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Research article



# Development and Reproduction of *Brethesiella* cf. *abnormicornis* (Girault) (Hymenoptera: Encyrtidae), a Parasitoid of the Colombian Fluted Scale, *Crypticerya multicicatrices* Kondo & Unruh (Hemiptera: Monophlebidae)

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## Abstract

Studies on the development and reproduction of *Brethesiella* cf. *abnormicornis* (Girault) (Hymenoptera: Encyrtidae), a parasitoid of the Colombian fluted scale, *Crypticerya multicicatrices* Kondo & Unruh (Hemiptera: Monophlebidae), were conducted under laboratory conditions. The duration of the life cycle, adult longevity, fecundity, and population parameters were determined. The parasitoid takes about 29 d to complete a single generation (from egg to adult). In addition, population parameters show that one female wasp is replaced by nearly 32 females, with a female-to-male sex ratio of 2:1. Population parameters estimated in this study can be considered baseline information for a mass-rearing protocol. This is the first detailed study of the biology of a species of *Brethesiella*, where the duration of all larval stages of the parasitoid is determined, being an important contribution for further biological studies of endoparasitoids.

## Resumen

Estudios sobre el desarrollo y reproducción de *Brethesiella* cf. *abnormicornis* (Girault) (Hymenoptera: Encyrtidae), un parasitoide de la escama acanalada de Colombia, *Crypticerya multicicatrices* Kondo y Unruh (Hemiptera: Monophlebidae), se llevaron a cabo bajo condiciones de laboratorio. Se determinó la duración de ciclo de vida, longevidad de adultos, fecundidad y parámetros poblacionales. El parasitoide tardó aproximadamente 29 días para completar una sola generación (de huevo hasta adulto). Además, los parámetros poblacionales mostraron que una avispa hembra es sustituida por cerca de 32 hembras con una proporción entre hembras: machos de 2:1. Los parámetros poblacionales estimados en este estudio pueden considerarse información de base para un protocolo de cría del parasitoide. Este es el primer estudio detallado de la biología de una especie de *Brethesiella*, en el cual se determina la duración de todos los estados larvales del parasitoide, por lo tanto esta investigación se constituye un aporte importante para estudios biológicos posteriores de endoparasitoides.

**Palabras clave**: *Brethesiella* cf. *abnormicornis*, parasitoides, enemigos naturales, control biológico

**Key words**: *Brethesiella* cf. *abnormicornis*, parasitoid, natural enemy, biological control, life history

The Colombian fluted scale, *Crypticerya multicicatrices* Kondo & Unruh (Hemiptera: Monophlebidae), is a polyphagous pest native to mainland Colombia (Kondo and Unruh 2009). This insect invaded San Andres and Providencia islands, Colombia, where it became a polyphagous pest in 2010 (ICA 2010, Kondo et al. 2012). *Crypticerya multicicatrices* have been recorded on a total of 147 different plant species (Kondo et al. 2014). On San Andres Island, the most common hosts of *C. multicicatrices* include essentially all palm species (Arecaceae); breadfruit, *Artocarpus altilis* (Parkinson) Fosberg (Moraceae); *Citrus* spp. (Rutaceae); guava, *Psidium* spp. (Myrtaceae); all leguminous trees and weeds (Fabaceae); *Ficus* spp. (Moraceae); *Mammea americana* (Calophyllaceae); *Melicocca bijuga* (Sapindaceae); and *Spondias* spp. (Anacardiaceae) (Kondo et al. 2012).

Damage is usually more severe in times of drought and host seedlings are particularly susceptible and experience dieback when populations are high (Kondo et al. 2012). In addition, *C. multicicatrices*

can cause cosmetic damage when fruits or other marketable parts of the plant host are affected; and in severe attacks, it can cause defoliation and death of the plant (Kondo et al. 2012). Furthermore, it produces honeydew, a sugary liquid produced by the insect that promotes the development of fungi that cause sooty mold on the host plant surface. The growth of sooty mold on commercial parts of the plant results in a cosmetic damage that reduces the quality of the product, and mold may decrease the photosynthetic rate of affected plants (Kondo et al. 2012). Damage caused by *C. multicicatrices* leads to loss of competitiveness and profitability of the agricultural sector of the islands, diminished quality of life of farmers and loss of self-sufficiency in terms of food security, as well as causes a cosmetic deterioration of the islands (Kondo et al. 2014).

The chemical control strategy use to control insect pests is very limited on the islands of San Andres and Providencia, because these islands were declared part of the Seaflower marine biosphere reserve by UNESCO in 2000 (UNESCO 2011). The reserve is situated in the Archipelago of San Andrés, Providencia, and Santa Catalina, in the southwestern Caribbean, and covers approximately 10% of the Caribbean Sea (Coralina–Invemar 2012). To reduce *C. multicicatrices* outbreaks and lower economic losses, there is an important need to implement a classical biological control strategy.

Classical biological control has proven effective against invasive species of scale insects, with parasitoids playing an important role in controlling invasive pest species (Noyes and Hayat 1994). Many natural enemies of *C. multicicatrices* have been reported in the literature, namely, *Delphastus quinculus* Gordon, *Diomus seminulus* (Mulsant) (González et al. 2012.), *Anovia punica* Gordon (González and Kondo 2014; Kondo et al. 2014, as *Anovia* sp.), *Rodolia cardinalis* (Mulsant) (Pinchao et al. 2015) (Coleoptera: Coccinellidae), *Syneura cocciphila* (Coquillett) (Gaimari et al. 2012) (Diptera: Phoridae), two species of unidentified parasitoid wasps (Hymenoptera: Encyrtidae) (Kondo et al. 2012), *Chrysoperla* sp. and *Ceraeochrysa* sp. (Neuroptera: Chrysopidae) (Kondo et al. 2014), and *Isaria* sp. (Eurotiales: Trichocomaceae) (Kondo et al. 2012, 2014; Silva-Gómez et al. 2012).

Worldwide, parasitoid species belonging to the Encyrtidae (Hymenoptera: Chalcidoidea) are of great economic value due to their important contribution as biological control agents (Grissell and Schauff 1990, Noyes and Hayat 1994, Trjapitzin et al. 2008a). Currently, Encyrtidae is composed of 3,735 described species grouped in 460 genera (Noyes 2014). Some successful biological control programs involving the introduction of encyrtid parasitoids include the control of the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), by *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) in Africa (Neuenschwander 2001); the control of the mango mealybug, *Rastrococcus invadens* Williams (Hemiptera: Pseudococcidae), by *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae) in Africa (Boavida and Neuenschwander 1995); the control of the Rhodesgrass mealybug, *Antonina graminis* (Maskell) (Hemiptera: Pseudococcidae), by *Neodusmetia sangwani* (Subba Rao) (Hymenoptera: Encyrtidae) in the USA (Dean et al. 1979); and the parasitoids *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae) and *Gyranusoidea indica* Shafee, Alam & Agarwal (Hymenoptera: Encyrtidae) to control the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), in the Caribbean (Kairo et al. 2000, Meyerdirk 2000, Noyes 2000, Evans et al. 2012). Furthermore, in neotropical countries, approximately 40 encyrtid species have been introduced in classical biological control programs (Fernandez and Sharkey 2006).

There are two parasitoids of *Crypticerya brasiliensis* (Hempel) (a closely related species of *C. multicicatrices*), namely, *Brethesiella abnormicornis* Girault (Girault 1917) and *Brethesiella longipes* Blanchard (Blanchard 1940) (Hymenoptera: Encyrtidae). *Brethesiella longipes* was reported in Argentina (Blanchard 1940, De Santis 1964) and *B. abnormicornis* was reported in Barbados (De Santis and Fidalgo 1994, Noyes and Hayat 1994, Trjapitzin et al. 2004), Bermuda (De Santis and Fidalgo 1994), Brazil (Sao Paulo) (Girault 1917, De Santis 1980), Saint Tome and Principe (Noyes and Hayat 1994), Trinidad and Tobago (Trjapitzin and Triapitsyn 2006), and Uruguay (Herting 1972, De Santis 1979). In mainland Colombia, at least two hymenopteran parasitoids have been reared from *C. multicicatrices* adults (Kondo et al. 2012). Of the two species, one is considered to be the true parasitoid of *C. multicicatrices* and was tentatively identified as a species of *Brethesiella* (Hymenoptera: Encyrtidae) based on photographic records; the other species is likely a hyperparasitoid, identified as *Cheiloneurus* sp. by Dr. James Woolley (Texas A&M) (Kondo et al. 2012). The species of *Brethesiella* in this study was recently identified as *Brethesiella* cf. *abnormicornis* (Girault) by Dr. John Noyes (Natural History Museum, London, UK). Kondo et al. (2012) suggested that due to the known geographic range of the parasitoid, it was possible that *B. abnormicornis* may occur in Colombia as a parasitoid of *C. multicicatrices*. Field observations show that *Brethesiella* cf. *abnormicornis* (as *Brethesiella* sp.) is a parasitoid of nymphs and young adults of *C. multicicatrices*, and is considered the primary parasitoid of this pest in mainland Colombia (Kondo et al. 2012). Here we report the life cycle as well as adult longevity, reproductive potential, and lifetime fecundity of the parasitoid *B. cf. abnormicornis* using *C. multicicatrices* as the host.

## Materials and Methods

### Study Location

The study was conducted 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.

### Insect Sampling

#### Host Rearing

*Crypticerya multicicatrices* used in all studies were collected from urban trees found in the city of Palmira, Colombia, where natural infestations of *C. multicicatrices* were observed. Plant material was visually inspected for the presence of *C. multicicatrices* and collected from February to November 2014, with field trips carried out once a week. Host plants of *C. multicicatrices* were mainly *Caesalpinia pluviosa* var. *peltophoroides* (Benth.) G.P. Lewis, *Pithecellobium dulce* (Roxb.) Benth., *Pithecellobium saman* (Jacq.) Benth. (Fabaceae), *Washingtonia* sp. (Arecaceae), *Mangifera indica* L. (Anacardiaceae), *Dypsis lutescens* (H. Wendl.) Beentje & J. Dransf. (Arecaceae), *Psidium guajava* L. (Myrtaceae), *Syzygium jambos* (L.) Alston (Myrtaceae), and *Lagerstroemia speciosa* (L.) Pers. (Lythraceae). *Crypticerya multicicatrices*-infested twigs were cut, placed in large-sized petri dishes (14.5 cm in diameter), and taken to the laboratory for examination. Samples collected in the field had individuals from all growth stages of *C. multicicatrices* as well as associated parasitoids and predators. From the collected samples, only first-instar nymphs (crawlers) of *C. multicicatrices* were selected and bred to stages that were needed in the different studies because this stage is not parasitized by the encyrtid. *Crypticerya multicicatrices* were reared

under laboratory conditions (25.9 ± 1.7°C, with 67.8 ± 8.1% RH, and natural light regimen, approximately 12:12 [L:D] h), where data were measured with a Datalogger (CEM, DT 171, Shenzhen Everbest Machinery Industry Co., Ltd, Shenzhen, China).

#### Voucher Specimens Sent for Identification

Colombia, Valle del Cauca, Palmira, 03° 32′40″ N, 76° 18′53″ W, 1015 m a.s.l., 19.ix.2013, reared from *Crypticerya multicicatrices* on twigs and leaves of *Caesalpinia peltophoroides*, voucher No. 443, three specimens in alcohol, deposited at the Natural History Museum, London (BMNH). Other reared specimens used in the experiment are preserved in 70% alcohol and deposited at the Museo de Entomología, Corporación Colombiana de Investigación Agropecuaria, Centro de Investigación Palmira, Palmira, Valle del Cauca, Colombia (MECP).

#### Parasitoid Rearing Experiment

*Crypticerya multicicatrices* individuals belonging to the second, third, and fourth stage of development were maintained under laboratory conditions (same as earlier) in sealed plastic containers (35 by 25 by 15 cm) with two circular holes (10 cm in diameter) on the lid, which were covered with a fine mesh for ventilation. The parasitoids collected were reared under laboratory conditions (same as earlier and fed with 50% diluted honey). Parasitoids then were used for different studies including the determination of the length of all developmental stages, oviposition preference on different developmental stages of its host, reproduction type, longevity, and fecundity.

### Biological Studies on *Brethesiella* cf. *abnormicornis*

#### Length of Developmental Stages of *Brethesiella* cf. *abnormicornis*

The method used for the biological studies was based largely on the methods described by Rojas (2010) and Daane et al. (2005), with some modifications. To determine the duration of the development time of the immature stages of the parasitoid, six yellow acacia *Caesalpinia pluviosa* var. *peltophoroides* seedlings were selected. Each plant was covered with a cylindrical cage made of 12 gauge acetate (80 cm in diameter by 50 cm in height with two side holes 15 cm in diameter covered with fine mesh). The top of the cage was covered with a veil to fit the cylinder. Each plant was infested with 100 second-instar nymphs of the host *C. multicicatrices*. In each cage, 35 females and 15 males of the parasitoids *B.* cf. *abnormicornis* were released to parasitize the host for 48 h, after which the parasitoids were removed from the cages. Every day, 20 nymphs of *C. multicicatrices* were dissected to monitor and identify the stages of the parasitoid and measure the duration of the different stages of development.

#### Type of Reproduction

The type of reproduction for *B.* cf. *abnormicornis* was determined according to the method proposed by Edwards and Hoy (1998), with some modifications. Six yellow acacia seedlings were selected and covered with a cylindrical cage made of acetate as described earlier. Each plant was infested with 50 second-instar nymphs of *C. multicicatrices*. Three of the six plants were exposed to 10 virgin *B.* cf. *abnormicornis* females and the other three plants were exposed to 10 pairs (male and female) of *B.* cf. *abnormicornis* adults for 48 h. Emerged adults were counted and sexed and the percentage of emerged females and males was determined.

#### Oviposition Preference on Different Developmental Stages of the Host

Oviposition preference of *B.* cf. *abnormicornis* was evaluated according to the method proposed by Edwards and Hoy (1998), with some modifications. Three yellow acacia seedlings were caged inside cylindrical containers made of acetate as described earlier. These plants were infested with 30 first-instar nymphs (crawlers), 30 second-instar nymphs, 30 third-instar nymphs, 30 young adults, and 30 mature adults (those with an ovisac) with the insect host, *C. multicicatrices*. These insects were exposed to 12 females and 6 males of *B.* cf. *abnormicornis* parasitoids for a period of 24 h. The parasitoids were removed from the cage after that time. A week later, individuals of *C. multicicatrices* were dissected and the percentage of parasitism of each stage of development of the host was determined.

#### Longevity and Fecundity

*Brethesiella* cf. *abnormicornis* longevity was evaluated according to the method proposed by Edwards and Hoy (1998), with some modifications. Thirty females and 30 males of the parasitoid were randomly chosen from rearing cages less than 16 h after they had emerged as adults. Parasitoids were individually placed in 35-ml glass vials covered with fine mesh for ventilation. Adult parasitoid wasps were fed with 50% diluted honey. The honey was changed every 24 h and the vials were replaced once a week to avoid contamination. To determine the average life span of the parasitoid wasps, daily observations were made by recording whether wasps were alive or dead, until the day of the death of the last individual.

To determine the fecundity of *B.* cf. *abnormicornis*, 30 pairs of parasitoid wasps were evaluated by placing six parasitoid couples in a container (35 by 25 by 15 cm), with five repetitions (five containers in total). The experiment began with adult individuals of 12–16 h post-emergence. The plastic containers had two circular holes (10 cm in diameter) on the lid, which were covered with a fine mesh for ventilation, and inside of the container, 50% diluted honey was provided as food and for hydration. In addition, wasps were provided with small twigs of Saman tree, *P. saman*, infested with approximately 50 second-instar nymphs of *C. multicicatrices*. A freshly cut twig infested with *C. multicicatrices* was provided every day until the day of the death of the last individual. After each exposure, the nymphs of *C. multicicatrices* were placed in 70% alcohol, and then dissections were performed to determine the presence of parasitoid eggs.

#### Statistical Analysis

Data were transformed to meet the assumptions of normality and heteroscedasticity when necessary. The number of eggs from the different host developmental stages (factor) were log10 $(x+1)$ transformed and compared using a one-way analysis of variance; post hoc testing was conducted using Tukey's HSD test ($\alpha = 0.05$). The survivorship of female and male parasitoids was analyzed using a product-limit survival estimate based on the Kaplan–Meier survival platform (SAS Institute 2012). Median survival time in days and 95% confidence intervals (CIs) were also estimated for both sexes using the survival analysis procedure. Independent-sample *t*-test was performed on adult wasp body characteristics to obtain size differences between female and male wasps. Population parameters were calculated based on fecundity and longevity data by using a SAS platform to estimate associated life-table parameters (Maia et al. 2000). The parameters were calculated using the equations formulated by Andrewartha and Birch (1954), Southwood (1978), and Maia et al. (2000): net reproductive rate ($R_o$) = $\Sigma$

$m_x \times l_x$, where $m_x$ = fecundity for female at day x and $l_x$ = female longevity at day x; mean generation time (T) = $\Sigma$ $x \times l_x \times m_x$/Ro, where x = time interval; intrinsic rate of natural increase ($r_m$) = log (Ro)/T; finite rate of increase ($\lambda$) = Antilog $r_m$; and doubling time (Dt) = $l_n$ (2)/$r_m$. It is not possible to work with variances to perform the statistical analysis of population parameters (synthetic) because these parameters (Ro, $r_m$, T, DT, and $\lambda$) compile the information from the development time of immature stages, reproduction, and survival as a single value. In that case, the variances can be calculated by Monte Carlo methods such as randomization tests, jackknife, and bootstrap (Maia et al. 2000). Our particular interest is the jackknife technique, as it is a parametric method that reduces the bias in the estimate of the population for a statistical parameter and generates a standard error for the same (Maia et al. 2000). Statistical analysis and calculations were conducted using SAS 9.3 (SAS Institute, 2012).

## Results

### Length of *Brethesiella* cf. *abnormicornis* Developmental Stages

Average duration of *B.* cf. *abnormicornis* life cycle was 32.94 ± 1.23 d (range = 31–35 d, *n* = 78; Fig. 1). Several distinct developmental stages of *B.* cf. *abnormicornis* were categorized during our investigation as follows: egg stage, four larval stages, pupal stage, and adult.

#### The Egg

Eggs encyrtiform, translucent, with two bodies connected by a narrow tube and with the presence of an aeroscopic plate as previously reported for encyrtid wasps (Maple 1947, Saakian-Baranova 1968, Trjapitzin et al. 2008b). The eggs were oviposited within the host body and were found mostly in the dorsal side of the posterior margin of nymphs and adults of *C. multicicatrices*.

#### First-Instar Larva

Larva encyrtiform, legless, with soft, smooth surface, and bright yellow body, found between the cuticle and fat bodies of its host. Body length 0.53 ± 0.12 mm (range = 0.36–0.84, *n* = 60).

#### Second-Instar Larva

Larva encyrtiform, legless, with soft and slightly segmented body, with whitish, creamy, or slightly pinkish coloration. Body length 0.84 ± 0.26 mm (range = 0.34–1.43, *n* = 60).

#### Third-Instar Larva

Larva well-developed, encyrtiform, legless, body 12-segmented, of a soft consistency, with bright red coloration. Body length 1.98 ± 0.48 mm (range = 0.62–3.18 mm, *n* = 60).

#### Fourth-Instar Larva

Larva well-developed encyrtiform, 12-segmented, legless, of a soft consistency, with cream-yellow or slightly pinkish coloration. Head located inside the first body segment. Body length 1.58 ± 0.28 mm (range = 0.86–2.21 mm, *n* = 60).

The larvae of the early stages of *B.* cf. *abnormicornis* were of the encyrtiform type, found within the content of the host liquid, which is consistent with that described by Trjapitzin et al. (2008a, 2008b). In addition, *B.* cf. *abnormicornis* larvae have 12 segments, which

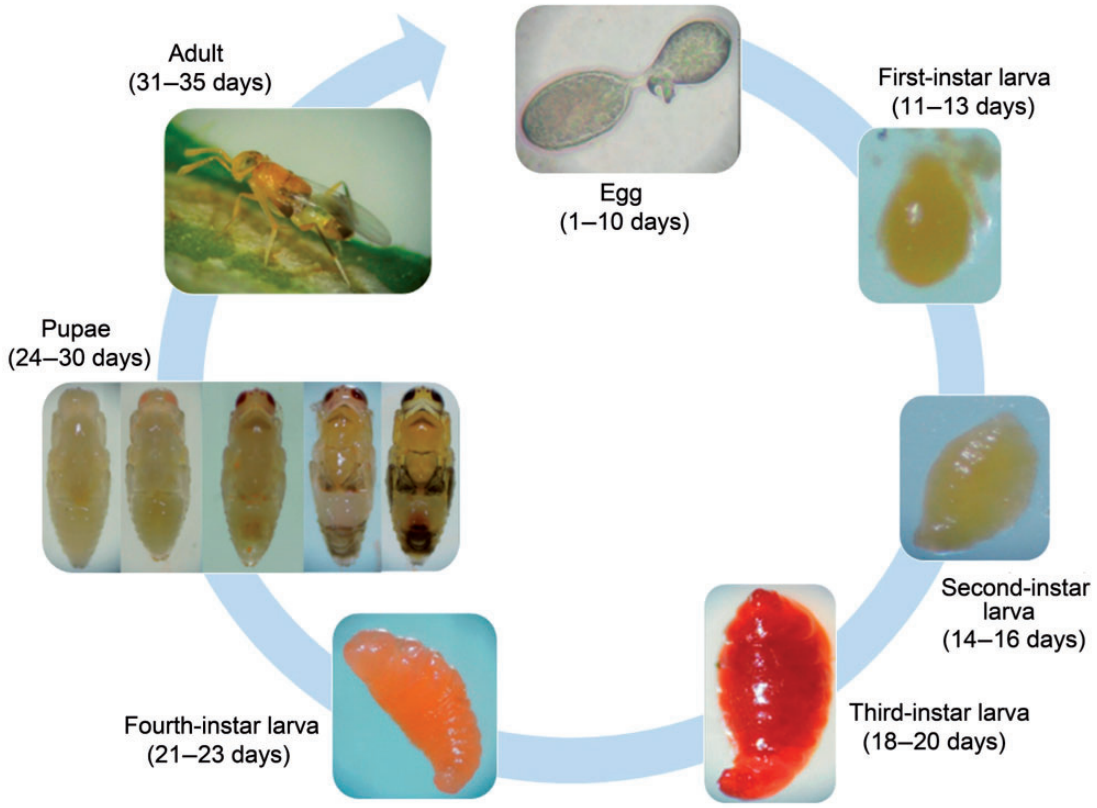


**Fig. 1.** Duration of developmental stages of *Brethesiella* cf. *abnormicornis* (*n* = 78 individuals).

**Table 1.** Mean ± SEM size differences between *Brethesiella* cf. *abnormicornis* females versus males

| Dimensions | Female | Male | *t*-test | *P* |
|---|---|---|---|---|
| Body length | 1.65 ± 0.18 | 1.37 ± 1.15 | 7.54 | 0.0001 |
| Head width | 0.43 ± 0.04 | 0.39 ± 0.04 | 3.87 | 0.0003 |
| Wing length | 1.56 ± 0.10 | 1.43 ± 0.14 | 4.31 | 0.0001 |
| Wing width | 0.56 ± 0.04 | 0.61 ± 0.09 | 2.74 | 0.0009 |

$n = 30$ individuals per gender.

differs from that described for *Microterys nietneri* (Motschulsky) (Encyrtidae), which has 11 segments (Trjapitzin et al. 2008b).

#### Pupa

The pupa was formed within a cell. The newly formed pupa had a bright creamy-white color, with a soft consistency. After about 3 or 4 d, the eyes and ocelli turned to a red-brown color. Most external morphological characteristics of the pupa agreed with those reported by Doutt (1947) in *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidae). Two to three days later, the pupae showed a yellow coloration, ranging from a pale yellow to a strong yellow coloration. The average time for pupation was 6–7 d. The average body length of the pupae was 1.47 ± 0.24 mm (range = 0.92–2.12, $n = 140$), while the average head width was 0.39 ± 0 08 mm (range = 0.22–0.92, $n = 140$). The morphological changes and development time from the larval to pupal stage in *B.* cf. *abnormicornis* were similar to previously reported studies for other encyrtids (Doutt 1947; Avila and Redolfi 1990; Trjapitzin et al. 2008a, b), where changes in color and the determination and definition of structures coincide.

#### Adults

*Brethesiella* cf. *abnormicornis* adults have well-defined sexual dimorphism (Table 1). The shape of the antenna was used to distinguish gender. In addition, females have longer wings and bodies and broad heads relative to males, while males have wider wings (Table 1). In general, *B.* cf. *abnormicornis* is characterized by an elongated body, dark brown-black compound eyes, hyaline wings, the ocelli form an equilateral triangle, and the tibiae of the mesothoracic legs have an extended spur (Fig. 2G). Adult females have a slightly stronger shiny yellow color, whereas males are almost white pale yellow (Fig. 2A and D) in the ventral area; generally, coloration in females is darker than in males (Fig. 2B and E). The female antennae are characterized by a distinct three-segmented antennal club that is obliquely truncate, funicle five-segmented (Fig. 2C), while the male antennae are filiform (Fig. 2F).

### Type of Reproduction

*Brethesiella* cf. *abnormicornis* virgin females give birth to males (arrhenotoky), whereas eggs need to be fertilized for the production of females, which is consistent with other studies of hymenopteran parasitoids (Moreno 1982, Avila and Redolfi 1990).

### Oviposition Preference of *Brethesiella* cf. *abnormicornis* on Different Developmental Stages of *C. multicicatrices*

*Brethesiella* cf. *abnormicornis* female wasps had a clear oviposition preference on three developmental stages of the host ($F_{4, 149} = 16.86$, $P < .0001$; Fig. 3). Forty-one percent of the total eggs laid by the females were found on third-instar nymphs, 39% were found on second-instar nymphs, and 20% on young adults of *C. multicicatrices* individuals (individuals without an ovisac). No eggs were found on first-instar nymphs (crawlers) or mature adults.

### Longevity and Fecundity

Life span in females and males of the parasitoid was significantly different (log-rank $\chi^2 = 37.76$ df = 1, $P < .0001$) under laboratory conditions when fed 50% diluted honey. The median survival time was 20 d for females (95% CI 18–22 d), with a maximum of 31 d ($n = 30$), while the median survival time for males was 10.5 d (95% CI 9–13 d), with a maximum of 16 d ($n = 30$) (Fig. 4).

Over the entire life span, each reproductive female *B.* cf. *abnormicornis* ($n = 30$) produced a mean of 54 (± 3.44 SEM) progeny, with a maximum of 77 and a minimum of 14 progeny. In total, five females (17%) failed to produce progeny and were not included in the fecundity calculation. *Brethesiella* cf. *abnormicornis* females showed a reproductive peak in the first 5 d after adult emergence, with 61% of progeny being produced during this period (Fig. 5). This result may suggest that *B.* cf. *abnormicornis* females are proovigenic. The sex ratio of all progenies produced during the course of the study was approximately 2:1 female to male.

The mean value for the net reproductive rate ($R_0$) was estimated as 32.4 (95% CI: 28.93–35.87; female daughters replacing mothers in one generation under controlled conditions); the mean value for the intrinsic rate of natural increase ($r_m$) was 0.118 female/day (95% CI: 0.117–0.12); the mean value for the mean generation time (T) was 29.37 d (95% CI: 28.66–30.08); the mean value for the doubling time (Dt) was 5.86 d (95% CI: 5.76–5.94); and the mean value for the finite rate of increase (λ) was 1.125 female/day (95% CI: 1.123–1.128).

A second analysis of the raw data was carried out to evaluate the importance of older parasitoid females. In this analysis, the complete life span of the parasitoid was compared with the first 10 d of data (shortened) for the females (Table 2). Results of this comparison were interesting in the sense that only two ecological parameters showed significant differences between the complete and short life span of the parasitoids, the net reproductive rate ($R_0$) and the generation time. However, no differences were observed for the rates of increase and the doubling time.

## Discussion

Results from our study show that *B.* cf. *abnormicornis* takes close to one month to complete a single generation (from egg to adult; generation time T = 29.37 d) under laboratory conditions (25.9 ± 1.7°C, with 67.8 ± 8.1% RH, and natural light regimen, approximately 12:12 [L:D] h). Given that the annual temperature range in the tropics varies little throughout the year, with changes only observed in terms of precipitation (i.e., dry and wet season), we suggest that *B.* cf. *abnormicornis* likely has multiple generations per year. In addition, population parameters show that one *B.* cf. *abnormicornis* female is replaced by nearly 32 females (Ro = 32.4 daughters/mother), with a female-to-male sex ratio of 2:1 over a life span. In line with this, several species of parasitoids have shown that young females are more fertile than older females (Riddick 2003, Escudero 2011). It is the same case for female *B.* cf. *abnormicornis*, where it was observed that the daily oviposition rate of females was greater during the first 7 d and then decreases to zero. This information is confirmed by the doubling time (Dt) obtained with a value of 5.86 d, meaning that a parasitoid generation will double in numbers after only 6 d. Furthermore, studies carried out on *Trichogrammatoidea bactrae* Nagaraja (Riquelme and Botto 2010), *Muscidifurax* sp., and

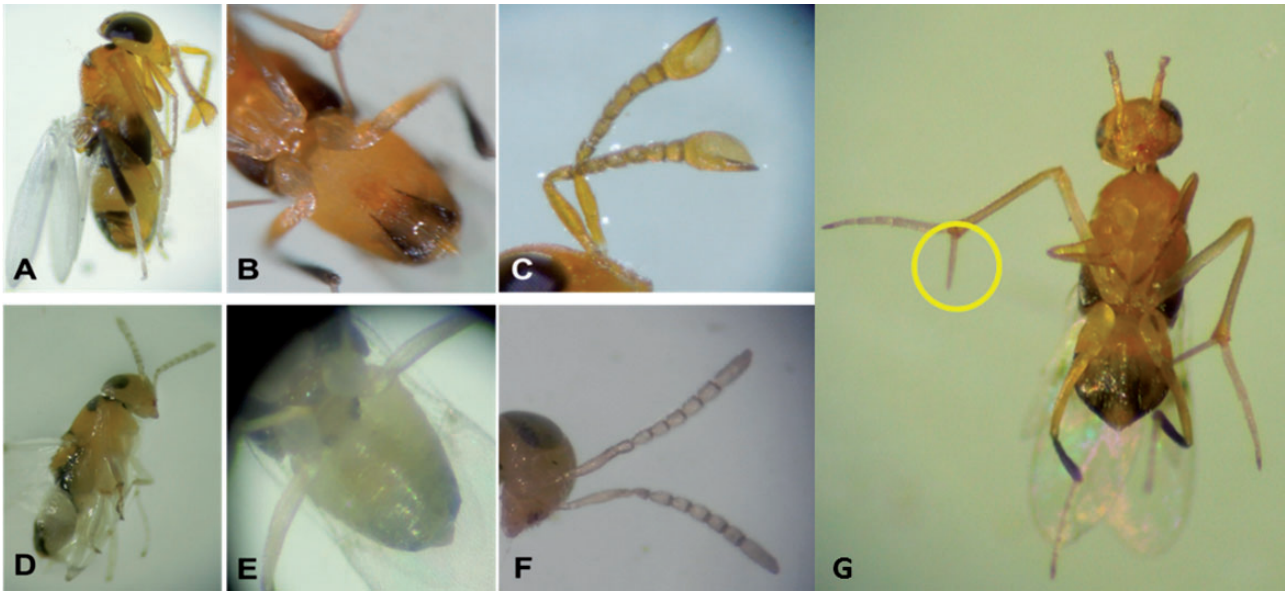


**Fig. 2.** *Brethesiella* cf. *abnormicornis* adults. **A.** Female. **B.** Darker female abdomen. **C.** Clavate female antenna. **D.** Male. **E.** Translucent male abdomen. **F.** Filiform male antennae. **G.** Elongate midtibial spur.

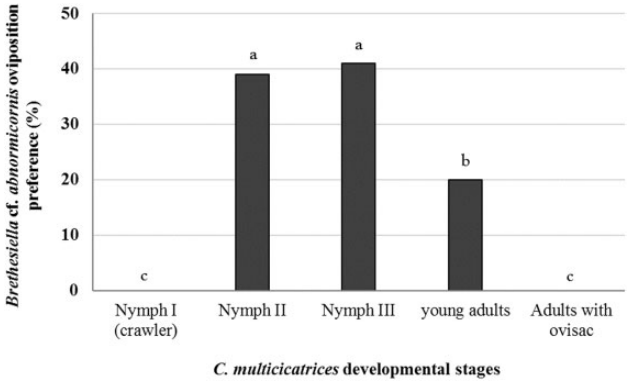


**Fig. 3.** *Brethesiella* cf. *abnormicornis* oviposition preference on different developmental stages of *C. multicicatrices* ($n = 30$ host individuals/stage).

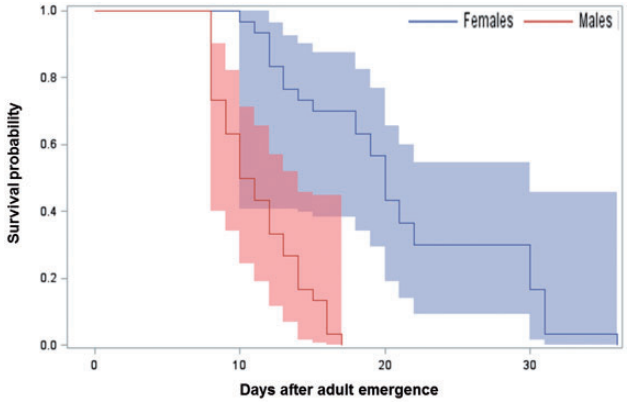


**Fig. 4.** Survivorship of adults of *Brethesiella* cf. *abnormicornis* ($n = 30$ for both sexes) on a daily basis for the duration of their life span.

*Spalangia endius* (Moreno 1982) showed similar results in terms of early oviposition. The parasitoid shows preference in terms of oviposition, with a rate of utilization (parasitization) close to 87% on second-instar nymphs. In addition to host preference, the population

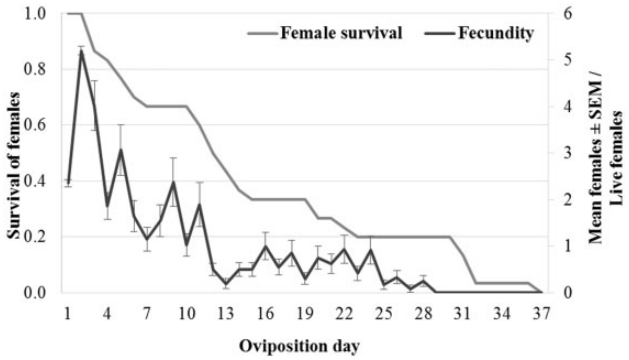


**Fig. 5.** Survival and fecundity of *Brethesiella* cf. *abnormicornis* females. Fecundity corresponds to mean females ± SEM that replace live females ($m_x$) ($n = 25$ females).

parameters estimated in this study can be considered baseline information for a mass-rearing protocol of this parasitoid. The intrinsic rate of natural increase $r_m$ had a mean value of 0.118 female/day, which is close to the mean value for the finite rate of increase ($\lambda$) of 1.125 per day. In both cases, the rate of growth is presented in the decimal part of the obtained values. The natural increase is a value that describes the growth potential of a population established in climatic and nutritional conditions and is the baseline to get accurate estimates of the expected populations under specific climatic conditions and feeding regimen offered in laboratory conditions. The comparison between the complete and the shortened life span data for the female parasitoids showed that females older than 10 d make a negligible contribution to the rate of natural increase, the finite rate of increase, and the doubling time. Such information can also be used to devote more attention in the critical age of females under a scheme of mass-rearing protocol or for field release purposes.

The high reproductive potential, together with multivoltinism, short life span, and the female-biased progeny sex ratio of *Brethesiella* cf. *abnormicornis*, are life history traits that may provide an advantage in the establishment of this parasitoid as a successful biological control agent against the Colombian fluted scale.

**Table 2.** Jackknife estimates and Student *t*-test for pairwise group comparison between normal (27 d) and shortened (10 d) life span for females of the parasitoid *Brethesiella* cf. *abnormicornis* ($n = 25$ females)

| Ecological parameter | Normal life span Mean ± SD | Shortened life span Mean ± SD | Probability |
|---|---|---|---|
| Doubling time (Dt) | 5.860 ± 0.0456 | 5.827 ± 0.0406 | 0.70765 |
| Finite rate of increase ($\lambda$) | 1.125 ± 0.0010 | 1.126 ± 0.0009 | 0.70713 |
| Intrinsic rate of natural increase ($r_m$) | 0.118 ± 0.0009 | 0.119 ± 0.0008 | 0.70715 |
| Net reproductive rate ($R_0$) | 32.400 ± 1.6814 | 27.024 ± 0.9069 | 0.00780 |
| Generation time (T) | 29.369 ± 0.3442 | 27.721 ± 0.0903 | 0.00008 |

This is the first detailed study of the biology of a species of *Brethesiella* where the length of all larval instars and its main ecological parameters were determined. This study contributes to the knowledge of parasitoids and can be used as a model for further biological studies of other species of endoparasitoids. Finally, it is necessary to continue biological studies of other natural enemies that have the potential to be implemented in biological control programs against *C. multicicatrices* and other insect pests.

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