Biology and Life History of Atanycolus cappaerti (Hymenoptera: Braconidae), a North American Larval Parasitoid Attacking the Invasive Emerald Ash Borer (Coleoptera: Buprestidae)

Authors: Duan, Jian J., and Schmude, Jonathan

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Biology and life history of *Atanycolus cappaerti* (Hymenoptera: Braconidae), a North American larval parasitoid attacking the invasive emerald ash borer (Coleoptera: Buprestidae)

Jian J. Duan and Jonathan Schmude*

Abstract

Atanycolus cappaerti Marsh & Strazanac (Hymenoptera: Braconidae) is a native North American parasitoid that has been found parasitizing late instars of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), a serious invasive pest of ash trees (*Fraxinus* species; Oleaceae). In this study, we determined the longevity, weekly oviposition rate, realized lifetime fecundity, diapause pattern, and development time of *A. cappaerti* for late instars of *A. planipennis* larvae at 25 ± 1.5 °C, 65 ± 10% RH, and a 16:8 h L:D photoperiod in the laboratory. Our study showed that adults of *A. cappaerti* had a median survival time of 9 to 11 wk, and females lived approximately 2 wk longer than males. The weekly progeny production by female parasitoids peaked at 3 wk after emergence, with a mean of 5.4 progeny per female. Throughout their life span, females produced a mean (± SE) of 28 (± 3.07) progeny. *Atanycolus cappaerti* larvae molted 5 times to reach the 6th instar in silk cocoons in a median of 8.3 d. Approximately 43% of the 6th instars continued development to adult wasps and the rest diapaused. The median development time from eggs to adults for non-diapaused parasitoids was 20 d. For diapausing *A. cappaerti*, however, the development stopped when larvae entered obligatory diapause in 6th instars. The median time for diapause-terminated larvae to adults was 28 d after being chilled for 2 to 4 mo at 2 to 4 °C. These findings provide critical information on the biology and life history of *A. cappaerti* that is important to the development of a potential mass-rearing protocol for augmentative biocontrol of *A. planipennis.*

Key Words: *Agrilus planipennis*; reproduction; diapause; new association; invasive insect

Resumen

Atanycolus cappaerti Marsh & Strazanac (Hymenoptera: Braconidae) es un parasitoide nativo de América del Norte que se ha encontrado parasitando estadios tardíos de la broca esmeralda del fresno, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), una plaga invasiva grave de los fresnos (*Fraxinus* spp.; Oleaceae). En este estudio, se determinó la longevidad, la tasa de oviposición semanal, la fecundidad total, el patrón de diapausa y el tiempo de desarrollo de *A. cappaerti* en los ultimos estadios de larvas de *A. planipennis* a 25 ± 1,5 °C, 65 ± 10% de humedad relativa, y un fotoperíodo de 16: 8 h L: D en el laboratorio. Nuestro estudio mostró que los adultos de *A. cappaerti* tenían un tiempo mediano de sobrevivencia de 9 a 11 semanas, y las hembras vivieron aproximadamente 2 semanas más que los machos. La producción semanal de progenies de las hembras del parasitoide alcanzó su punto máximo a las 3 semanas después de la emergencia, con un promedio de 5,4 progenie por hembra. A lo largo de su ciclo de vida, las hembras produjeron un promedio de (± SE) de 28 (± 3,07) progenie. Las larvas de *Atanycolus cappaerti* mudaron 5 veces para alcanzar el 6º estadio en capullos de seda en un promedio de 8,3 dias. Aproximadamente el 43% de las larvas del 6º estadio continuaron su desarrollo a avispas adultas y el resto entró en diapausa. El tiempo mediano de desarrollo de huevo hasta el adulto para los parasitoides no en diapausa fue de 20 dias. Sin embargo, para los *A. cappaerti* en diapausa, el desarrollo se detuvo cuando las larvas entraron en diapausa obligatoria en el 6º estadio. El tiempo mediano de larvas que terminaron la diapausa hasta el estadio del adulto fue de 28 dias después de haber sido enfriadas durante 2 a 4 meses a 2 a 4 °C. Estos resultados proveen información crítica sobre la biología y historia de vida de *A. cappaerti* que es importante para el desarrollo de un protocolo potencial de la cría en masa para el control biológico aumentativo de *A. planipennis*.

Palabras Clave: *Agrilus planipennis*; reproducción; diapausa; nueva asociación; insecto invasor

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), accidentally introduced from northeast Asia in the 1990s, is a devastating invasive pest of ash (*Fraxinus* species; Oleaceae) trees in North America (Herms & McCullough 2014). It has now established in 28 U.S. states and 2 provinces in Canada, killing tens of millions of North American ash trees. The cost of treating, or removing and replacing affected ash trees in urban areas has been estimated to be billions of dollars in the U.S. (Sydnor et al. 2007; Kovacs et al. 2010). Although some insecticides containing the active ingredients imidacloprid, dinotefuran, or emamectin benzoate can be used to protect ash trees from *A. planipennis* (Herms et al. 2009; McCullough et al. 2011), classical biological control via the introduction and establishment of natural enemies from the pest's native range appears to be the only viable tool for managing *A. planipennis* populations in natural forests (Bauer et al. 2015).

United States Department of Agriculture, Agricultural Research Service, Beneficial Insects Introduction Research Unit, 501 S. Chapel St, Newark, Delaware 19713, USA; Email: Jian.Duan@ars.usda.gov (J. J. D.), Jonathan.Schmude@ars.usda.gov (J. S.)
*Corresponding author; Email: Jian.Duan@ars.usda.gov (J. J. D.)

Three species of parasitoids were introduced from China in 2007, including 1 egg parasitoid *Oobius agrili* Zhang & Huang (Hymenoptera: Encyrtidae), and 2 larval parasitoids *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Spathius agrili* Yang (Hymenoptera: Braconidae) (Bauer et al. 2015). Currently, *O. agrili* and *T. planipennisi* have established self-sustaining populations and begun to play an important role in suppressing *A. planipennis* populations in some sites of previous releases such as central Michigan in the U.S., where these agents were released between 2007 and 2010 (Duan et al. 2013a, 2015; Abell et al. 2014). In contrast, *S. agrili* is not establishing in most release sites. Most recently, a new larval parasitoid *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae) from the Russian Far East was approved by the U.S. Regulatory Agency for field releases against *A. planipennis* in North America (Federal Register 2015). Large-scale field releases of this new larval parasitoid in the U.S. have started in the summer of 2016 (J. S. & J. J. D., unpublished).

Besides classical biocontrol, however, field studies in the U.S. and Canada show that *A. planipennis* larvae are also parasitized by a complex of native North American species of hymenopteran parasitoids in both the newly infested area (e.g., Maryland, Pennsylvania, Ohio, and Kentucky) and the epicenter of invasion (Michigan and Ontario). These native or resident North American parasitoids include *Atanycolus* spp., *Heterospilus* sp., *Spathius floridanus* Ashmead (as *S. simillimus* Ashmead), *Spathius laflammei* Provancher, *Leluthia astigma* (Ashmead) (Braconidae), and *Phasgonophora sulcata* Westwood (Chalcididae) (Bauer et al. 2004, 2005; Lyons 2008; Cappaert & McCullough 2009; Duan et al. 2009, 2012, 2013b; Kula et al. 2010; Davidson & Rieske 2015). Parasitism rates of *A. planipennis* larvae by these native parasitoids have been generally very low (<5%) in North America except for the complex of braconid parasitoids, *Atanycolus* spp. The *Atanycolus* species complex is consistently observed attacking 9 to 71% of *A. planipennis* larvae in Michigan, the epicenter of the *A. planipennis* invasion (Cappaert & McCullough 2009; Duan et al. 2012, 2015).

This native *Atanycolus* complex includes *A. disputabilis* Cresson, *A. nigropyga* Shenefelt, *A. simplex* Shenefelt, *A. tranquebaraicae* Shanefelt, *A. hicoriae* Shenefelt, and *A. cappaerti* Marsh & Strazanac, with the latter 4 species found in Michigan and the former 2 in Pennsylvania and other states (Cappaert & McCullough 2009; Duan et al. 2012, 2013b; Davidson & Rieske 2015). Among the species complex, *A. cappaerti* is the dominant species (accounting for >93% of the total complex abundance) and has contributed significantly to the reduction of *A. planipennis* population growth in Michigan (Duan et al. 2012, 2015). Like other congener species, *A. cappaerti* has a much longer ovipositor (4.5–5.6 mm) than that of the currently introduced larval parasitoid *T. planipennisi* (1.5–2.5 mm) and thus can parasitize host larvae in larger trees with diameter at breast height (DBH) up to 57.4 cm (Abell et al. 2012).

The new association of *A. cappaerti* with *A. planipennis* therefore may be a great addition to the current classical biocontrol program for protection of large ash trees from *A. planipennis*. In the present study, we determined the adult longevity, weekly oviposition rate, and realized lifetime fecundity of *A. cappaerti* when reared on *A. planipennis* larvae infesting ash sticks under standard rearing conditions (25 ± 1.5 °C, 65 ± 10% RH, and 16:8 h L:D photoperiod) in the laboratory. In addition, we characterized the life cycle of both diapausing and non-diapaused individuals by determining the development time of various stages of *A. cappaerti*. Information from this study will provide insights into the new host association of *A. cappaerti* with *A. planipennis*, and can be useful for developing effective methods to rear this parasitoid for biological studies or potential field releases against *A. planipennis*.

Materials and Methods

PARASITOIDS

Atanycolus cappaerti used for this study were originally collected in the fall of 2012 and 2013 from central Michigan as diapausing mature larvae (in cocoons) from parasitized *A. planipennis* larvae infesting green ash (*Fraxinus pennsylvanica* Marshall) or white ash (*F. americana* L.) trees. These specimens were identified as *A. cappaerti* by using the *Atanycolus* key provided by Paul Marsh (North Newton, Kansas, formerly with United States Department of Agriculture, Agricultural Research Service, Systematic Entomology Laboratory) and confirmed by comparing the *A. cappaerti* voucher specimens collected from the same locations and identified by Paul Marsh.

Throughout the investigation, we utilized only naïve wasps of F0 to F5 generations reared on *A. planipennis* larvae infesting green ash or tropical ash (*Fraxinus uhdei* [Wenzig] Lingels) bolts (1.5–5 cm in diameter, 10–15 cm long). Green ash and greenhouse grown tropical ash were used in previous studies as an effective rearing medium depending on the season when the experiment was carried out (Duan et al. 2011). Before experiments, adult parasitoids were housed in ventilated clear-polystyrene crisper boxes (each 17.6 × 12.6 × 10 cm, State Plastics, Latonia, Kentucky) and maintained in environmental chambers (AR-66L2, Percival Scientific, Perry, Iowa) at 25 ± 1.5 °C, with 65 ± 10% RH and a 16:8 h L:D photoperiod. A water source was provided inside each crisper box (containing approximately 10 female and 10 male parasitoids) via a 10 dram clear plastic vial (US Plastics, Lima, Ohio) fitted with a 10 cm braided cotton dental wick (Richmond Dental, Charlotte, North Carolina) and replaced weekly; pure clover honey was streaked on the ventilation screen of rearing containers as a food source for adult parasitoids. Newly emerged adult parasitoids (<1 wk old), from solitary cocoons dissected from ash, were used for adult longevity, oviposition, lifetime fecundity, and progeny diapause experiments. Adult parasitoids of up to 4 wk of age after emergence were used in the primary parasitoid exposure trials for the larval development study.

HOST LARVAE

All host larvae used in the study were reared in freshly cut green ash or tropical ash bolts according to the method described in Duan et al. (2011, 2013c). Briefly, freshly cut ash bolts (2–3 cm in diameter, 10–25 cm long) were infested with *A. planipennis* eggs laid on coffee filter paper. Egg density per bolt varied with the diameter and length of the stick: 5 eggs for a small bolt (~2 cm in diameter, 10–15 cm long) and 12 eggs for a large bolt (~3 cm in diameter, 20–25 cm long). After infestation with *A. planipennis* eggs, ash bolts were placed in water-soaked floral foam bricks (OASIS®, Smithers-Oasis Company, Hent, Ohio) inside large plastic containers (58.4 × 41.3 × 31.4 cm WLH, Sterilite, Sterilite Corporation, Townsend, Massachusetts), and incubated in an environmental chamber at 27 ± 2 °C, 65 ± 10% RH, and a 16:8 h L:D photoperiod for 4.5 to 5 wk to produce 3rd to 4th instars of *A. planipennis* larvae or for more than 10 wk to produce mature J-shaped (4th instar) larvae for use in different experiments.

ADULT LONGEVITY, OVIPOSITION RATE, FECUNDITY, AND PROGENY DIAPAUSE

All adult parasitoids used for this experiment originated from diapaused larvae in cocoons that had been chilled at 2 to 4 °C for at least 3 mo. Newly emerging *A. cappaerti* adults (<1 wk old) were reared in single pairs (1 female and 1 male per container) on late (3rd to 4th) instars of *A. planipennis* infesting tropical ash sticks. On a weekly basis,

pairs of newly emerging female and male parasitoids (n = 16) were exposed to ash bolts hosted in clear acrylic cylinders (5 cm in diameter × 15 cm in height; Consolidated Plastics, Stow, Ohio), each with 4 screened cuts on the side or lid of the container for ventilation. Pure clover honey was streaked on the screen of containers and maintained throughout the study as a food source for parasitoids. Each parasitoid pair was given 1 or 2 ash bolts containing 4 or more late instars of *A. planipennis* larvae twice a week until the death of the female parasitoid. On a weekly basis, mortality or survivorship of adult parasitoids was recorded.

All parasitoid-exposed ash bolts were immediately placed in fresh containers and incubated at 25 ± 2 °C, 65 ± 10% RH, and a 16:8 h L:D photoperiod for adult wasp emergence. Adult parasitoids normally emerged from parasitized host larvae within 3 to 4 wk after the primary exposure to parental parasitoids. One wk after the last parasitoid emerged, all exposed ash sticks were dissected to determine the fate and stage of each viable host larva (parasitized or not parasitized) as well as parasitoid cocoons and/or larval cadavers. The number of progeny produced weekly for each pair was calculated as the sum of parasitoid adults emerged and diapausing parasitoid larvae for each week, and used as a measurement of weekly oviposition rate by the pair. Non-emerged parasitoid cocoons (with no exit holes) containing mature live larvae were classified as diapausing parasitoid progeny. These diapausing parasitoid larvae could resume development to adults only after were they chilled at 2 to 4 °C for 2 to 4 mo. The number of diapausing (non-emerged) progeny produced by each pair was recorded upon dissection of each exposed ash stick. Throughout the study, we observed a few dead parasitoid cadavers as larvae (n = 12) and excluded these dead parasitoid progeny (~3% of the total progeny) from data analysis.

DEVELOPMENT STAGE AND TIME OF IMMATURE LARVAE

We observed immature stages (eggs, larvae, and cocoons) of *A. cappaerti* and their development time using a similar procedure described for the braconid parasitoid *S. galinae* in Duan et al. (2014). This procedure involved using mature (J-shaped) host larvae artificially inserted into green ash sticks (2 larvae per stick), which were then exposed to gravid female parasitoids to obtain parasitism. The procedure for insertion of host larvae into ash sticks was described in detail by Duan et al. (2014). Briefly, grooves (0.3 mm deep × 5 mm wide × 30 mm long) with bark flaps were first fashioned on each stick with a utility knife and a palm-handled straight groover (Style # 11, 3 mm in size, Woodcraft Supply LLC, Parkersburg, West Virginia). A single host larva was then placed in the groove and covered with the bark flap. All host larvae inside the grooves of sticks were secured with bands of Parafilm at the top and bottom of the flap and exposed to adult parasitoids at a host-to-parasitoid (female) ratio of 1:2 in a crisper box as described previously.

Approximately 24 h after parasitoid exposure, exposed bolts were removed and immediately examined under a dissection microscope for parasitism of host larvae by *A. cappaerti* (i.e., presence of parasitoid eggs). Exposures continued until 20 parasitized host larvae, each with an *A. cappaerti* egg, were obtained from 24 h of the primary parasitoid exposure. After observation of the parasitism of each host larva, ash sticks with parasitized host larvae were placed into 200 mL plastic cups with a moist paper towel and a screened lid and returned to the rearing environmental chamber for daily observations of immature stages by just opening the flap and observing the parasitoid stage under a dissection microscope. To assist in the determination of instars of *A. cappaerti*, we made a small dot on each parasitoid larva before each molt by using a fine-point blue permanent marker (Sharpie, Newell Rubbermaid Office Supplies, Oak Brook, Illinois). When each larva molted to the next stage, the dot was seen on the shed exuvia, or was absent from integument, and the next-stage larva was then marked.

Because of the diapause behavior in mature *A. cappaerti* larvae, our observation of larval development stopped once mature larvae completed formation of cocoons. However, we set up additional parasitoid exposure trials as described previously and determined the development time from eggs to adult emergence of non-diapaused *A. cappaerti*. For diapausing *A. cappaerti*, we chilled the mature larvae in cocoons at 2 to 4 °C for approximately 2 to 4 mo. This period of chilling was effective in terminating the diapause of *A. cappaerti* larvae (J. J. D., unpublished data). Following diapause termination, we then placed the cocoons under the normal rearing conditions described previously and determined their development time from diapaused mature (6th instar) larvae to adult emergence.

DATA ANALYSES

The median survival time (wk from eclosion) and 95% confidence interval (CI) as well as the mean survival time (± SE) of both sexes were estimated with survival analysis based on the Kaplan–Meier survival platform. The realized lifetime fecundity was estimated by the total number of progeny produced by each female parasitoid over her lifetime, and oviposition rates were estimated based on the mean numbers of progeny (± SE) produced per wk by each female parasitoid. The relationship between the probability of progeny entering diapause and the age of their parental parasitoids was analyzed with nominal logistic regression analysis. Median development times (in d) and 95% CI as well as the mean survival time (in d ± SE) for recognized immature stages including eggs, 1st to 5th instars, cocoons (6th instars), and adults were estimated by the same survival analysis procedure described previously. All statistical analyses and calculations were carried out with JMP Pro Version 12.1.0 (SAS Institute 2015).

Results

ADULT LONGEVITY, OVIPOSITION RATE, FECUNDITY, AND PROGENY DIAPAUSE

The median survival time of *A. cappaerti* adults was 9 wk (95% CI = 3–9 wk) for males and 11 wk (95% CI = 8–12 wk) for females (Fig. 1A). The mean (± SE) survival times varied slightly from the medians, with the males living an average of 7.4 wk (± 0.89) and females living an average of 10.5 wk (± 1.09). The median survival time differed between male and female parasitoids (log rank test, $\chi^2 = 5.42$, df = 1, $P = 0.0199$). The oviposition rate peaked at week 3 with 5.4 (± 1.7) progeny produced per parental female (Fig. 1B). Over the entire 19 wk period, 3 female parasitoids failed to produce any progeny, and the rest of the females (n = 13) produced a mean of 28 (± 3.1) progeny over their lifetime with a minimum of 8 and maximum of 42 progeny. The host utilization (parasitism) rate peaked at week 8 with 22% (± 7.1%) and then declined to 0% in week 13 (with spikes in weeks 14 and 17 and a large spike to 57% in week 19 with 1 remaining female parasitoid).

Throughout the entire period (19 wk) of progeny production (n = 352), approximately 43.2% of progeny developed to adult parasitoids, whereas the remaining 56.8% of the progeny diapaused as mature larvae in cocoons. The proportion of diapausing progeny varied with parental age (log likelihood ratio test: $\chi^2 = 5.56$; df = 1; $P = 0.0184$). As they aged, parental parasitoids produced a higher proportion of diapausing progeny (Fig. 2).

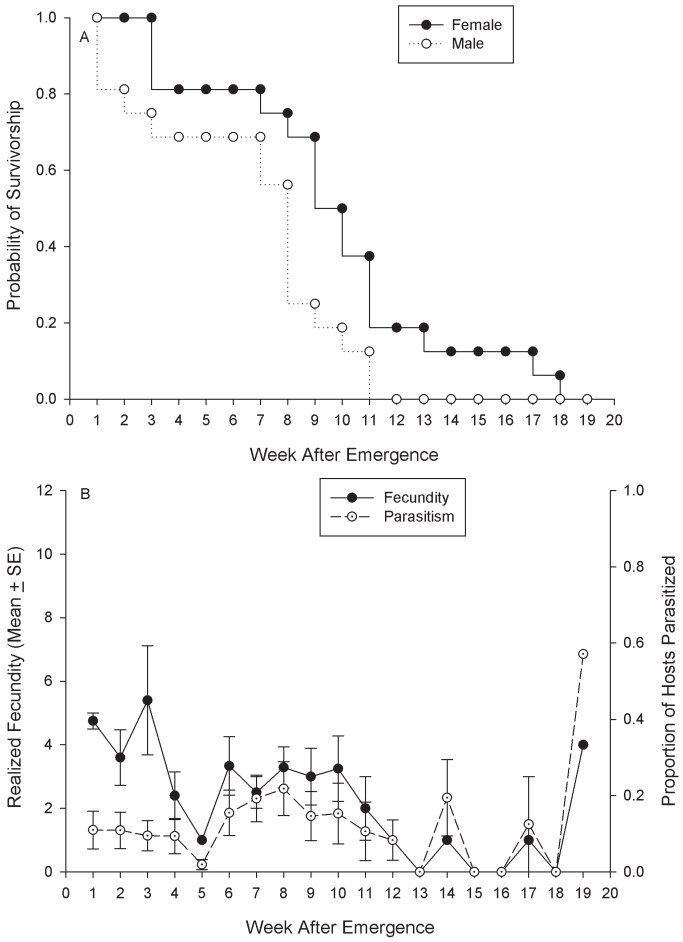

Fig. 1. (A) Longevity and (B) realized fecundity and host utilization (parasitism) rate of adults of *Atanycolus cappaerti* when reared in single mating pairs (n = 16 for both sexes) and continually provided with emerald ash borer larvae on a weekly basis for their lifespan.

DEVELOPMENT TIME OF IMMATURE LARVAE

We observed 6 instars after egg eclosion (Fig. 3A–G) of *A. cappaerti*, with the last (6th) instars forming solitary silk cocoons (Fig. 3H). Different instars of larvae had similar morphology and differed mainly in size as measured with a micrometer; however, the 1st instar had noticeable mandibles and antennal appendages that disappeared from its successive stages. Median and mean development times for each instar or stage can be found in Table 1. Eggs developed fairly quickly with a 50% hatch rate by 1.6 d after the host had been parasitized. The 1st instar molted 5 times into the 6th instar in approximately 4.8 d, and 50% of 6th instars formed solitary cocoons in another 1.9 d (8.3 d after 1st instars had hatched).

Once cocoons were formed, a proportion of the mature larvae continued development to adult parasitoids and the rest diapaused. The median development time from egg to adult for non-diapaused parasitoids was 20 d and differed between female and male parasitoids (log rank test, $\chi^2 = 0.45$, df = 1, $P = 0.5037$; Table 2). Diapausing larvae from separate experiments resumed development to adult parasitoids in 28 d (± 2 d 95% CI) after being chilled for 2 to 4 mo at 2 to 4 °C. There was no difference in the development time of the diapaused larvae to adults between female and male parasitoids (log rank test, $\chi^2 = 1.76$, df = 1, $P = 0.1847$; Table 2).

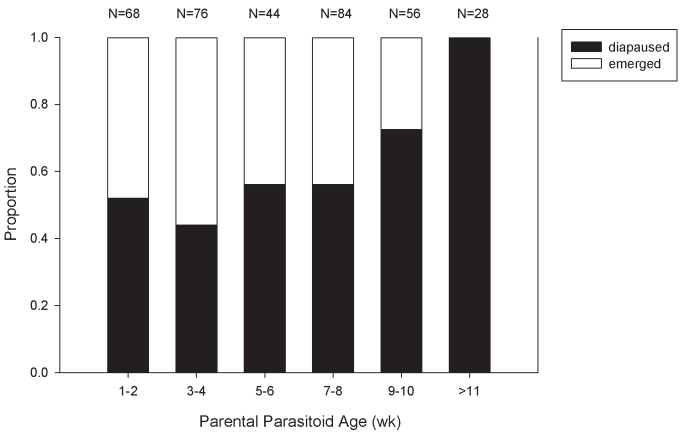

Fig. 2. Diapause behavior of *Atanycolus cappaerti* progeny when reared in normal rearing conditions (25 ± 2 °C, 65 ± 10% RH, and a photoperiod of 16:8 h L:D).

Discussion

Based on an outdoor cage study, Cappaert & McCullough (2009) reported that the mean longevity of *A. cappaerti* was 31.7 d for females and 14.8 d for males in the early season from May to Jun in Michigan. Cappaert & McCullough (2009) also reported that the longevity for late-season females of *A. cappaerti* collected from the field was much shorter, averaging 10.3 ± 1.0 d (range: 1–55). In contrast, the survival time of newly emerging *A. cappaerti* adults in our laboratory study was twice as long, with the median longevity being 11 wk (77 d, same as the mean) for females and 9 wk (63 d, mean = 7.4 wk or 52 d) for males. The large discrepancy in the adult longevity of *A. cappaerti* between our laboratory study and the previous field cage study was likely due to different environmental conditions (e.g., ambient temperature or RH) for maintaining the test insects.

The mean (± SE) realized lifetime fecundity (28 ± 3.1 progeny per female) of *A. cappaerti* from our study appears to be comparable to that of *S. agrili* (averaging 19.7–51.2 progeny per female) and *S. galinae* (31–47 progeny per female), both of which are braconid parasitoids introduced to the U.S. from northeast Asia for biocontrol of *A. planipennis* (Gould et al. 2011; Duan et al. 2014). However, both *S. agrili* and *S. galinae* are gregarious, laying a clutch of 9 to 15 eggs per host larva, whereas *A. cappaerti* is solitary, normally laying 1 egg per host larva. It is likely that even with comparable levels of reproductive potential, *A. cappaerti* females may attack more *A. planipennis* larvae than *S. agrili* or *S. galinae* females in the field. Future studies are needed to assess the potential efficacy in attacking *A. planipennis* and the outcome of interspecific competition between *A. cappaerti* and the introduced biocontrol agents such as *S. agrili* and *S. galinae*. This aspect is particularly important because all 3 braconid parasitoid species attack similar stages of *A. planipennis* larvae (3rd to 4th instars).

Cappaert & McCullough (2009) suggested that the *A. cappaerti* population in the field includes both univoltine and bivoltine individuals. Results from our laboratory study showed that *A. cappaerti* females produced ~57% diapausing individuals under normal rearing conditions. Those diapausing individuals would result in a univoltine population in the field, as they would resume reproduction the next growing season (Jun to Sep) after overwintering (or chilling). However, our laboratory observation showed that non-diapausing individuals of *A. cappaerti* took approximately 20 d to complete a life cycle (from egg to adult) at 25 ± 1.5 °C, with 65 ± 10% RH and a 16:8 h L:D photoperiod, indicating that non-diapausing parasitoids would be able to have multiple (>2) generations during a growing season (Jun to Sep) in the

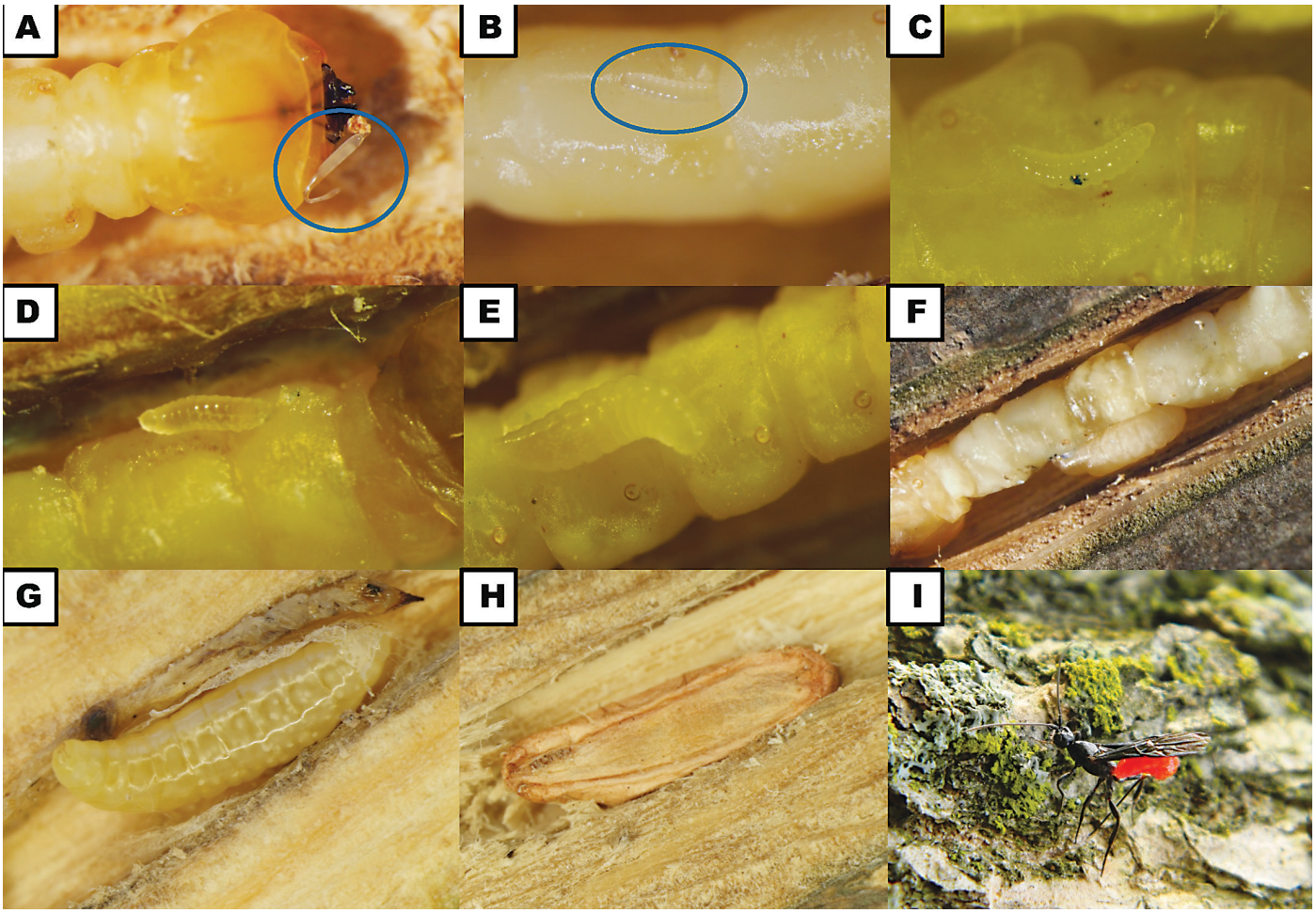

Fig. 3. Immature stages of *Atanycolus cappaerti*. (A) Egg on *Agrilus planipennis* larva at 3× magnification; (B) 1st instar at 3× magnification; (C) 2nd instar at 3× magnification; (D) 3rd instar at 3× magnification; (E) 4th instar at 3× magnification; (F) 5th instar at 1.8× magnification; (G) 6th instar at 1.8× magnification; (H) pupal cocoon at 1.8× magnification; (I) eclosed adult.

Midwest and northeast U.S., where the average ambient temperatures normally are around or above 25 °C in these months. Therefore, the diapause behavior of *A. cappaerti* observed in our study predicts the dichotomy of voltinism in *A. cappaerti* observed under field conditions in the midwestern U.S.

Currently, it is not known if and when non-diapausing *A. cappaerti* adults produce diapausing progeny for overwintering or temporal risk spreading in the field. Results from our laboratory observation showed that diapausing *A. cappaerti* adults (which originated from diapaused larvae after being chilled at 2–4 °C for 2–4 mo) produced an increasing proportion of diapausing progeny even under the favorable laboratory rearing conditions (warm temperature and long-day photoperiod) as they became older. This finding indicates that a proportion of *A.cappaerti* populations in the field may use diapause as temporal risk spreading or bet hedging against unpredictable environmental conditions such as the availability of suitable stages of host larvae. A recent laboratory study has shown that the emerald ash borer egg parasitoids *O. agrili* and *O. primorskyensis* Yao & Duan have similar diapause behavior, which is strongly affected by the parental parasitoid's age and short (8:16 h L:D) photoperiod (Hoban et al. 2016; Larson & Duan 2016). Future studies should determine environmental cues regulating the diapause induction and termination and their impacts on temporal

Table 1. Development stage and time of *Atanycolus cappaerti* larvae with emerald ash borer used as a host under normal rearing conditions (25 ± 1.5 °C, 65 ± 10% RH, and a photoperiod of 16:8 h L:D).

Development stage from egg to	No. of individuals observed (*n*)	Median (mean ± SE) (d)[a]	Lower 95% CI (d)[b]	Upper 95% CI (d)[b]
First instar	20	1.6 (1.8 ± 0.06)	—	—
Second instar	20	2.5 (2.3 ± 0.19)	2.5	3.5
Third instar	19	3.4 (3.3 ± 0.23)	3.5	4.5
Fourth instar	19	4.5 (4.6 ± 0.26)	4.5	5.5
Fifth instar	19	5.6 (5.6 ± 0.26)	5.5	6.5
Sixth instar	17	6.4 (7.5 ± 0.30)	4.5	7.5

[a]Estimated by nonparametric (product-limit) survival model as time of 50% of individuals reaching the target stage.
[b]Lower and upper 95% confidence intervals (CI) estimated by nonparametric survival model.

Table 2. Development time of both non-diapaused and diapausing *Atanycolus cappaerti* parasitizing larvae of emerald ash borer under normal rearing conditions (25 ± 1.5 °C, 65 ± 10% RH, and a photoperiod of 16:8 h L:D).

Parasitoid population	Initial stage	Sex	Number of individuals observed (*n*)	Median (mean ± SE) (d)	Lower 95% CI (d)[b]	Upper 95% CI (d)[b]
Non-diapaused	eggs	male	16	19.0 (18.8 ± 0.64)	18.0	25.0
	eggs	female	11	21.0 (21.3 ± 0.66)	18.0	25.0
	eggs	combined	27	20.0 (20.0 ± 0.65)	18.0	25.0
Diapausing	diapaused larvae	male	25	28.0 (27.0 ± 0.76)	26.0	30.0
	diapaused larvae	female	16	24.5 (26.2 ± 0.82)	20.0	30.0
	diapaused larvae	combined	41	28.0 (26.6 ± 0.81)	24.0	30.0

[a]Estimated by nonparametric (product-limit) survival model as time of 50% of individuals reaching the target stage.
[b]Lower and upper 95% confidence intervals (CI) estimated by nonparametric survival model.

risk spreading and population growth of the emerald ash borer parasitoids. Information from such investigations will be important for the development of effective mass rearing and augmentation programs with *A. cappaerti* for biocontrol of *A. planipennis* in North America.

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