Biology and rearing of *Cleruchoides noackae* (Hymenoptera: Mymaridae), an egg parasitoid for the biological control of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae)

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Short Title: Biology and rearing of *Cleruchoides noackae*

ABSTRACT   *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae) is a solitary egg parasitoid of *Thaumastocoris peregrinus* Carpintero and Dellapé (Hemiptera: Thaumastocoridae). The parasitoid was first described in 2009 and its biology and rearing are poorly understood. A key obstacle to the use of *C. noackae* as a biological control agent has been the ability to consistently rear it under quarantine conditions. This study reports on a series of experiments conducted in quarantine to rear *C. noackae* and to examine the effects of diet on longevity, per capita reproduction and progeny sex ratio, as well as to determine development time, and preference and suitability of host eggs of different ages. When supplemented with honey solution, the longevity of *C. noackae* females increased significantly

by 2.4 days and that of males by 1.7 days, relative to the unfed adults. Mean per capita reproduction for the honey-fed wasps was 7.7 offspring per female, with progeny sex ratio slightly skewed towards males. Mean percentage parasitism was 32.2 %. *Cleruchoides noackae* was capable of parasitizing and completing development from oviposition to adult eclosion within 15.7 days in host eggs between zero and five days old. The ability of *C. noackae* to parasitize a wide range of host egg ages increases the period of vulnerability of *T. peregrinus* to attack, increasing its potential efficacy as a biological control agent. The methods and results reported here represent a crucial step in the on-going efforts to develop this potential biological control system.

KEY WORDS   Biological control, *Cleruchoides noackae*, Mymaridae, per capita reproduction, *Thaumastocoris peregrinus*

Introduction

Global trade together with the widespread planting of common non-native tree species has resulted in an increased diversity of insect pests relevant to plantation forestry (Wingfield et al. 2008). Among these, the Australian bug *Thaumastocoris peregrinus* Carpintero and Dellapé (Hemiptera: Thaumastocoridae) is a serious and widespread pest of *Eucalyptus* species and commercially propagated hybrid clones (Carpintero and Dellapé 2006, Noack et al. 2011, Soliman et al. 2012). This insect is commonly referred to as the winter bronze bug due to the characteristic reddening and yellowing of the canopy foliage followed by bronzing and drying of leaves. These symptoms are a result of sap-sucking and puncturing of the leaves and twigs which leads to chlorosis and eventual reduction in tree growth and yield (Wilcken et al. 2010, Soliman et al. 2012). The insect has been recorded on various species of

the family Myrtaceae, including 13 species of the genus *Eucalyptus* and one species of the genus *Corymbia* (*C. citriodora* (Hook) K.D. Hill and L.A.S Johnson ssp. *citriodora*) (Noack et al. 2011).

Thaumastocoris peregrinus was first recorded outside its native range in South Africa in 2003 (Jacobs and Neser 2005). By 2009, it had spread northwards as far as Kenya (Noack et al. 2011, Nadel and Noack 2012). In South America, the insect has also spread rapidly since its first detection in Argentina in 2005, in Uruguay and Brazil in 2008 and Chile in 2006 (Wilcken et al. 2010, Nadel et al. 2010, Soliman et al. 2012). In 2011, *T. peregrinus* was detected in Italy, marking the first report of a Thaumastocoridae species in Europe (Laudonia and Sasso 2012). In March 2012, *T. peregrinus* was reported on *E. nicholii* Maiden and Blakely in New Zealand (Sopow et al. 2012).

Thaumastocoris peregrinus is a relatively new pest of *Eucalyptus* in plantations and, therefore, management options are still being developed. Pesticides such as imidacloprid are effective on a small scale, but its use is neither economically viable nor ecologically acceptable as a long-term management strategy (Noack et al. 2009). Host resistance is also unlikely to be a viable management option as *T. peregrinus* has been reported to attack a wide variety of *Eucalyptus* species, with no clear patterns of host resistance (Noack et al. 2011, Soliman et al. 2012). In Brazil, general natural enemies such as the green lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae), a predatory bug *Atopozelus opsimus* Elkin (Hemiptera: Reduviidae) and entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin and *Zoophthora radicans* (Brefeld) Batko (Entomophthorales: Entomophthoraceace) have been observed attacking or infecting nymphs and adults of *T. peregrinus* (Wilcken et al. 2010, Laudonia and Sasso 2012, Mascarin et al. 2012). Though such natural enemies may form part of an integrated pest management approach, they do not appear to be sufficient in controlling the populations to economically acceptable levels. As with many invasive forestry pests,

classical biological control using a host-specific parasitoid ranks as a highly desirable management option.

Cleruchoides noackae Lin and Huber (Hymenoptera: Mymaridae) is a solitary endo-parasitoid of *T. peregrinus* eggs, and is currently being considered in South Africa and in other parts of the world for release as a biological control agent (Lin et al. 2007, Nadel and Noack 2012). This minute mymarid wasp, which is approximately 0.5 mm long and commonly known as a "fairy fly" due to its lace-like wing structure, was recovered from *T. peregrinus* eggs in 2009 during systematic searches for biocontrol agents in New South Wales, Australia, where the insect appears to be a relatively common egg parasitoid (Lin et al. 2007, Nadel et al. 2012). The parasitoid has since been imported into South Africa and Brazil for biology and host specificity studies in quarantine (Nadel and Noack 2012). In Chile, *C. noackae* was imported from Australia, reared in quarantine and 230 adults and 3937 parasitized eggs were released into the field in 2009 (Nadel and Noack 2012). This was the first field release of *C. noackae* into a non-native *T. peregrinus* infested-landscape, but no information regarding its establishment or impact has been published.

Very little is known regarding the life history of *C. noackae* or its interaction with *T. peregrinus* (Cross 2009). Despite this, *Eucalyptus* growers are hopeful that this parasitoid could play a key role in reducing losses due to *T. peregrinus*. In Sydney, Australia, laboratory and field studies with *C. noackae* conducted by Cross (2009) showed a 25% and 21% parasitism rate, respectively. The parasitoid is capable of attacking several species within the Thaumastocorinae and has not been reported to attack hosts outside this sub-family (Lin et al. 2007, Nadel et al. 2010). The wasp has emerged from newly discovered *Thaumastocoris* eggs collected in Perth, Western Australia (host species unknown) and has also been reared from *Baclozygum depressum* (Thaumastocorinae) on *Eucalyptus globosus* and *E. nicholii* leaves (Cross 2009, Nadel et al. 2010). The apparent specificity to the Thaumastocorinae

enhances the potential to use *C. noackae* as a biological control agent against *T. peregrinus* in Africa and South America since no native Thaumastocorinae are known to occur in these regions.

Biological control is increasingly becoming one of the more favored and feasible approaches to deal with insect pests in plantation forestry (Garnas et al. 2012). Unfortunately, developing and implementing a successful classical biological program is a difficult task involving many complex interacting factors (Hokkanen 1985, Vorsino et al. 2012). One of the factors that can potentially inhibit the introduction of new biological control agents, is the ability to rear the insect in quarantine conditions. Challenges related to this include obtaining knowledge on life history parameters such as fecundity, feeding and oviposition behavior, developmental rate and longevity in order to guide the rearing, release and establishment efforts. This paper reports on a series of experiments designed to capture key elements of the basic biology of *C. noackae*, including aspects that are relevant to laboratory rearing and potential procedures for release. The specific objectives of this study were to elucidate the characteristics of the life history of *C. noackae* such as the effect of nutrient provision on adult longevity, development time, sex ratio, percentage parasitism, per capita reproduction and the stage at which host eggs are suitable and preferred for parasitism.

Materials and methods

Parasitoid colony establishment

Adults and nymphs of *T. peregrinus* (approximately 300 individuals) were collected from infested trees around the Experimental Farm at the University of Pretoria, South Africa and transferred using a camel-hair brush to cut twigs of the *Eucalyptus grandis* W. Hill ex Maiden clone TAG5 for oviposition. *Eucalyptus grandis* and *E. urophylla* S. T. Blake have been identified, among others, as the most suitable for *T. peregrinus* development and reproduction (Soliman et al. 2012). The population of

T. peregrinus adults was allowed to lay eggs for 24 hours after which the entire population was transferred to a new batch of twigs. The eggs were collected by cutting away excess leaf tissue. This strategy was feasible given that *T. peregrinus* has a strong propensity to lay egg clusters to which multiple females contribute. Eggs were stored in Petri dishes (9.0 cm diameter), placed inside a plastic container (27 cm x 27 cm x 15 cm in size) for a maximum number of 10 days at 6-8°C and 70-80% relative humidity for slow development. These eggs were then used for parasitoid mass rearing and also in the life history studies.

The culture of *C. noackae* was established from over 1500 parasitized *T. peregrinus* eggs collected from infested *Eucalyptus* trees at Olympic Park, Sydney, Australia and shipped to the Biological Control Centre of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Rearing conditions were similar to those for host egg production: 24 ± 2ºC, $70 \pm 10\%$ RH with a photoperiod of 12L: 12D hours. Emerged *C. noackae* adults were transferred into glass vials (7.5 cm long by 2.5 cm wide) containing *T. peregrinus* eggs to establish a continuous breeding colony of the parasitoid. This was achieved by joining two glass vials, one containing the emerging *C. noackae* and the other with unparasitized *T. peregrinus* eggs. The vial-to-vial joint was made of a cork with central hole through which a narrow white paper strip was passed to allow easy movement of the emerging *C. noackae* adults to the other vial containing the host eggs. The use of cork between the two vials made it easy to separate the vials when required. The vial with the emerging *C. noackae* eggs was covered with an opaque sleeve of black masking tape to establish a dark environment. This accelerated the movement of the *C. noackae* to the vial exposed to light and that contained the *T. peregrinus* eggs to be parasitised.

Effect of diet on parasitoid adult longevity, sex ratio and per capita reproduction

A single breeding pair of <12-hour-old *C. noackae* adults were exposed to twenty 0-1 day-old eggs of *T. peregrinus* in a glass vial (7.5 cm long, 2.5 cm wide). Three diet treatments were evaluated to determine their influence on adult parasitoid longevity. The diet treatments were: (i) 50% honey solution, (ii) water and (iii) no food. The honey solution was provided by painting a thin strip (< 0.5 mm wide) of commercially purchased honey inside the vial with a fine brush, while water was provided through a soaked cotton-wool plug enclosed in a muslin cloth and placed inside the lid of the vial. A total of 58 *C. noackae* pairs were observed on each of the three diet treatments. Vials were monitored daily for 25 days and male and female longevity, per capita reproduction and offspring sex ratio recorded.

Host egg age class preference and suitability

To determine whether the age of the host egg influences female oviposition, preference and suitability as a developmental substrate for *C. noackae*, choice and no-choice experiments were conducted. Host eggs that had been collected within 24 hours (0-1 day) of oviposition were used directly to develop to 2-3 or 4-5 day old in a rearing room. Choice tests were conducted to determine whether *C. noackae* has a preference for a particular age class of *T. peregrinus* eggs. A total of 20 eggs each of the three age classes (0-1, 2-3 and 4-5 day-old eggs) were labeled with a permanent marker on the cut leaf segments (cut disc of < 2 cm) and placed in vials. Three males and three females of *C. noackae* were exposed to each vial containing 60 host eggs for 24 hours. The parasitoids were provided with a honey-water solution as before. After 10 days, the marked eggs were separated according to age category and monitored for emergence over 25 days. After 25 days, the unhatched eggs were dissected to determine the proportion of infertile eggs (eggs with no evidence of development), dead *C. noackae* and dead *T. peregrinus* nymphs. The experiment was replicated eight times.

No-choice tests were conducted to determine whether there is a certain host egg age class that is more suitable for development of *C. noackae*. This experiment was identical to the choice test except that the different egg age classes were kept in separate vials, with a total of 20 host eggs per vial. There were eight replicates for the 0-1 and 2-3 day-old egg age class, and six replicates for the 4-5 day-old egg age class. Measurements were as above. In both choice and no-choice tests, percent parasitism was calculated as the total number of *C. noackae* progeny (emerged and dead inside the egg) divided by total number of viable *T. peregrinus* eggs.

Thaumastocoris peregrinus eggs older than five days were not compared in the choice and no-choice tests, because most of these eggs hatched into *T. peregrinus* nymphs after five days and therefore sufficient replication for the specific experimental set-up (that needed 180 eggs per egg age class) could not be obtained. Small numbers of eggs older than five days were obtained irregularly. These were used separately to test the ability of *C. noackae* to oviposit and develop fully in host eggs older than five days using a total of 14 replicates consisting of 224 eggs that included 5-6 day-old eggs (n=120, replicates = 6), 6-7 day-old eggs (n= 72, replicates = 6) and 7-8 day-old eggs (n=32, replicates = 2). Each replicate was exposed to a pair (male and female) of *C. noackae* adults supplemented with honey-water solution. The number of offspring that emerged and the time of development were enumerated and unhatched eggs dissected after 25 days of exposure to the parasitoids.

Mode of reproduction, oviposition and mating behaviour

To determine the mode of reproduction and the mating behavior of *C. noackae*, two sets of experiments involving unmated and mated adult females were conducted. In the first experiment 20 (0-1 day-old) host eggs were placed in similar vials as those used in the longevity studies above, and exposed to 10 virgin female parasitoids. In the second experiment, 20 eggs in the 0-1 day age class were exposed to 10 pairs of *C. noackae*. The parasitoids were fed on a thin strip of honey-water solution painted inside

the vials and were allowed to parasitize the eggs until the insects died. Parasitoid oviposition behavior was observed in all the replicates while mating behavior was monitored in the paired experiments. The sex of the emerging progeny was determined and unhatched eggs were dissected after 25 days to identify dead *T. peregrinus* nymphs, dead *C. noackae* in the eggs and infertile (non-viable) eggs.

Data analysis

We examined adult longevity as a function of the diet and gender. Survival analysis was done using a Cox proportional hazard model where pairwise comparisons were performed using log-rank tests and adjusting *p*-values for multiple tests using Holm's procedure (Holm 1979). To evaluate the relative importance of the diet and *C. noackae* adult longevity on per capita reproduction, we used hurdle models as the data were not amenable to traditional ANOVA or regression due to non-constant variance and a large number of zeros. Hurdle models are useful in dealing with zero-inflated datasets as they partition analyses into a binary (zero v. nonzero) and a continuous component (Zeileis et al. 2008, Zuur et al. 2009). For the continuous component, a negative binomial error distribution (with a logit link) was used because it provided the best fit for the data. To determine the relative contribution of longevity and diet to offspring production, we compared full and reduced hurdle models containing these terms as predictors of offspring production using Akaike's Information Criterion (AIC) (Akaike 1974, Burnham 1998). The simplest model containing only diet (and not longevity, either as a crossed or additive term) was preferred (AIC=262.1, ∆AIC between 1.1 and 3.2). As such, longevity was dropped from subsequent analyses. We examined the relationship between the diet and the frequency of producing offspring (offspring produced: yes or no) using contingency analysis and performed a nonparametric Kruskal-Wallis rank sums test on total offspring count (including zero values). Pairwise comparisons for the former test were again adjusted using Holm's correction and in the second analysis using methods described in Siegel and Castellan (1988). Similarly, Kruskal-Wallis tests were used to

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compare preference and suitability among egg age treatments in the choice and no choice tests. Finally,

we used linear regression to explore the negative relationship between offspring number and egg age in

our choice tests, as the data conformed to required assumptions.
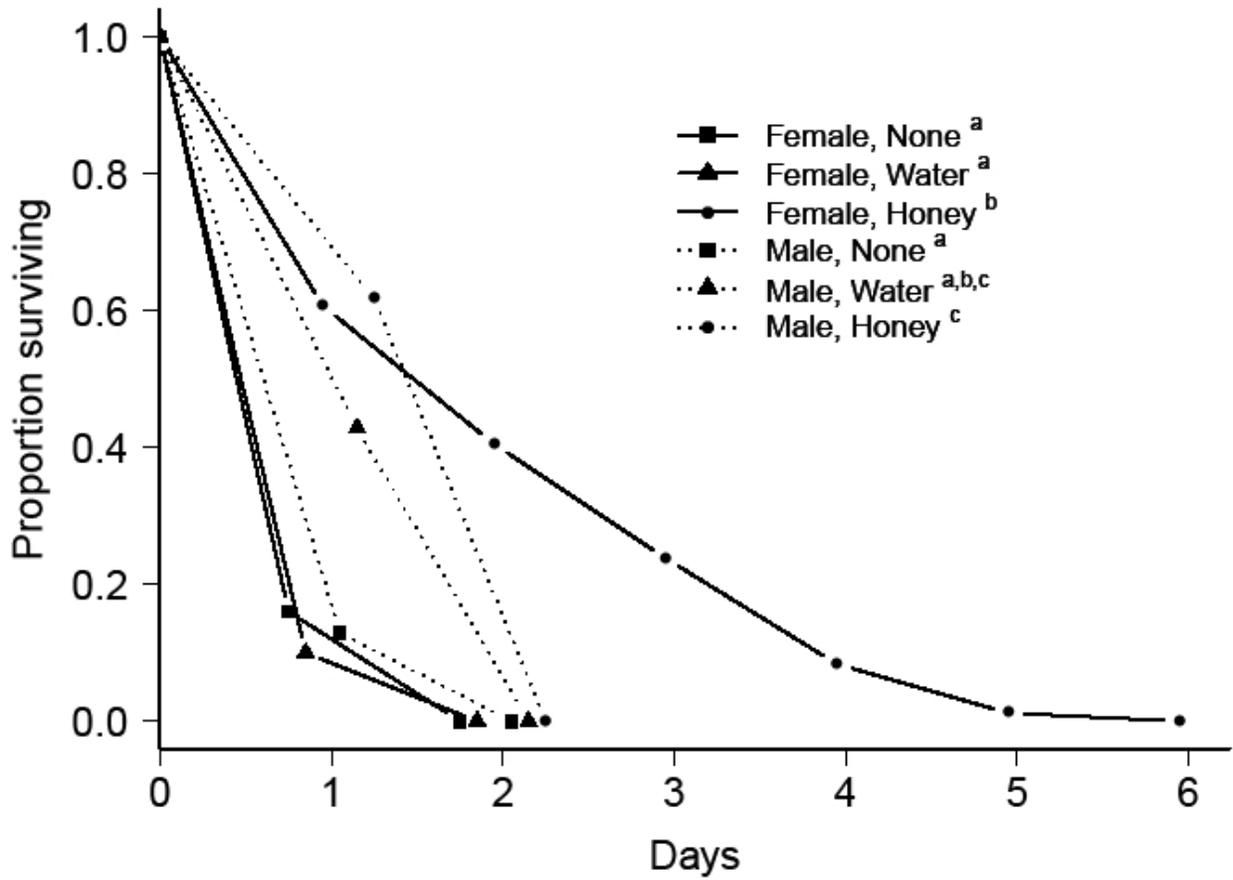
Figure 1 Survivorship curves of *C. noackae* female and male adults on different diets. Curves with the same letters do not

differ statistically at $\alpha = 0.05$ (adjusted for multiple tests using Holm's correction).

Results

Effect of diet on parasitoid adult longevity, sex ratio and per capita reproduction

Survival of adult *C. noackae* differed as a function of sex and diet (Likelihood ratio test = 62.2, df = 5, $P < 0.0001$, R^2 = 0.26) (Figure 1). There was a significant effect of sex (χ^2 = 15.0, df = 1, P = 0.0001), diet (χ^2 = 37.4, df = 2, $P < 0.0001$) and their interaction (χ^2 = 9.9, df = 2, P = 0.007). Overall, female survival was significantly higher than that of males (females 2.0 ± 1.3 days, males 1.4 ± 0.5 days; χ^2 = 14.0, df = 1, P = 0.0002). Pooling males and females, all corrected pairwise differences in survival among diet treatment were significant. Parasitoids that were unfed, fed on water or fed on honey lived for 1.2 ± 0.4, 1.1 ± 0.4 and 2.1 ± 1.1 days, respectively. Honey-supplemented females lived for as long as six days compared to all other diet treatments where the maximum longevity was two days. Survival for these females differed significantly from all other treatments with the exception of males supplied with water.

Diet significantly influenced whether or not offspring were produced by *C. noackae* (χ^2 = 19.6, df = 2, $P < 0.0001$). Pairwise comparisons showed that the proportion of unfed females producing no offspring (68%) differed significantly from water- (10%) and honey-provided females (13%). The number of unhatched eggs was small (1.6%).

Diet also significantly influenced the number of offspring produced (Kruskal-Wallis χ^2 = 24.5, df = 2, $P < 0.0001$). The post hoc test (α = 0.05) revealed significant differences between none- and water-fed females and also between none- and honey-fed females. There was no significant difference between water- and honey-fed females on the number of offspring produced. The number of offspring produced ranged between 0-16 with a mean of 4.4 ± 0.3 when data for the three diets were pooled. The largest number of offspring was recorded from the honey-fed females (7.7 ± 0.4) followed by the water-

fed females (4.8 ± 0.4) and the females that received no food or water recording the lowest (1.2 ± 0.4). Comparing unfed females with honey- and water-fed females, there was a six and four-fold increase in the number of offspring produced, respectively.
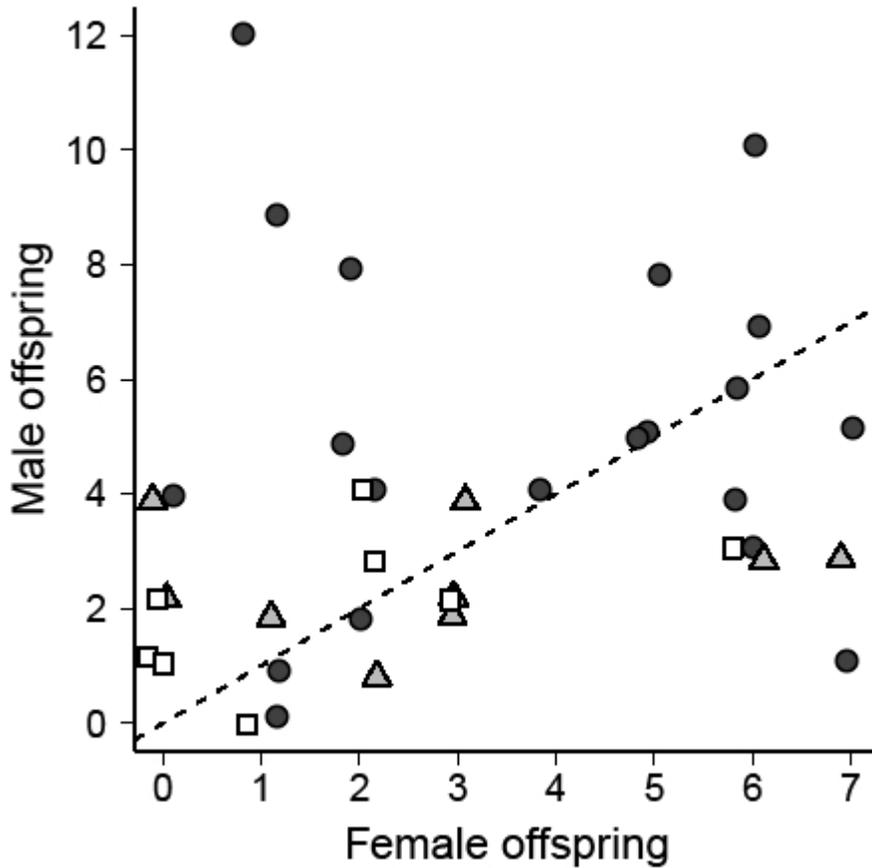
Figure 2 Offspring sex ratio as a function of diet: (■) unfed, (▲) water- and (●) honey-supplemented females, Dotted line represents a 1:1 male:female offspring ratio. There was no significant difference in the sex ratio among diet treatments; $\chi^2 = 1.6$, df = 2, $P = 0.4$

There was no significant difference ($\chi^2 = 1.6$, df = 2, $P = 0.4$) in the sex ratio of offspring produced between the three diet treatments (Figure 2). The 37 parental females produced a total of 256

offspring, of which 65.5% were male. There was a high level of variation in sex ratio of the offspring produced in the three diet treatments. The mean sex ratio of 1.2:1.0 (male: female) observed was not significantly different from a 1:1 female to male ratio ($\chi^2 = 3.1$, df $= 1$, $P = 0.08$).
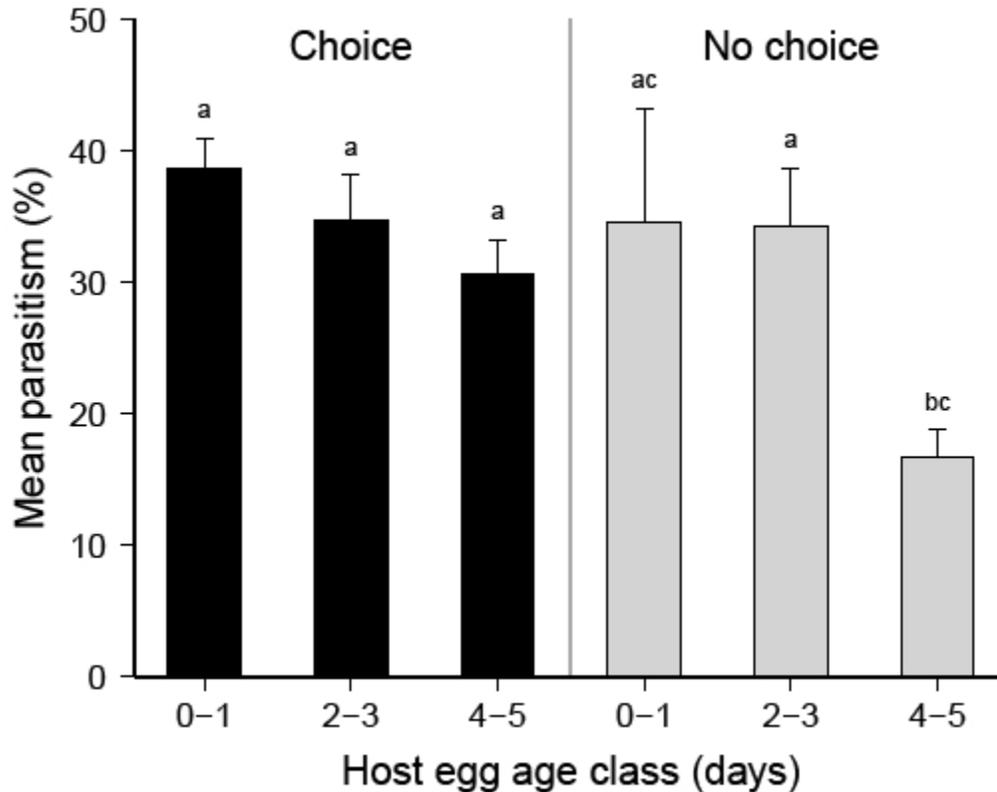
Figure 3 Mean parasitism (±SE) by egg age class in choice (left) and no-choice (right) tests. There was no significant difference in the in choice tests; $\chi^2 = 4.8$, df $= 2$, $P = 0.097$, but there was significant difference in no-choice tests, $\chi^2 = 6.37$, df $= 2$, $P = 0.04$. Treatment with same letters in the same test do not differ statistically at $\alpha = 0.05$.

Host egg age class preference and suitability

There was a significant difference among the three egg age classes in suitability (no-choice tests) to *C. noackae* ($\chi^2 = 6.37$, df $= 2$, $P = 0.04$) (Figure 3). Kruskal-Wallis multiple comparison test ($\alpha = 0.05$) revealed that there was a significant difference in parasitism between 2-3 and 4-5 day-old eggs,

but no significant difference between 0-1 and 2-3 day treatments or between 0-1 and 4-5 day-old eggs. The highest rate of parasitism was observed in 0-1 day-old eggs (34.1 ± 8.6%) with the lowest parasitism on 4-5 day-old eggs (16.6 ± 2.2%). Although *C. noackae* females were observed in an oviposition posture on eggs older than 5 days, no parasitoid offspring emerged from those eggs after 25 days of observation. Nymphs of *T. peregrinus* emerged from 215 eggs (96%, n=224). The remaining nine unhatched eggs were dissected after 25 days, five eggs (2.2%) had dead *T. peregrinus* nymphs inside, and four eggs (1.8%) were found not to be viable.

Considering egg age as a categorical variable (as above) there was no significant difference in parasitism rate among the three egg ages (χ^2 = 4.8, df = 2, *P* = 0.097) (Figure 3). However, the data were amenable to linear regression and it was possible to detect a moderate negative trend. Parasitism rate declined moderately with egg age (*F* = 4.5, df = 1, 22, *P* = 0.045; R^2 = 0.2) from 38.8 ± 2.8% in the 0-1 day old age class to 30.6 ± 2.6% in the 4-5 day age class.

There was no significant difference (*F* = 0.4, df = 2, *P* = 0.51) in the offspring development time between the three egg age classes when data from choice and no-choice were pooled. The mean development time of *C. noackae* was 15.7 ± 0.1 days across all host egg age classes. The *C. noackae* male adults emerged concurrently with females.

Mode of reproduction, oviposition and mating behaviour

Parasitoid mating was observed to occur immediately after the males encountered the females (n=8). The males showed no courtship behavior and mating occurred within 2-5 seconds. Once mated, females were not receptive to the males. Oviposition was observed immediately after parasitoids came into contact with the host eggs, irrespective of mating status. The ovipositing females tapped the eggs with their antennae several times before and after oviposition. Repeated oviposition in the same egg was

rare (observed three times). Only a single parasitoid was observed to emerge per egg. The duration of oviposition in the mated and unmated females ranged between 1.9-4.1 (mean±SE; 2.3 ± 0.1; n=19) minutes per egg.

The ten replicates of the paired mated females produced a total of 95 offspring; 51 males and 44 females from the 200 host eggs that had been exposed. Of these, seven replicates produced mixed progeny, two replicates produced only male progeny and one replicate produced only female progeny. Overall, the ratio of males to females was 1.2: 1.0. The unmated females produced a total of 87 offspring from a total of 200 host eggs exposed in ten replicates. All the ten replicates produced only male offspring. Unhatched eggs in the two experiments were dissected and no signs of undeveloped parasitoid embryos were observed.

Discussion

This study provides important information regarding the rearing and biology of *C. noackae* with bearing on its use as a potential biological control agent of *T. peregrinus*. Methods developed to effectively rear the parasitoid will be critical for host specificity tests that must be undertaken to determine the environmental suitability of *C. noackae* in potential areas of release. Knowledge generated in this study regarding the biology of *C. noackae* is also important to guide and evaluate its field release and assessment of establishment.

Cleruchoides noackae adult has a short life span (range; 1– 6 days, mean±SE; 1.7 ± 0.1 days, n = 204) that is strongly influenced by provision of honey. The considerable increase in *C. noackae* longevity due to provision of carbohydrates and water is similar to that reported for other mymarids (Cronin and Strong 1990, Jervis et al. 2001). For example, females of *Anagrus delicatus* Dozier (Hymenoptera: Mymaridae), an egg parasitoid of the leaf planthopper, *Prokelisia marginata* (Van

Duzee) (Homoptera: Delphacidae), lived for 3.3 days when supplemented with a honey solution and 2.0 days when provided only with water (Cronin and Strong 1990). Likewise, adults of the mymarid *Gonatocerus deleoni* Logarzo and Virla lived three times longer when provided with a honey solution than when provided with water or no-food supplement (Cronin and Strong 1990, Lytle et al. 2012). Differences in longevity between females and males has also been observed in other mymarid species such as *Stethynium* sp. and *A. delicatus*, where males were found to have shorter life-spans than females (Cronin and Strong 1990, Jacob et al. 2006). It is thus important to consider the food source that *C. noackae* might utilize in the field as this will most likely influence the establishment success of the parasitoid, as it has been seen for other parasitoids (Jervis et al. 2001, Irvin and Hoddle 2005). These food sources can include honeydew from sap-sucking insects, floral nectar or pollen and in some cases, host haemolymph (Jervis et al. 1996, Lopez et al. 2004, Jacob et al. 2006).

Thaumastocoris peregrinus adults can live for approximately 40 days and females can lay up to 60 eggs (Noack and Rose 2007). Development time from oviposition to adult emergence of *C. noackae* is about 16 days and is not influenced by host egg age, for eggs less than five days old. One *T. peregrinus* egg laying duration of 40 days could thus potentially be exposed up to approximately three generations of *C. noackae* adults. This short generation time of the *C. noackae* could contribute to sustained suppression of *T. peregrinus* population. However, additional ecological studies of the host and parasitoid life cycle are required to better understand the effects of other interacting factors such as climate, season or host quality.

Feeding regime clearly influenced both the probability of producing offspring and also the number of *C. noackae* offspring produced. Similar studies with the mymarid *A. delicatus* also showed that females provided with honey solution produced larger numbers of offspring compared to water or unfed females which the authors attributed to increased lifespan and oviposition rate within the first two

days after female emergence (Cronin and Strong 1990). Immediate oviposition post-emergence, as was observed in *C. noackae*, is associated with a pro-ovigenic life history in parasitoids, where no egg maturation period is required (Jervis et al. 2001). From this study with *C. noackae*, it was difficult to conclude whether this influence was due to females having more time to lay eggs because they lived longer, or because the honey-fed females acquired more energy for egg-laying. Incorporating longevity as an explanatory variable with diet did not improve model fit and there was no correlation between adult longevity and offspring number after correcting for diet. While longevity co-varied with diet and progeny production, it does not appear that the additional lifespan strongly influences total egg placement, further supporting hypothesis that *C. noackae* is pro-ovigenic. Rather, it appears likely that honey-supplemented females had more energy to fuel oviposition, as is the case with other mymarids (e.g., *Stethynium* species; Jacob et al. 2006).

The age of *T. peregrinus* eggs influenced egg suitability, but preference was broad for eggs of age class <5 days old in relationship to *C. noackae* parasitism rate. The incubation period of *T. peregrinus* eggs at room temperature (24±2 ℃) is six days (Soliman et al. 2012), so it is likely that older host eggs (< 5 days old) which are at pre-embryonic stage provide minimal resources because nutrients are metabolized into substances that may be less readily assimilated by developing parasitoids (Godin and Boivin 2000). Similar results showing younger eggs to be more suitable for parasitism have been recorded in studies of other mymarid species such as *Gonatocerus triguttatus* Girault parasitizing eggs of *Homalodisca vitripennis* (Germar) (*syn. coagulata*) (Hemiptera: Cicadellidae) (Irvin and Hoddle 2005). Mymarids such as *Gonatocerus ashmeadi* Girault have also been observed to have lower parasitism on older eggs of the host *H. vitripennis*, possibly due to egg nutrient availability or inhibition of parasitiod development (Irvin and Hoddle 2005). The lack of parasitism by *C. noackae* observed in *T. peregrinus* eggs older than five days could also be due to the hardening of the chorion, as reported in

Patasson lameeri Debauche (Irvin and Hoddle 2005). Alternatively, inhibition could be associated with the developing *T. peregrinus* nymph in the older eggs.

The sex ratio of *C. noackae* progeny was slightly skewed towards males, but did not significantly differ from a 1:1 ratio. This matches findings for other mymarid species (Chen et al. 2006, Jacob et al. 2006). The offspring sex ratio was also unaffected by the diet. In this study, arrhenotokous parthenogenesis in *C. noackae* is confirmed, as is common for hymenopteran parasitoids. Mated females produce both male and female offspring, while unmated females produce only haploid males, which is common in the Mymaridae (Jervis et al. 2001). Male and female *C. noackae* offspring emerged simultaneously and mated immediately (Jervis et al. 2001). Immediate mating after eclosion is expected for short-lived, pro-ovigenic parasitoid species such as *C. noackae*.

The life history traits of *C. noackae* determined in this study suggest that this parasitoid has many characteristics that could make it a successful biological control agent against *T. peregrinus*. The short life cycle coupled with broad host egg age preference would enable *C. noackae* populations to increase rapidly with multiple generations capable of attacking each generation of *T. peregrinus*. Furthermore, the mating and oviposition behavior, as well as the broad host egg age preference is likely to positively influence population establishment, spread and persistence in the introduced habitat. *Cleruchoides noackae* is currently the only biological control candidate for *T. peregrinus* and knowledge of its biology such as reported in this study, together with the rearing technique, thus provides an important step towards the management of *T. peregrinus*. Additional ecological studies on host specificity, host-parasitoid interactions, and impacts on host populations across a range of field conditions are required to fully assess and improve a future biocontrol program using *C. noackae*.

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