# *Biological Data on* Anovia punica *Gordon (Coleoptera: Coccinellidae), a Predator of* Crypticerya multicicatrices *Kondo & Unruh (Hemiptera: Monophlebidae)*

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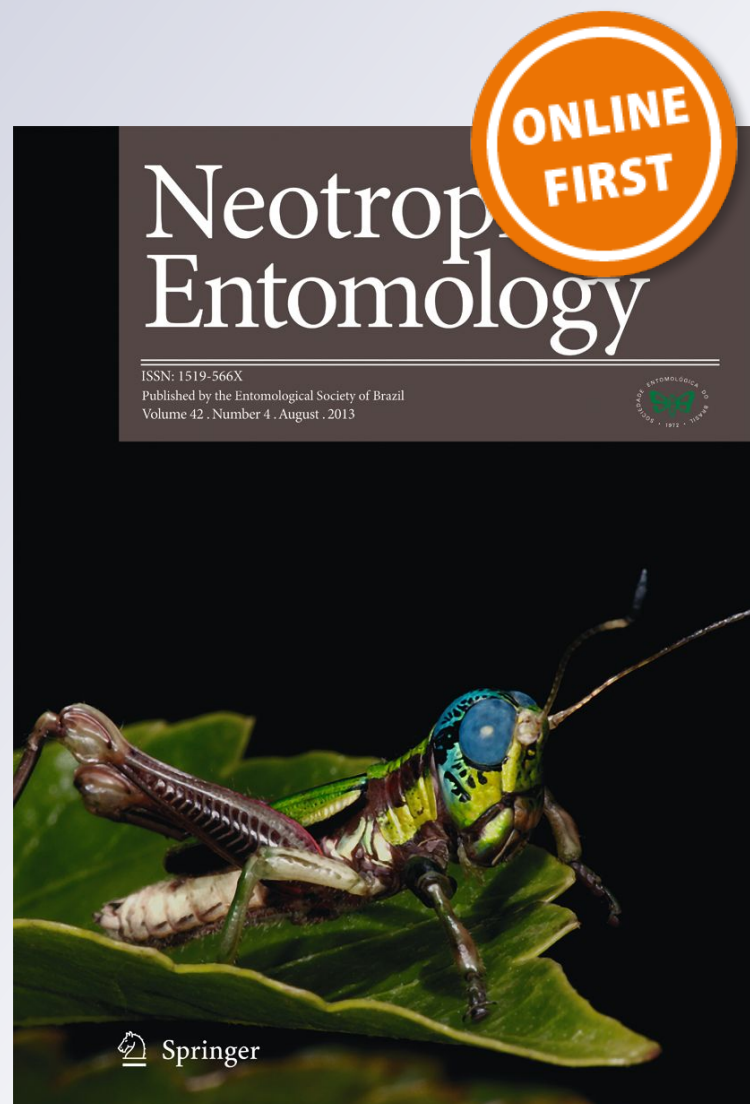
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**Abstract**

The coccinellid beetle *Anovia punica* Gordon (Coleoptera: Coccinellidae: Noviini) is an important predator of the Colombian fluted scale, *Crypticerya multicicatrices* Kondo & Unruh (Hemiptera: Monophlebidae). In order to gather information on the biological traits of *A. punica*, we conducted a series of studies, including of the developmental time of each life history stage, estimation of life table parameters, and predation rates under laboratory conditions [25.1 ± 1.6°C, with 70.5 ± 7.3% RH, and natural light regime, approx. 12:12 (L:D) h]. Developmental stages of *A. punica* were categorized as follows: egg stage, four larval instars, prepupal instar, pupal instar, and adult. Developmental time from egg to adult emergence averaged 29.41 ± 1.85 days, and 47.6% of the eggs developed to adulthood. Female and male survival was 94.42 and 90 days, respectively. Life table parameters show that one female of *A. punica* is replaced by 86 females ($R_o$), the intrinsic growth rate ($r_m$) was 0.1115, the average generation time ($T$) was 40 days, and the doubling time ($D_t$) was 6.2 days. The life table parameters suggest that *A. punica* can be used as a potential predator of *C. multicicatrices* and, more importantly, provided baseline information for a mass-rearing protocol. This is the first detailed study on the biology of *A. punica* that reports the potential of this predator as a biological control agent for scale insects of the tribe Iceryini.

## Introduction

The Colombian fluted scale, *Crypticerya multicicatrices* Kondo & Unruh (Hemiptera: Coccomorpha: Monophlebidae) (Kondo & Unruh 2009), is a phytophagous and polyphagous insect native to continental Colombia, and reported as an invasive pest on the islands of San Andrés and Providencia from 2010–2013 (Kondo *et al* 2012). Due to the lack of natural enemies on the island, *C. multicicatrices* showed a rapid population growth that affected more than 150 species of plants, including some

species of economic importance such as avocados, *Persea americana* (Lauraceae); coconut, *Cocos nucifera* (Arecaceae); soursop, *Anona muricata* (Annonaceae); guava, *Psidium guajava* (Myrtaceae); and mango, *Mangifera indica* (Anacardiaceae) (Kondo *et al* 2012, 2014, Silva-Gómez *et al* 2013).

*Crypticerya multicicatrices* sucks the sap from leaves, branches, and fruits of the host plant and produces honeydew that promotes the development of sooty molds. The presence of sooty molds over the commercial parts of plants causes damage that reduces the quality of the product, decreases the

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photosynthetic rate, and in severe attacks may cause the death of the plant (Kondo *et al* 2012). These same authors also discussed the need to implement a classical biological control program as a way to mitigate the environmental impact of this invasive pest, which led to the search for natural enemies of *C. multicicatrices* in continental Colombia. Fortunately, in 2013, *C. multicicatrices* on San Andrés island was controlled by a coccinellid, initially identified as *Anovia* sp. (Coleoptera: Coccinellidae), through a fortuitous and efficient classical biological control event (Kondo *et al* 2014). This voracious predator, now identified as *Anovia punica* Gordon (González & Kondo 2014), was first found in February 2013 on the island of San Andrés and only 8 months from its first sighting, this predator had spread throughout the island, decimating the populations of *C. multicicatrices* to the point that it was very difficult to find even a specimen (Kondo *et al* 2014). All known preys of *A. punica* are scale insects that belong to the tribe Iceryini (Hemiptera: Coccomorpha: Monophlebidae) (González & Kondo 2014). Besides *C. multicicatrices*, *A. punica* has been previously reported feeding on *Icerya purchasi* Maskell (Monophlebidae) and *Crypticerya montserratensis* (Riley & Howard 1890) (Gordon 1972) (Monophlebidae). Thus, it is likely that this predator may feed on other species of fluted scales in other regions of Central and South America (González & Kondo 2014).

Coccinellids, or lady beetles, of the tribe Noviini (Coleoptera: Coccinellidae) are known for their effectiveness in the control of scale insects, and most of the literature related to Noviini focuses on this aspect (Forrester 2008). Larvae and adults are voracious predators exclusively of scale insects of the family Monophlebidae (Forrester 2008). In addition, the tribe Noviini is diverse and consists of approximately 80 species divided into three genera: *Anovia* Casey, *Novius* Mulsant, and *Rodolia* (Mulsant) (Forrester *et al* 2009), many of which have a great importance as biological control agents. For example, the most famous reported case is the introduction of *Rodolia cardinalis* (Mulsant) to the State of California (USA) in 1889, to control the cottony cushion scale *I. purchasi*, an iceryine scale that caused large losses to the citrus industry in California (Caltagirone & Doutt 1989). Another example is the release of *R. cardinalis* to control *I. purchasi* in the Galapagos Islands in 2002 (Causton 2004). After 3 months, the population of *I. purchasi* decreased by 99–100%, possibly together with the effect of high rainfall in the period of study. Another noviine coccinellid, *Anovia circumclusa* (Gorham), has been reported as a natural enemy of the introduced pest *Crypticerya genistae* Hempel (Monophlebidae) in the State of Florida, USA (Forrester *et al* 2009).

Currently, there are no reports or studies on the life history of *A. punica*, and to our knowledge, the present work is also the first detailed study of the biology of *A. punica*.

Although it has been documented that *C. multicicatrices* was controlled successfully by *A. punica* on San Andrés Island (Kondo *et al* 2014), the process behind this case of classical biological control has not been assessed. The present study aimed to confirm the efficacy of this predator by assessing the following: developmental time of each growth stage, adult longevity, reproductive potential, fecundity, and other ecological parameters of the predator *A. punica* when offered individuals of *C. multicicatrices* as prey. Such biological information on this noviine coccinellid will be of great importance when assessing the efficacy of this predator in biological control efforts against fluted scale insects and other monophlebids.

## Materials and Methods

*Study location.* The study was conducted between May and December 2014 at the Colombian Corporation for Agricultural Research (Corpoica), Palmira Research Station, located in the municipality of Palmira, Valle del Cauca, Colombia, 03°31′17″N, 76°18′25″W, ca. 1000 m a.s.l. We conducted several biological studies, including diagnoses of the different developmental stages and their duration, life cycle, estimation of life table parameters, and predation rates under laboratory conditions [25.1 ± 1.6°C, with 70.5 ± 7.3% RH, and natural light regime, approx. 12:12 (L:D) h]. Environmental data were measured with a Datalogger (CEM, DT 171, Shenzhen Everbest Machinery Industry Co., Ltd., Shenzhen, China).

*Rearing of Anovia punica.* Sealed plastic containers (33 cm length × 23 cm width × 13 cm height) with two circular holes (10 cm in diameter) on the lid and covered with fine mesh for ventilation were used as rearing chambers. Individuals of *A. punica* and its host, *C. multicicatrices*, were collected from urban trees in the city of Cali, Colombia, where the presence of both prey and predators were observed. Collecting field trips were carried out once a week from May to December 2014. Larvae and pupae of *A. punica* were placed in rearing chambers to monitor the emergence of adults. Emerged adults of *A. punica* were collected and transferred to new rearing chambers, where 60–80 female and male individuals were placed per chamber to allow the oviposition of the female lady beetles. Twigs infested with *C. multicicatrices* were placed within each container to serve as food and oviposition substrate for the beetles. Every week, adults of *A. punica* were transferred to new rearing chambers with fresh prey and new substrate for oviposition, until all adults of *A. punica* had died. Eggs collected from the containers were monitored until the beetles reached the adult stage and these were separated into additional rearing chambers.

*Developmental stages and life cycle duration of A. punica.* In order to determine the development time of the immature stages of *A. punica*, 20 pairs of newly emerged adults were transferred to rearing chambers, which contained twigs of *Caesalpinia pluviosa* var. *peltophoroides* (Fabaceae) infested with second- and third-instar nymphs of *C. multicicatrices*. Due to the similarity between the eggs of *A. punica* and *C. multicicatrices*, no adults of the host iceryine were used as food and/or as an oviposition substrate in order to avoid confusion between the eggs of both insect species. The body surface of each nymph of *C. multicicatrices*, which is covered by white wax, was visually checked for the presence of eggs of *A. punica*. Infested nymphs were transferred to Petri dishes containing moistened filter paper. A cohort of 105 eggs of *A. punica* was used to study development and survival. Eggs, larvae, and adults were monitored daily to determine the survival and length of each developmental stage and the sex ratio of emerging adults. Observations were done under a stereoscope with a camera (Leica EZ4 HD Leica Microsystems, Wetzlar, Germany) to obtain measurements and photographic records that were processed when necessary using the software Adobe ® Photoshop ® (Adobe Systems, Inc., San Jose, CA).

*Fecundity and longevity of A. punica.* To determine the fecundity of *A. punica*, 85 pairs were evaluated by placing them in Petri dishes containing moistened filter paper and a twig of *C. pluviosa* var. *peltophoroides* infested with nymphs of *C. multicicatrices*. Three to eight pairs of *A. punica*, each less than 16 h after emergence, were placed in each Petri dish. Infested twigs were replaced daily, and eggs of *A. punica* were counted from the surface of the hosts on the removed twigs. Oviposition data from *A. punica* were recorded daily until the death of the last female coccinellid. To determine the average lifespan of the predator, daily observations were made by recording whether coccinellids were dead or alive, until the day of the death of the last individual.

*Life table parameters of A. punica.* The life table parameters of *A. punica* were estimated based on the data of fecundity and longevity using the jackknife method according to Maia *et al* (2000). The jackknife technique is a parametric method that reduces the bias in the estimation of the population and generates standard errors for a statistical parameter (Maia *et al* 2000). The life table parameters were calculated as follows: net reproductive rate $R_O = \Sigma l_x m_x$, where $m_x =$ fecundity for female at day $x$ and $l_x =$ female longevity at day $x$; mean generation time $T = \Sigma x l_x m_x / R_O$, where $x =$ time interval; intrinsic rate of increase $r_m = \ln R_O / T$; and doubling time $D_t = \ln 2 / r_m$ (Andrewartha & Birch 1954, Silveira-Neto *et al* 1976, Rabinovich 1978, Southwood 1978, Maia *et al* 2000).

*Predation rate of A. punica.* This study was conducted in order to establish the consumption rate of the larvae and adults of *A. punica* on eggs of *C. multicicatrices* offered as prey. A pretest was carried out to define the number of eggs in the experiment. Two thousand four hundred eggs of *C. multicicatrices* were offered to five adults (1200 eggs) and five larvae (3–4th instar) (1200 eggs) of *A. punica* in the pretest (60 eggs/adult/day + 60 eggs/larva/day), and the numbers of eggs consumed were scored during the 4 days of observations. Both adults and fourth-instar larvae of *A. punica* consumed about the same number of eggs and never more than 20 eggs/day; thus, both stages were supplied with 25 eggs/day in order to provide them with plenty of eggs. Among the different larval stages, fourth-instar larvae consumed the most eggs because of their larger size.

Second-, third-, and fourth-instar larvae and adults of *A. punica* ($n = 12$ for each instar) were placed individually in Petri dishes covered with moistened filter paper and were fed with 25 eggs of *C. multicicatrices*. Information on egg consumption and larval development was daily recorded. Data recording on consumption by the larvae ended when the second- or third-instar larvae started molting or when the fourth-instar larvae reached the prepupal stage, since at this point they ceased feeding. Recently emerged adults of *A. punica* ($n = 12$) also were placed individually in Petri dishes and were supplied with 25 eggs of *C. multicicatrices* per day. Non-consumed eggs by both larvae and adults were discarded and replaced with a new batch of eggs each day. Given the difficulty of assessing the sex of adults of *A. punica*, the sex of individuals in the predation study was not taken into account. Data on predation of first-instar larvae was unavailable due to the high mortality at this stage. First-instar larvae are very small and fragile and were likely injured during manipulation. For the above reasons, the predation study was conducted using second- and older-instar larvae. However, it was noted that predation ranged from 3 to 6 eggs/day in three first-instar individuals that survived, and thus, the overall predation rate is actually higher than herein reported. With the recorded data, we calculated the age-specific predation rate ($k_x$), which is defined as the mean number of prey consumed by *A. punica* at age $x$. Following Chi & Yang (2003) and Yu *et al* (2005, 2013), with information on survival rate, we were able to calculate the age-specific net predation rate ($q_x$) defined as the weighted number of prey consumed by a predator of age $x$ taking into consideration the survival rate of the predator at age $x$, calculated as $q_x = k_x l_x$.

*Statistical analyses.* All the statistical analyses were done with the statistical program SAS (SAS Institute Inc. 2011). We used the post-hoc Tukey–Kramer test to compare the difference among treatments when necessary. Development time is expressed in units per day and changes in the development of the insects are assumed to occur at the midpoint of the

Table 1  Developmental time of *Anovia punica* under laboratory conditions [temp 25.6 ± 1.7°C, HR 67.8 ± 8.1%, and natural light regime, approx. 12:12 (L:D) h].

| Developmental stage | N | Length of developmental stages (days) | | Survival (%) |
|---|---|---|---|---|
| | | Mean ± SEM | Range | |
| Egg | 105 | 5.27 ± 0.67 | 4–6 | 74.3 |
| Larva I | 78 | 3.49 ± 0.77 | 2–5 | 60.0 |
| Larva II | 63 | 2.84 ± 0.85 | 2–5 | 55.2 |
| Larva III | 58 | 2.59 ± 0.65 | 2–5 | 52.4 |
| Larva IV | 55 | 1.92 ± 0.70 | 1–3 | 51.4 |
| Prepupa | 54 | 5.27 ± 1.28 | 4–9 | 49.5 |
| Pupa | 52 | 8.14 ± 1.01 | 6–11 | 47.6 |
| Adult—female | 26 | 94.42 ± 4.03 | 81–124 | 47.6 |
| Adult—male | 24 | 90.00 ± 4.16 | 79–121 | 47.6 |

interval of 24 h. Data on survival for males and females were analyzed using the LIFETEST procedure of SAS, through log-rank test (SAS Institute Inc. 2011).

## Results and Discussion

*Length and diagnoses of the developmental stages of A. punica.* The average duration of the life cycle of *A. punica* from laying of an egg to emergence of an adult was 29.41 ± 1.85 days (Table 1, Fig 1). The developmental stages of *A. punica* were categorized during our study as follows: egg stage, four larval instars, prepupal stage, pupal stage, and adult (Figs 1, 2, 3).

*The egg.* Eggs are elongated and oval in shape (Fig 2A) (0.64 ± 0.04 mm length and 0.32 ± 0.04 mm width, $n = 32$), being larger than the eggs of a closely related species, *A. circumclusa* (Gorham) as described by Forrester *et al* (2009). The eggs have a red coloration, with a granular surface and are covered by waxy exudate as reported for *A. circumclusa* (Forrester *et al* 2009). Prior to hatching, the eggs become whitish in color (Fig 2B), caused by the separation of the chorion from the embryo, forming an air space as reported for eggs of *Scymnus* (*Neopullus*) *sinuanodulus* Yu and Yao (Coleoptera: Coccinellidae) (Lu *et al* 2002). Eggs are oviposited on the body surface of second- and third-instar nymphs and adults of the host, *C. multicicatrices*, or on its ovisac (Fig 2C). In some cases, eggs were found on the surface of the leaves or twigs of the host plant of *C. multicicatrices*. Eggs were oviposited singly and it was common to find 1–3 eggs per individual of *C. multicicatrices*, although in a rare case, an individual with up to 11 eggs on its surface was observed. The eggs of *A. punica* have a very similar appearance to the eggs of *C. multicicatrices* and are very difficult to differentiate with the naked eye.
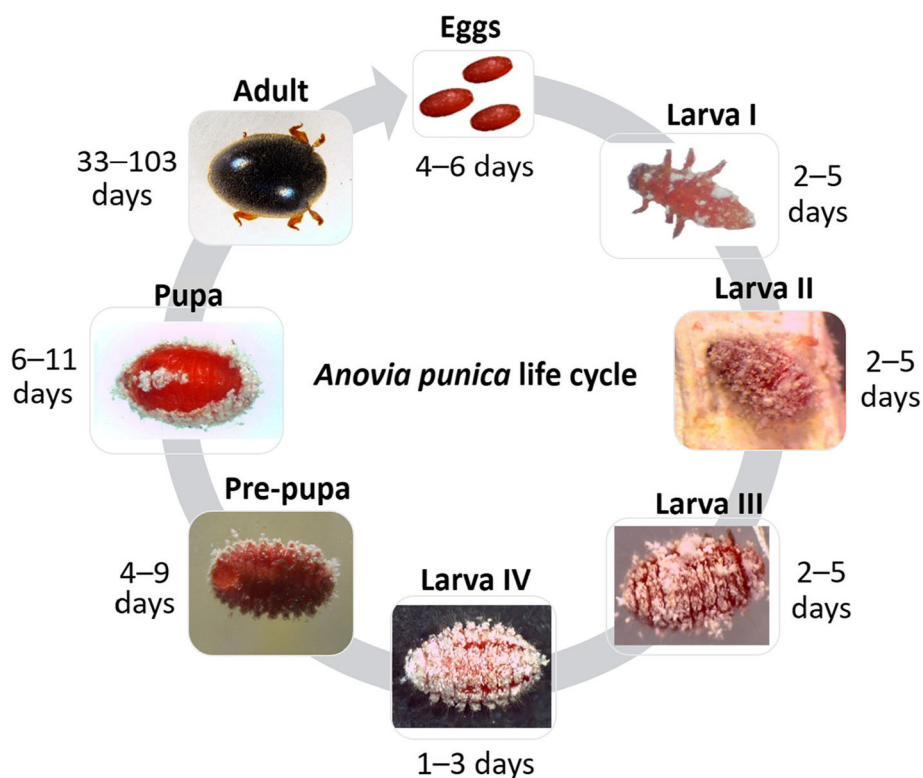


Fig 1  Duration of the developmental stages of *Anovia punica* (initial cohort = 105 individuals). Photos: C. Pinchao.
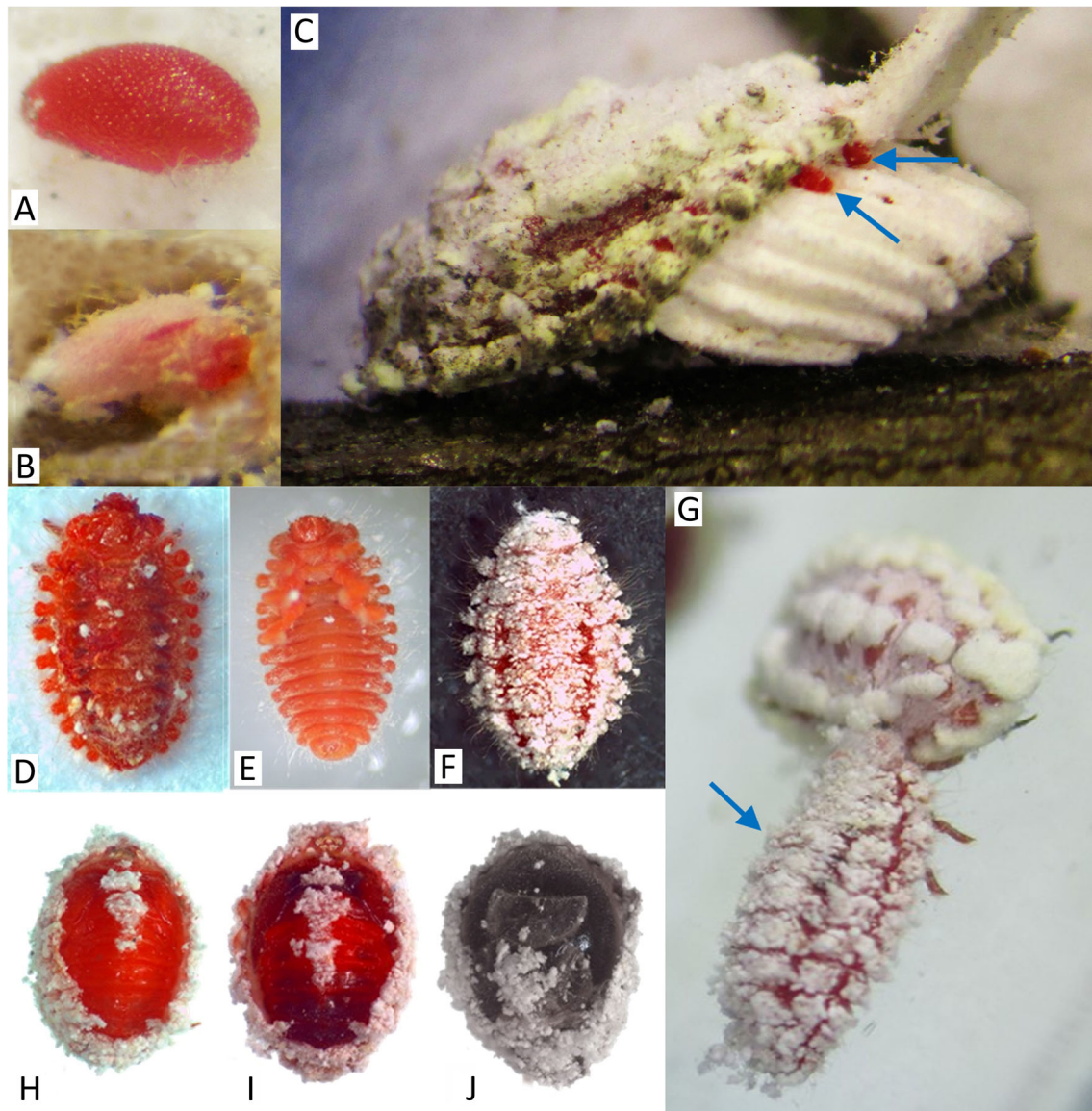
Fig 2 Immature stages of *Anovia punica*. **A** A recently oviposited egg of *Anovia punica*. **B** An egg close to hatching. **C** Eggs of *A. punica* laid between the body margin and ovisac of an adult individual of *Crypticerya multicicatrices* (see arrows). **D** Newly emerged fourth-instar larva, still devoid of wax; dorsal view. **E** Newly emerged fourth-instar larva; ventral view. **F** Fully developed fourth-instar larva with typical white wax; dorsal view. **G** Third-instar larva of *A. punica* (lower left) preying on a *C. multicicatrices* nymph (upper right). **H** Newly formed pupa. **I** Intermediate pupal stage. **J** Late pupal stage, showing same coloration as adults. Photos: C. Pinchao.

*Larvae. Anovia punica* has four larval instars, which do not show marked morphological differences except for size (Table 2, Fig 1). The characteristics of the larval instars are as follows: larvae campodeiform and red in color; body convex and ovoid, widest in the middle part (Fig 2D–G), membranous, with 10 segments (Fig 2E); dorsal surface lightly covered with setae and waxy exudate (Fig 2F, G), which hide most aspects of their external anatomy, as previously reported for *A. circumclusa* (Forrester *et al* 2009). In some species of coccinellids, wax threads are apparently exudates from the base of a spicule, which are used also as a support when wax threads grow upwards (Pope 1979). Wax threads in coccinellids may also have evolved as a physical defense against ant attacks (Pope 1979). In line with this, Majerus *et al* (2007) compiled evidence that suggests that waxy threads in coccinellid larvae may act as a barrier against ants trying to bite them. In addition, the sticky property of the wax can cause the ants to clean their mouthparts and therefore decrease the frequency of attacks (Völkl & Vohland 1996). In the same way, mimicry with their prey will be another adaptive function for avoidance of ant attacks, since the tending ants will ignore or will not be able to distinguish between the hemipterans that they tend and the coccinellid predators (Bach 1991).

Manipulation of larvae (also pupae and adults) causes the release of a red fluid, possibly containing carminic acid. The
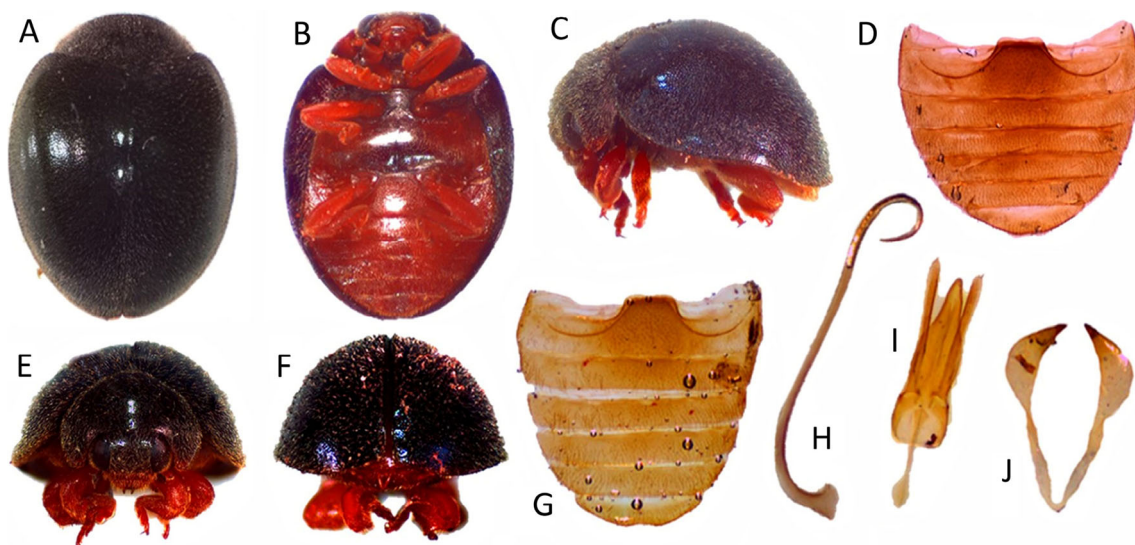
Fig 3 Adult of *Anovia punica*. **A** Dorsal view. **B** Ventral view. **C** Lateral view. **D** Female abdomen. **E** Frontal view. **F** Posterior view. **G** Male abdomen. **H** Male genitalia, sipho. **I** Male genitalia, tegmen. **J** Female genitalia, coxites. Photos: C. Pinchao.

same response has been observed in larvae and adults of *R. cardinalis*, wherein the secreted fluid is used as a chemical defense (Forrester 2008). The secretion of unpleasant fluids, which are composed mainly by alkaloids, histamines, and pyrazines, is a well-studied defense mechanism against vertebrate and invertebrate predators of Coccinellidae (Majerus *et al* 2007). The newly emerged larvae (at each molt) lack the waxy processes and are completely red in color (Fig 2D, E), but after a few hours, they become fully covered with wax.

Larvae of *A. punica* and nymphs of *C. multicicatrices* may be mistaken at first glance due to similar body size and the white wax common to both species (Fig 2G). Larvae and adults of *A. punica* prefer feeding on eggs of *C. multicicatrices*, but may feed on other nymphal stages (Pinchao *et al* 2015). On the other hand, both larvae and adults of *R. cardinalis* feed on eggs and crawlers, and on the underside of young prey (Quezada & DeBach 1973, Pinchao *et al* 2015), with no apparent preference for eggs.

*Prepupa.* After the fourth instar, the beetle larva enters a prepupal stage (Fig 1). At this point, individuals secrete a red exudate to adhere the apex of the abdomen to the

substrate, as has been reported for other species of the tribe Noviini (Vandenberg 2002, Forrester 2008). In addition, individuals in the prepupal stage lack movement and do not feed.

*Pupa.* The exarate pupa (4.17 ± 0.19 mm long and 3.04 ± 0.12 mm wide, *n* = 33) is partially covered by the last larval exuviae at the body margins (Fig 2H–J), as reported by Phuoc & Stehr (1974) for the Noviini tribe. The pupa is sedentary and attaches to the substrate using the exudate previously released in the prepupal stage. In the dark color morph used in the present study, the pupae have an initial red coloration and gradually darken to black (adult color) (Fig 2H–J). Individuals in the pupal stage do not feed.

*Adults.* Adults (Fig 3A–C, E, F) have an average length of 2.95 ± 0.18 mm (range 2.5–3.3 mm) and an average width of 2.47 ± 0.15 mm (range 2.1–2.8 mm) (*n* = 52). Body is oval and hemispherical in shape; head is not visible from above (Fig 3A); semi-decumbent pubescence (Gordon 1972); abdomen with incomplete post-coxal line; with six ventrites, progressively closer; the last ventrite in males with slightly emarginate apex (Fig 3G), apex of females not emarginate (Fig 3D) (Gordon 1972). Basal lobe of male genitalia abruptly narrows at one third of its length from distal end; basal lobe shorter than parameters (Fig 3I) (González & Kondo 2014). The curvature of the tube of the sipho is quite variable, ranging from almost absent (as illustrated in Gordon 1972) to highly curved, forming a letter "C" shape (Fig 3H); however, the curvature of the sipho may be affected by the dissection treatment (González & Kondo 2014). Coxites in *A. punica* females have sclerotized apices that are used to scrape off the wax of its host (e.g., the ovisac of *C. multicicatrices*) in

Table 2 Mean length and width (±SE) for the different larval instars of *Anovia punica*. N = 30 individuals per stage.

| Larval instar | Length (mm) | | Width (mm) | |
|---|---|---|---|---|
| | Mean ± SE | Range | Mean ± SE | Range |
| I | 0.95 ± 0.17 | 0.69–1.25 | 0.38 ± 0.03 | 0.32–0.42 |
| II | 2.54 ± 0.39 | 1.63–2.83 | 1.30 ± 0.21 | 0.88–1.61 |
| III | 3.26 ± 0.27 | 2.93–3.66 | 1.93 ± 0.28 | 1.56–2.40 |
| IV | 4.20 ± 0.19 | 3.88–4.80 | 2.62 ± 0.24 | 2.00–2.97 |

order to aid in the anchoring of oviposited eggs to the surface of the host. This novel function of the coxites has not been reported previously in Coccinellidae.

Adults of *A. punica* are extremely polymorphic, ranging from light brown, dark brown, purple, black opaque, or black with tinges of shiny green or blue; with color patterns composed of reddish or brown areas on black background or black areas on brown or reddish background; these patterns are usually found on the front edge and the lateral third of the pronotum and scutellum, and the lateral edges of the elytra; often forming a band of variable width and length submarginally or marginally on the elytra or sometimes found at the basal area of the elytra near the scutellum (González & Kondo 2014). Specimens used in the present study have a black design with shiny blue elytra.

*Longevity, fecundity, and life table parameters of Anovia punica.* Fifty individuals reached the adult stage, representing 47.6% of the initial cohort (*n* = 105 eggs). The adults lived on average 71.24 ± 22.4 days under laboratory conditions (Table 1). The female to male sex ratio was 1:1. The population of *A. punica* exhibited a type III survival curve with a high mortality rate (about 40%) during the egg and first-larval (larva I) developmental stages. According to Rabinovich (1980), in this model, animal populations with well-defined larval instars often suffer from relatively high mortalities at very young stages of development or in the transitions of one way of life to another. After surviving the first-larval instar, the probability of reaching the adult stage increases. This information is very useful for the implementation of a mass-rearing program for *A. punica*, since improvement of rearing conditions at early developmental stages would lead to a decrease in the mortality of *A. punica* populations.

Longevity among adult males and females of *A. punica* was not significantly different (log-rank $\chi^2$ = 30.3658, df = 1,

$p < 0.5453$) under laboratory conditions. The average survival time for females was 94.42 days [95% confidence interval (CI) 81–124 days, *n* = 26] and the average male survival time was 90 days (95% CI 79–121 days, *n* = 24).

The maximum fecundity was reached during the first month after emergence as adult females (Fig 4). Over the entire lifespan, a female laid an average of 366 ± 114 eggs. During the first 15 days, females had low mortality (10%) and then (up to day 40) had a recorded mortality of 80% (Fig 4).

Life table parameters provide an overall description of the development, survival, and fecundity of a certain population. Within the evaluated parameters, the intrinsic rate of increase is the most important, since it allows comparison of the population growth potential under specific climatic and food conditions (Southwood & Henderson 2000). In this study, the mean value for the intrinsic rate of natural increase ($r_m$) was 0.1115 female/day (95% CI 0.09–0.13), and this value coincides with that of lambda 1.12 (95% CI 1.09–1.13). By comparing this ecological value with that recorded by Sotelo & Kondo (2016) ($r_m$ = 0.05) for *C. multicicatrices*, we can suggest that *A. punica* would potentially act as an efficient biological control agent since there is a twofold increase in the $r_m$ parameter compared to its host. Likewise, the mean value for the net reproductive rate ($R_o$) was estimated as 86.1 (95% CI 63.6–108.6). This means that, on average, each female is replaced by 86 females in one generation under controlled conditions. The value for the mean generation time (*T*) was 39.9 days (95% CI 35.1–44.5) which means that *A. punica* would have an average of 9 generations per year, which is very important in terms of biological control efficacy against *C. multicicatrices*, since this species was recorded to have a total life cycle of 5 months (Sotelo & Kondo 2016). In addition, prey eggs in ovisacs will be available as food resource for *A. punica* for at least 3 months (Sotelo & Kondo 2016). Finally, the mean value for the
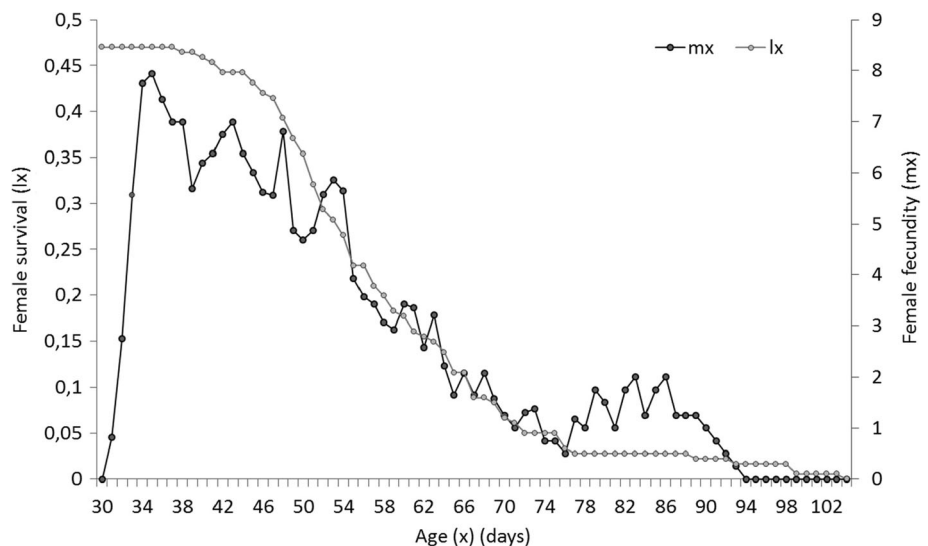


Fig 4 Survival (*l_x*) and fecundity (*m_x*) of adult females of *Anovia punica*. Initial cohort, *n* = 85 females, under laboratory conditions [temp 25.6 ± 1.7°C, HR 67.8 ± 8.1%, and natural light regime, approx. 12:12 (L:D) h].

Table 3   Mean consumption rate ± SE for the different larval instars of *Anovia punica* on eggs of *Crypticerya multicicatrices*. N = 12 individuals per stage.

| Larval instar | Daily egg consumption | | Total egg consumption | |
|---|---|---|---|---|
| | Mean ± SE | Range | Mean ± SE | Range |
| II | 9.1 ± 5.9b | 0–18 | 26.6 ± 6.6b | 14–36 |
| III | 11.4 ± 4.6b | 4–18 | 32.3 ± 7.5b | 17–39 |
| IV | 17.1 ± 2.5a | 11–19 | 42.7 ± 9.6a | 26–56 |

Means followed by different letters in a column are significantly different. Tukey post-hoc test, $F_{2, 35}$ = 24.45; $P < 0.0001$.

doubling time ($D_t$) was 6.18 days (95% CI 5.20–7.16), meaning that the original population will double in number after 1 week.

Life history parameters have been extensively studied for aphidophagous coccinellids, including *Hippodamia variegata* (Goeze) (Farhadi *et al* 2011), *Harmonia dimidiata* (Fabricius) (Yu *et al* 2013), *Scymnus apetzi* Mulsant, *S. subvillosus* (Goeze), *Exochomus nigromaculatus* (Goeze) (Atlihan & Kaydan 2002), and *Lemnia biplagiata* (Swartz) (Yu *et al* 2005). In the case of biological control efforts against scale insects, there are numerous studies on the success of *R. cardinalis* as a biological control agent. More specifically, an extensive review by Van Driesche *et al* (2010) focused on how *R. cardinalis* has been repeatedly and extensively used to control the polyphagous cottony cushion scale, *I. purchasi*, since the coccinellid develops exclusively on monophlebines (Hemiptera: Monophlebidae), principally *Icerya* species (Causton *et al* 2004). However, no information is currently available on the biological control efficacy for any species of *Anovia*. Results of this study will contribute to a better understanding of biological aspects and life history parameters

of *A. punica*. We include information that may be useful in estimating the potential success of *A. punica* when used as a biological control agent, measured in terms of predation.

*Predation rate.* Measurements of daily consumption of eggs of *C. multicicatrices* by the larvae of *A. punica* increased as a result of the size of the larvae. Fourth-instar larvae presented the highest daily consumption average compared to larvae of earlier instars ($F$ = 24.45, $P < 0.001$) (Table 3). Larval predation rate increased significantly from age 9 to 11 days. Second-instar larvae that were molting to the third instar ceased feeding (C. Pinchao, personal observation). However, the same behavior was not observed for third-instar larvae molting to fourth-instar larvae. Third- and fourth-instar larvae showed a significant increase in the predation rate from age 13 to 19 days. In general, this coccinellid has three non-predatory periods, namely, the egg stage, second- to third-instar larval molting period, and the collective prepupal and pupal stages. These non-predatory periods formed three gaps in the predation rate (Fig 5).

Regarding the total eggs consumed by each larval instar, fourth-instar larvae consumed more eggs compared to smaller second- and third-instar larvae ($F$ = 12.26 $P < 0.001$) (Table 3). In addition, adults of *A. punica* consumed an average of 13.6 ± 2.3 eggs of *C. multicicatrices* per day. The first day after an adult's emergence, a consumption of 16.5 ± 2.1 eggs of *C. multicicatrices* was observed. Consumption rate increased gradually until day 5 after emergence and then stabilized at 12–13 eggs per day (Fig 5). During the entire larval period, an individual of *A. punica* consumed 101.5 ± 11.1 eggs of its host, *C. multicicatrices*.

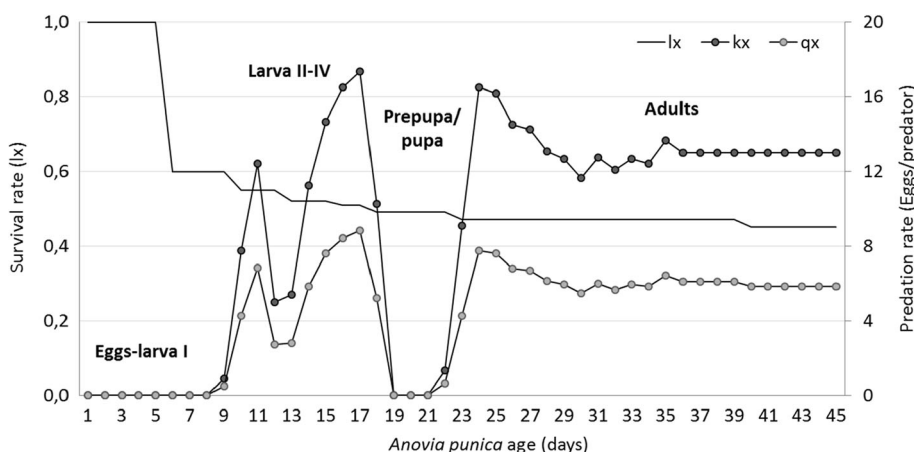Due to a lack of availability of eggs of the host reared under laboratory conditions (i.e., viable and non-parasitized



Fig 5   Age-specific predation rate ($k_x$), age-specific net predation rate ($q_x$), and survival ($l_x$) of several developmental stages of *Anovia punica* on eggs of its host, *Crypticerya multicicatrices*, under laboratory conditions [temp 25.6 ± 1.7°C, HR 67.8 ± 8.1%, and natural light regime, approx. 12:12 (L:D) h]. Age of the predator is counted from birth. ($l_x$) of several developmental stages of *Anovia punica* on eggs of its host, *Crypticerya multicicatrices*, under laboratory conditions [temp 25.6 ± 1.7°C, HR 67.8 ± 8.1%, and natural light regime, approx. 12:12 (L:D) h]. Age of the predator is counted from birth.

eggs), we limited the study to the first 15 days after the emergence of adults of *A. punica*, which prevented us from having an accurate comparison between larval and adult predation rate. By calculating the mean daily predation rate for the interval between age 0 to 45 days (counted from birth), we were able to estimate the net predation rate of *A. punica*. Chi & Yang (2003) calculated the net predation rate ($C_0$) as follows: $C_0 = \Sigma k_x l_x$. In this study, because the data on predation were recorded only to age 45 days, we obtained a partial net predation rate $C_0 = 174.5 \pm 9.3$ eggs of *C. multicicatrices* per individual beetle. However, the studies on development here presented show that individuals of *A. punica* can live close to 100 days (counted from birth); therefore, additional studies are required to estimate a more accurate predation rate. As mentioned before, larvae and adults of *A. punica* use the same food resource, preferring to feed on eggs of *C. multicicatrices*, which would represent a continuous control of the fluted scale. However, it is necessary to consider that *A. punica* may feed on other nymphal stages of *C. multicicatrices* (Pinchao *et al* 2015).

Although population growth parameters obtained here are indicative of the effect of the predator population on the prey population, it is necessary to consider a population dynamics study through the use of more complex models. Thereby, we expect to evaluate the prey population levels according to the predation level in terms of stage specific predation rate and also the specific vulnerability of the prey stage as suggested by several authors (Hassell 1978, Chi and Yang, 2003, Yu *et al* 2005, 2013). Aspects such as food resource availability for prey, predator searching efficiency, predator conversion (i.e., ability to turn food into offspring), and the predator's functional response (i.e., rate of prey capture as a function of prey abundance) also must be considered in future studies for this promising biological control agent.

## Conclusions

In this study, we obtained basic information about the morphology and duration of the developmental stages of *A. punica*, a coccinellid with larvae and adults that are voracious predators of the scale insect *C. multicicatrices*. Basic information provided by the life cycle, predation rate, and life table parameters obtained herein suggests that *A. punica* may be considered as a candidate for use in future control programs as a biological control agent against *C. multicicatrices* and other pestiferous iceryine scales. Further studies are needed to elucidate the predator-prey relationship of *A. punica* and *C. multicicatrices*, by looking at the predator preference at different developmental stages of the prey in order to have a better understanding of the predator-prey dynamics under field conditions.

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