tamarixia* was split off as a separate genus from *Tetrastichus* by Mercet (1924). All are parasitoids of Psylloidea (LaSalle 1994). Tang & Aubert (1990) described some distinguishing characteristics of *T. radiata*: about 1 mm long including head; eyes red in fresh specimens; head and body blackish and shining but without a metallic sheen, underside of gaster pale with a large whitish basal patch on the 5th tergite of dorsum; legs totally pale-white and wings transparent (Fig. 1).

Morphology and Sensory Perception

Male and female are the similar in color and body structure, except for antennae and a some-



Fig. 1. Female *Tamarixia radiata* ovipositing into an early 5th instar *Diaphorina citri* nymph. Photo by J. Lotz, FDOCS-DPI.

what darker abdomen in the male. The female antenna has 8 segments, both funicle and club with 3 segments covered with fine, short setae. The funicle is slender with the 1st segment longer than the 2nd and the 2nd segment longer than the 3rd. The length of the 3rd segment is almost equal to the width. The male antenna is more slender, and 9-segmented. The 4-segmented funicle is covered with long, slightly curved hairs, with a ventral scapal sensorium near the base of the scape (Tang & Aubert 1990). Male antennae possess a number of olfactory multiporous trichoid sensilla which could function for perception of mating-related volatile cues, whereas female antennae are characterized by more multiporous placoid sensilla suggesting greater sensitivity to host-related volatile cues (Onagbola et al. 2009). Olfactometer experiments verified that female wasps use volatiles emanating from *D. citri* nymphs for host location and that male wasps are attracted to a volatile pheromone emitted by female conspecifics (Mann et al. 2010).

Life Cycle of *Tamarixia radiata*

The four life stages of *T. radiata* were described by Chien et al. (1991a). The egg is translucent, ivory, and reniform, with one end adhered to the host. There are four larval instars, each distinguished by head capsule width: 0.06 mm, 0.09 mm, 0.14 mm, and 0.22 mm for 1st, 2nd, 3rd, and 4th instar larva respectively (Chien et al. 1991a). Development of the immature stages was reported by Xu & Tang (1993) and Chien et al. (1991a). The newly-enclosed larva sucks fluids externally from the site where it is closely attached to the nymph's integument (Husain & Nath 1923; Tang & Huang 1991). The third instar crawls to the ventral side of the host thorax to feed (Chien et al. 1991a). The parasitized nymph continues to live and secrete honeydew for some time (Husain & Nath 1923). All contents of the nymph are consumed by the time the parasitoid molts to the fourth instar and the nymph turns to a dark-brown mummy (Husain & Nath 1923; Chien et al. 1991a). The mature larva ceases to feed as it progresses to the prepupal stage which secures the mummy to the plant surface by means of silken threads (Chien et al. 1991a). After expelling the meconium, it molts to the pupal stage which turns yellow, with red ommatidia and ocelli (Chien et al. 1991a). As soon as the adult hardens, it makes its way out of the mummy by chewing a round hole of about 0.5 mm diameter in the region of thorax (Husain & Nath 1923; Chien et al. 1991a; Aubert 1987; Fig. 2). Over 80% of adults emerge between 5 a.m. to 10 a.m., with the peak of 7 a.m. to 8 a.m. (Chien et al. 1991a). The male emerges 1.5 h earlier than the female on average.

Mating of *Tamarixia radiata*

Males use their antennae to locate females. Once a receptive female was found, the male crawled onto the dorsum where he remained for 68 ± 7 s before actual mating for an additional 33 ± 3 seconds (Chien et al. 1991a). About 93% of females mated once and only once during the first day of emergence. The remaining 7% mated twice during the first 2 days following emergence. Fecundity and longevity of females were not affected by mating frequency. Males are capable of multiple matings over their lifetime (Chien et al. 1991a).

Oviposition of *Tamarixia radiata*

Eggs can be laid immediately after emergence by either mated or unmated females (Chien et al. 1991a). From 5 am to 10 am is the most active time of day for oviposition (Chu & Chien 1991). Host volatiles mediate host location (Mann et al. 2010). The female moves actively among *D. citri* nymphs using her antennae to search for suitable hosts (Husain & Nath 1923). She deposits an egg or occasionally 2 on the underside of the chosen nymph, usually next to a mid or hind coxa (Husain & Nath 1923; Aubert 1987; Chien et al. 1991a; Hall 2008b; Tang & Huang 1991; Fig. 1). Oviposition took 3 to 4 min according to Husain & Nath (1923), but only 61 ± 8 s according to Chien et al. (1991a). Chien et al. (1991a) reported that the female *T. radiata* injects venom into the host nymph through the ovipositor, immobilizing it for 4 to 8 min. If the egg was removed, the host nymph could not molt, and died 8 days later at 25 °C. First, second, or third *T. radiata* instars placed on an unparasitized 5th *D. citri* instar could not attach, and dropped off when the nymph began crawling, which demonstrated the importance of the venom for paralyzing *D. citri* nymphs (Chien et al. 1991a).

Host Preference of *Tamarixia radiata*

Studies on host selection have produced varying results. Chien et al. (1991a) and Chu & Chien (1991) reported that 5th instar *D. citri* nymphs were preferred for oviposition. Survival rates were 85% on 5th instars, compared with 33% and 71% on 3rd and 4th instars, respectively (Chien et al. 1991a). Body lengths of females and males also were greater among offspring from 5th instar hosts compared to offspring from 4th instar hosts, i.e., with regard to females, 1.12 mm compared with 0.91 mm; and with regard to males, 1.03 mm compared with 0.86 mm (male), respectively. This pattern was repeated with fecundity data: 215 eggs per female from 5th instar hosts compared to 120 eggs per female from 4th instar hosts. Further, longevities: of females and males also were

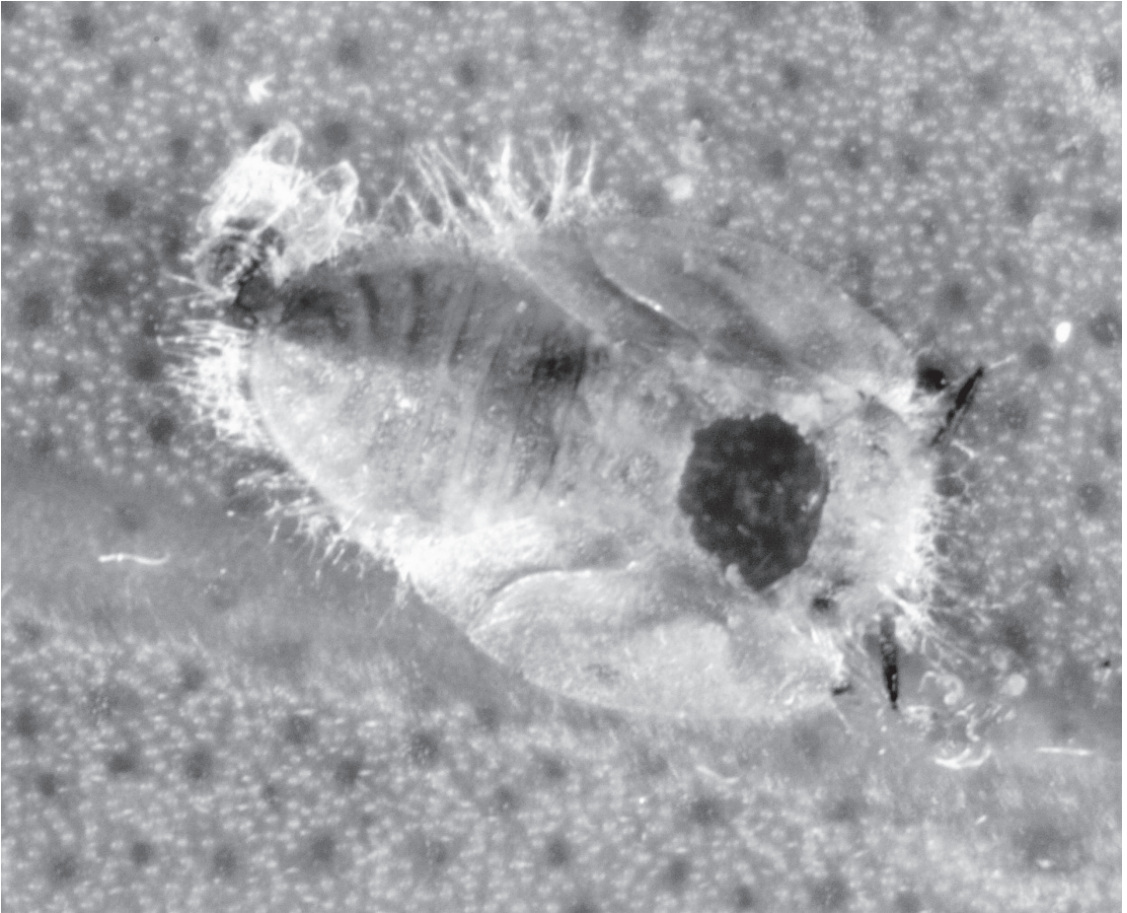


Fig. 2. Emergence hole left by *Tamarixia radiata* in 5th instar *Diaphorina citri* mummy. Photo by J. Lotz, FDOCS-DPI.

greater among offspring from 5th instar hosts compared to offspring from 4th instar hosts, i.e., with regard to females 18.0 days from 5th instar hosts compared to 14.4 days 4th instar hosts; and with regard to males, 11.6 days compared to 7.2 days (Chien et al. 1991). However, Tang & Huang (1991) reported that 4th instars are parasitized significantly more than either 3rd or 5th instars.

Host Feeding of *Tamarixia radiata*

Both genders feed on honeydew excreted by *D. citri* nymphs, and females use the ovipositor to puncture the nymphal cuticle for host feeding (Chien et al. 1991a; Skelley & Hoy 2004). Males also may feed on hemolymph from nymphs punctured by a female (X. Chen, personal observation). Like oviposition, host-feeding occurs in daytime and takes an average of 21 ± 2 s (Chien et al. 1991a). Nymphs die once fed upon, and females avoid laying eggs and feeding on the same host (Chien et al. 1991a; Tang & Huang 1991).

The ratio of the number of hosts on which *T. radiata* feed to the number on which it oviposits averages 5.6:1 and correlates with host density and parasitoid age (Chien et al. 1991a). Females younger than 4 days or older than 18 days laid one egg per an average 0.29 or 0.38 hosts that had been fed upon, respectively (Chien et al. 1991a). Approximately 80% of *D. citri* nymphal mortality in the laboratory resulted from parasitism with an additional 20% from host-feeding (Chien et al. 1991a, 1994a). Therefore, it has been estimated that a single female *T. radiata* could kill up to 500 nymphs during her lifetime (Chu & Chien 1991). However, temperature can influence the number of *D. citri* nymphs killed. Chien et al. (1993) estimated host-killing capacity (host feeding + parasitism) at 16, 25, 245, 196 nymphs per female at 15 °C, 20 °C, 25 °C and 30 °C, respectively. Skelley and Hoy (2004) reported that 36% of hosts were parasitized and 57% were fed upon in their colonies at 25 ± 2 °C, 30- 65% RH and 18:6 h L:D.

Sex Ratio of *Tamarixia radiata*

Tamarixia radiata is arrhenotokous, which means that unfertilized eggs develop into males, while fertilized eggs develop into females. The average number of eggs deposited by virgin and mated females in one study were 209.2 and 215.4 respectively (Chu & Chien 1991). The sex ratio of the progeny was highly correlated with the age of the female parasitoid (Tang & Huang 1991; Chu & Chien 1991). The proportion of female progeny increased as the mother aged, from 0.5 for a 1 day-old females to 0.77 for 22 day-old females (Chu & Chien 1991). Sex ratio was also correlated with host stage, although published results differ. Tang & Huang (1991) reported 88% females emerging from 5th instar hosts, 75% females from 4th instars and 41% from 3rd instars. However, Chu & Chien (1991) reported 67% females from 5th instar nymphs, compared with 16% from 4th instars. Skelley & Hoy (2004) found 64% and 67% females emerging from 4th instar nymphs in their Taiwanese and Vietnamese colonies of *T. radiata*, respectively.

Ovigeny in *Tamarixia radiata*

Tamarixia radiata has been characterized by an ovigeny index of 0.03, which is the proportion of the potential lifetime complement of eggs that is mature upon female emergence. Therefore *T. radiata* is synovigenic and the production of eggs throughout the female's entire lifespan requires host-feeding to mature most of the eggs (Jervis et al. 2001). Like most synovigenic parasitoids, *T. radiata* can resorb eggs when hosts are absent or scarce (Chien et al. 1994b), thereby maintaining reproductive resources and synchrony with the host population (Jervis et al. 2001). Egg resorption was observed in *T. radiata* at 15 °C and 25 °C, even when honey was provided, and occurred at rates positively correlated with host deprivation time (Chien et al. 1991a, 1994b; Chen & Stansly 2014).

Once suitable hosts are fed upon, new eggs can be matured and oviposition can occur (Chien et al. 1994b). Little or no effect on total fecundity (204 eggs) was observed after host deprivation for 10 days at 25 °C with honey provided as food, but there was a loss of fecundity after host deprivation for 20 days (156 eggs) (Chien et al. 1994b). Wasps stored for 10 to 20 days at 25 °C laid significantly more eggs (156 eggs) than wasps stored at 15 °C (98 eggs). Fecundity decreased greatly following host deprivation for 30 to 40 days at 15 °C (25-59 eggs).

Superparasitism

Female *T. radiata* can discriminate between parasitized and unparasitized hosts to avoid

superparasitism (Chien et al. 1991a). Husain & Nath (1923) observed superparasitism during Dec and Jan when hosts were scarce, but not when hosts were abundant. Chien et al. (1991a) observed superparasitism rates of up to 10.4% when host density was low (1:20) and active space was limited. Chen (2013) found superparasitism rates that averaged $37.9 \pm 0.03\%$ at a female: host density of 1:10 to less than 0.5% at host densities of 1:40 and above. However, only one *T. radiata* larva from a superparasitized host was found to develop to the adult stage.

Other Host Density Effects on Life History Parameters

Longevity, fecundity, sex ratio and ratio of host feeding to oviposition all correlate with host density (Chu & Chien 1991; Chien et al. 1991a, 1995). The relationship between host density and parasitoid longevity (both males and females) was described as following a domed parabolic response, i.e., female longevity and fecundity increase with host density to a peak and then decrease as host density continues to increase (Chien et al. 1995). Average longevity increased from 15.9 to 18.6 to 20.3 days when 10, 20 and 30 nymphs were provided daily to individual females. However, female longevity decreased from 23.6 to 17.2 to 11.2 days over a range of 40, 60, 80 hosts per day, respectively (Chien et al. 1995). Chu & Chien (1991) reported that females lived an average 23.6 days and males 14.8 days when forty 5th instars were provided at 25 °C, 14:10 h L:D photoperiod and 100% RH. Both daily and lifetime fecundity showed similar parabolic responses to host density, with the peak at 40 hosts per day (Chien et al. 1995). However Chen (2013) found the average number of 4th instars parasitized in 24 h to increase from 4 to 11 over densities of 10 to 40 hosts per female, and then to level off at higher densities, resulting in a Type II functional response. Searching efficiency was estimated at $0.442 \pm 0.036 \text{ day}^{-1}$, and handling time per host at $0.045 \pm 0.008 \text{ day}$ (Chen 2013). Sule et al. (2014) also reported a type II functional response but estimated searching efficiencies at 39.99 h^{-1} and 34.04 h^{-1} , and handling times at 0.60 h and 0.71 h for 4th and 5th instar *D. citri* nymphs respectively.

Influence of Food on Longevity, Reproduction and Survival of Progeny

Adults deprived of food or water survived from 1.0 to 1.7 days (Chien et al. 1994b). Chien et al. (1994b) found that all food supplements tested improved fecundity, longevity and progeny survivorship compared to total deprivation, but observed no significant differences in longevity among female adults deprived of hosts and fed

either honey alone (22.5 days), honey and pollen (23.0 days), or honey and yeast extract (23.4 days). Chen et al. (2013) found a survival rate of 97% when *T. radiata* was stored at 25 °C with honey for 14 days, which agreed to the results from Hall & Klein (2014). Adults fed on a diet of honey and yeast extract significantly decreased host-feeding, while maintaining or improving the intrinsic rate of increase (0.2976 to 0.3014 progeny per day) and the net reproductive rate (140 to 187 female eggs per female) (Chien et al. 1994b). Addition of hydrolyzed corn gluten (Nu-Lure®) to a diet of honey alone increased egg load, as did substitution of this same proteinaceous liquid for honey to supplement host feeding (Chen & Stansly 2014).

Developmental Times of Life Stages

Chien et al. (1991a) found the duration of one generation for *T. radiata* on orange jasmine (egg to adult emergence) to be around 11.4 days at 25 °C, 14:10 h L:D, and 100% RH. Development times for individual life stages were 45 h for the egg, 24 + 24 + 22 + 26 = 96 h for the 1st through 4th instars, and 14.4 h and 117.6 h for the prepupal and pupal stages, respectively. Xu & Tang (1993) reported a generation time of 12.6 days at 25 ± 1 °C, 14:10 h L:D, and 75 to 85% RH, using 3rd and 4th instar nymphs. This corresponded to 40 h for the egg and 119 h = 25, 28, 32 and 34 h for the 1st through 4th larval instar, respectively, 24 h for the prepupa and 120 h for the pupa. These differences could reflect the different humidity conditions under which the experiments were conducted.

Temperature and Humidity Effects

Tamarixia radiata completed development at 15 to 32 °C with an optimum temperature of 25 °C (Chien et al. 1993). Gomez-Torres et al. (2012) found parasitism rates to be highest at 25 and 30 °C (85.5% and 72.8%, respectively) compared to 23.1% and 40.2% at 15 and 35 °C respectively. They also found emergence rates to be highest (86.7% and 88.3%) at 25 and 30 °C respectively, compared to about 50% in the 15 to 20 °C range. At 70 ± 10% RH and 14:10 h L:D, they estimated maximum parasitism rates of 77.2% at 26.3 °C, whereas emergence was greatest (89.9%) at an estimated 30.8 °C. Pre-imaginal development was longer for females, varying from 489.6 h at 15 °C to 247.2 h at 35 °C compared to males from 343.2 h to 146.4 h at these same temperatures.

Longevity with access to pure honey was negatively correlated with temperature between 8 °C to 30 °C (Quilici & Fauvergue 1990). These authors found that adult longevity decreased from 34 days at 20 °C to 22 days at 22 °C, 10 days at 30

°C and 8 days at 35 °C. Chien et al. (1993) found longevity increased from 45.5 to 59.5 at 8 and 15 °C, but decreased to 22.5 and 9.6 days at 25 and 30 °C, respectively. Only 10% of *T. radiata* adults survived for 50 days when stored at 25 °C with access to honey and yeast extract (Skelley & Hoy 2004).

McFarland & Hoy (2001) reported that *T. radiata* adults from Vietnam survived longer without food and water compared to parasitoids from Taiwan over a range of RH from 7% to 97% at 25 °C and especially at 30 °C. They attributed this difference to the greater moisture requirements for *T. radiata* from Taiwan. Host-killing capacity also increased to peak at 25 °C and then to decrease at higher temperature as noted above. Gomez-Torres (2012) estimated intrinsic rate of increase (r_m), net reproductive rate (R_0), and mean generation time (T) for pairs of *T. radiata* provided 5th instar *D. citri* nymphs reared on *Murraya paniculata*. Results differed considerably, especially at low temperatures (Table 1). Chien et al. (1993) did their study at a host density of 20 per female and 100% RH with 5 replications, whereas Gomez-Torres et al. (2012) conducted their study at a host density of 30 per female and 70 ± 10% RH for 10 replications. Thus, different experimental conditions may have led to differing results, although inherent differences in the races of *T. radiata* tested from Taiwan and Brazil, respectively cannot be ruled out.

Skelley & Hoy (2004) showed that *T. radiata* stored for up to 35 days at 17 °C with honey and yeast suffered less than 5% mortality. Chien et al. (1994a) reported that females stored for 20 days at 25 °C were able to lay a total of 156 eggs, compared with 98 eggs when stored at 15 °C. We may conclude that less mortality was experienced at low temperature but that reproductive fitness suffered. Therefore, ideal storage temperature should be determined according to specific objectives (establishment or augmentation) and conditions (host availability).

Molecular Genetics and Origins of Strains

Barr et al. (2009) used sequence analysis of the internal transcribed spacer (ITS) region 1, ITS-2, and the 5' end of the cytochrome oxidase subunit 1 (CO1) gene to evaluate relatedness among laboratory colonies and field collections from China, Vietnam, Pakistan, Florida, Puerto Rico, Guadeloupe and Texas. They determined that all were the same species and identified 6 haplotypes, one of which was shared between some Florida collections and Texas, whereas other haplotypes were unique to a colony originating from Vietnam, one from south China, and collections made on the Caribbean islands of Puerto Rico and Guadeloupe. DeLeon & Setamou (2010) also analyzed sequences from ITS_1 and CO1 but found no

TABLE 1. DEMOGRAPHIC PARAMETERS OF *TAMARIXIA RADIATA* ESTIMATED BY CHIEN ET AL. (1993) AND GOMEZ-TORRES ET AL. (2012).

Parameter* at various temperatures, °C	Chien et al. (1993)	Gomez-Torres et al. (2012)
Generation time, $T_{15^{\circ}\text{C}}$	39.9 days	20.3 days
Generation time, $T_{20^{\circ}\text{C}}$	22.8 days	18.8 days
Generation time, $T_{25^{\circ}\text{C}}$	16.1 days	15.5 days
Generation time, $T_{30^{\circ}\text{C}}$	12.3 days	11.8 days
Generation time, $T_{35^{\circ}\text{C}}$	NA	10.4 days
Net reproductive rate, $R_{15^{\circ}\text{C}}$	2 nymphs/♀	9.9 nymphs/♀
Net reproductive rate, $R_{20^{\circ}\text{C}}$	6 nymphs/♀	23.6 nymphs/♀
Net reproductive rate, $R_{25^{\circ}\text{C}}$	140 nymphs/♀	126.8 nymphs/♀
Net reproductive rate, $R_{30^{\circ}\text{C}}$	90 nymphs/♀	58.6 nymphs/♀
Net reproductive rate, $R_{35^{\circ}\text{C}}$	NA	21.3 nymphs/♀
Intrinsic rate of increase per day, $r_{15^{\circ}\text{C}}$	0.0011 progeny per day	0.18 progeny per day
Intrinsic rate of increase per day, $r_{20^{\circ}\text{C}}$	0.0081 progeny per day	0.25 progeny per day
Intrinsic rate of increase per day, $r_{25^{\circ}\text{C}}$	0.31 progeny per day	0.37 progeny per day
Intrinsic rate of increase per day, $r_{30^{\circ}\text{C}}$	0.37 progeny per day	0.34 progeny per day
Intrinsic rate of increase per day, $r_{35^{\circ}\text{C}}$	NA	0.25 progeny per day

*T = generation time in days, R_0 = net reproductive rate as nymphs per female, and r = intrinsic rate of increase per day, i.e., the daily rate at which the population increased in size in the absence of density-dependent forces regulating the population.

common haplotypes between Texas and Florida, concluding, therefore, that their origins were different. McFarland & Hoy (2001) suggested that populations from Vietnam and Taiwan were different ecotypes based on their different responses to relative humidity. However the expected relationship was reversed given that the less drought tolerant wasps from Taiwan actually originated in the Punjab (now Pakistan) by way of Reunion Island where they had been established for biological control of *D. citri* (Aubert 1987). Given these origins, wasps from Taiwan would be expected to show better adaptation to low RH compared to the Vietnam population. Founder effects from the two introductions might provide a better explanation for the apparent lack of tolerance to low RH among wasps sourced from Taiwan.

Symbionts

Meyer & Hoy (2008) found molecular evidence of 3 bacterial symbionts in *T. radiata*: *Caulobacter* sp., *Methylobacterium* sp. and a species of Alcaligenaceae. However, none were found in eggs suggesting no vertical (transovarial) transfer, and thus only transient associations with the host parasitoid.

Impact of Toxic Chemicals

Hall & Nguyen (2010) reported that direct contact with sprays of the following insecticides were toxic to adult *T. radiata*: abamectin, carbaryl, chenopodium oil, chlorpyrifos, fenpropathrin, imidacloprid, fenpyroximate, phosmet, pyridaben and 435 petroleum spray oil. These sprays induced 80-100% mortalities within 24 h and 100%

within 72 h. Mortalities from 80% to 100% within 24 h was observed on leaves with freshly dried residues of abamectin, carbaryl, chlorpyrifos, fenpropathrin, imidacloprid, fenpropathrin, fenpyroximate, phosmet and 435 spray oil. Cocco & Hoy (2008) reported that, the survival rate of *T. radiata* was significantly influenced by imidacloprid (95% mortality) and abamectin (91% mortality) through residual effects.

DISCUSSION

Interest in *T. radiata* has waxed and waned over the years, commensurate with concern over *D. citri* as it invaded yet another citrus growing region of the world, bringing with it (sooner or later) the devastation of HLB. The consummate ability of this parasitoid to search out and successfully establish on its host is notable. It has done so wherever introduced, either expressly or inadvertently, tracking the spread of *D. citri* around the world (Grafton-Cardwell et al. 2013; Hall et al. 2013). On the other hand, the other known primary parasitoid of *D. citri*, *Diaphorocytus aligarhensis*, has shown less promise, failing to establish in Florida despite considerable effort (Rohrig et al. 2012; J. Qureshi, personal communication). Therefore, the best hope for an effective parasitoid against *D. citri* resides with *T. radiata*. Nevertheless, *T. radiata* together with a complex of natural enemies have not been sufficient to arrest HLB neither in Florida nor elsewhere (Qureshi & Stansly 2009; Hall et al. 2013). Therefore, an integrated approach is required to adequately manage the vector and the disease (Qureshi & Stansly 2007).

In regard to classical biological control, questions remain concerning the genetic diversity of *T. radiata* over its native range, and possible impacts on biology and behavior that could influence adaptation to particular environments and ultimately to the parasitoid's ability to adequately control *D. citri*. We have so far only scratched the surface in describing this genetic diversity, and even less in ascribing it to biological or behavioral characteristics. Thus more in-depth genetic and biological studies are needed, particularly in the Indian subcontinent and elsewhere in Asia to better evaluate genetic diversity and biological characteristics associated with different haplotypes of *T. radiata*.

Founder effects are inevitable when establishment is based on a few insects collected from a single location. Genetic diversity can also be lost in culture through drift or selection. Collections made over a wide geographical area are likely to include more different haplotypes than more localized collections. The suggestion has been made but rarely if ever followed that insects could be reared in isofemale lines to maximize homozygosity and then mixed before release to restore genotypic variation (Roush et al. 1990). Greater attention to genetic, biological and ecological diversity of *T. radiata* populations in its native range may pay dividends in the ultimate success of introductions to exotic locations.

In regard to augmentative biological control, much needs to be learned to improve the efficiency of mass rearing, storage, release, and ultimately, the ability of the parasitoid to drive the host to low numbers in diverse habitats. A better understanding is needed of functional and numerical responses, and the role of host marking and male-female interactions in searching and oviposition behavior both in colony and field. The objective would be to increase rearing efficiency by mitigating competitive interference and other maladaptive behaviors. Ultimately, it may be necessary to develop rearing systems based on artificial diets in order to economically produce the numbers necessary to achieve satisfactory control. Indeed, such efforts have been most successful with ectoparasitoids (Rojas et al. 1999; Ferkovich et al. 2000).

Millions of years of evolution have apparently provided a parasitoid superbly adapted to survive and thrive at the expense of a single species originally distributed on scattered host plants over a broad range of habitats. The challenge is adapting this resource to an intensively managed monoculture with the ultimate goal of a sustainable integrated management system for *D. citri*.

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