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Ecological traits of a new aphid parasitoid, *Endaphis fugitiva* (Diptera: Cecidomyiidae), and its potential for biological control of the banana aphid, *Pentalonia nigronervosa* (Hemiptera: Aphididae)

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ABSTRACT

Most parasitoids of aphids are small wasps belonging to the families Braconidae or Aphelinidae. However, some dipteran parasitoids are also known to use aphids as hosts. We describe and give life-history information on a new species of aphid parasitoid, *Endaphis fugitiva* Gagné and Muratori, found to parasitize the banana aphid, *Pentalonia nigronervosa* Coquerel, the vector of banana bunchy top virus. Field and laboratory observations show that fly eggs are laid on the leaves near an aphid colony, the newly hatched fly larvae search for hosts while crawling on the leaf, and then penetrate the aphid between the legs and thorax. They develop as koinobiont endoparasitoids, and eventually emerge from the aphid anus and fall to the ground to pupate in the soil. We provide data relevant to parasitoid rearing on patterns of emergence, adult longevity, and effects of pupation substrate on adult emergence rate, and we discuss the potential of this new species to contribute to the biocontrol of banana aphid in Hawaii.

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1. Introduction

The most important constraint for banana production in Hawaii is the damage caused by the Banana Bunchy Top Virus (BBTV) that is transmitted by the banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Sternorrhyncha: Aphididae) (Robson et al., 2007a). Banana aphids acquire the virus from infected plants and no vertical transmission occurs (Hu et al., 1996). BBTV is transmitted persistently (Hu et al., 1996), and alate aphids are presumably responsible for spread of the virus (Waterhouse, 1987). Therefore, suppression of the aphid population below the 'alate-production threshold' may limit the spread of the disease (Young and Wright, 2005).

Currently, foliar applications of diazinon and imidacloprid are the primary tools used for banana aphid management in Hawaii (Robson et al., 2007b). However, the tendency of aphids to remain hidden under leaf sheaths on banana plants may limit the efficacy of insecticide applications to reduce populations (Robson et al.,

2006). Host plants of *P. nigronervosa* besides banana in Hawaii include taro (*Colocasia esculenta* Schott), *Dieffenbachia* sp., and *Caladium* sp. [Araceae]; red ginger (*Alpinia purpurata* Schum) and cardamom (*Elettaria cardamomum* Maton) [Zingiberaceae]; and *Heliconia* spp. [Heliconiaceae] (Blackman and Eastop, 1984).

This aphid, originally from southeast Asia, but now largely pan-tropical in distribution, was first recorded from Oahu in 1922 (Timberlake, 1924). It now occurs on most of the main Hawaiian island (though there have been no reports from Niihau or Molo-kai: Culliney et al., 2003). In other Pacific Islands, several attempts at biological control of *P. nigronervosa* have been undertaken, including the introduction of *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae: Aphidiinae) and *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae) in Tonga (Stechmann and Volk, 1988; Wellings et al., 1994) and two species of coccinellids in Western Samoa (Waterhouse, 1987). However, in both cases the introduced species failed to establish and control the target host. The Hawaii Department of Agriculture Plant Pest Control Branch has tried to introduce the aphid predator, *Scymnus* sp. (Coleoptera: Coccinellidae) from Thailand to Hawaii (Culliney et al., 2003), but has not been successful. Some authors have proposed the use of new host-parasitoid associations for the control of *P. nigronervosa* (Stary and Stechmann, 1990). Before initiating

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a new biocontrol program, we assessed the status of extant natural enemies of the banana aphid in the state, about which little is currently known. Doing so, we found a new banana aphid parasitoid in the Hawaiian Islands. This is *Endaphis fugitiva* Gagné and Muratori, a new species of Cecidomyiidae (Diptera) described in this paper.

It is not commonly known that some cecidomyiids use aphids as obligatory hosts for larval development (Gagné, 2004). Five species of *Endaphis* Kieffer have been described so far: *E. perfida* Kieffer (Kieffer, 1896), *E. compitalis* Mamaev (Mamaev, 1973), *E. gregaria* Gagné (Gagné, 1981a), *E. aphidimyza* Shivpuje and Raodeo (Grover and Kashyap, 1988), *E. maculans* Barnes (Tang et al., 1994), but only scarce information is available on the ecological traits of these aphid parasitoids (Table 1). Unlike hymenopteran aphid parasitoids, these dipterans do not oviposit inside the aphid, but rather lay their eggs on the leaves near the aphid colony or occasionally directly on the aphid body. The newly hatched larva actively searches for aphids, crawls onto the aphid abdomen, and penetrates the aphid exoskeleton to develop as a koinobiont endoparasitoid. Once mature, the parasitoid larva emerges from the anus of the aphid, which dies a few seconds after parasitoid emergence. As in other cecidomyiid flies, the larva is able to perform repeated jumps by hooking the caudal end of the body on the anterior end of a specialized anterior dermal structure, the sternal spatula, and flipping itself some distance away (Gagné, 1994). The mature larva falls to the ground to pupate in the soil. For biological control purposes, cecidomyiids can be considered underutilized. An exception is the aphid predator *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) that is currently mass reared and sold by four companies to control aphids in greenhouses (van Lenteren et al., 1997), but its polyphagous habits make it less suitable than monophagous parasitoids for classical biological control programs.

The species described in the present study was previously found in Hawaii but misidentified as *Endaphis maculans* (Messing and Klungness, 2002). It is considered a fortuitous (accidentally) introduced biological control agent of unknown origin. This paper aims to (i) describe the new parasitoid species, (ii) provide information on life-history traits and (iii) discuss its potential as a biological control agent of aphid populations in Hawaii.

2. Materials and methods

2.1. Insect collection and rearing

Banana aphids, *P. nigronevosa*, were collected on red ginger flowers, *Alpinia purpurata*, in an ornamental garden in the Hanalei district (north shore, Kauai Island) and reared in a greenhouse on red ginger plants at 24.9 °C (range = 18.5–36.8 °C), 72% rh, ≈10L:14D. To collect *Endaphis* eggs, *A. purpurata* plants with mixed-age aphid colonies were exposed in an open banana field at the University of Hawaii Kauai Agricultural Research Center (KARC) for 24 h. With the use of a stereo-microscope, parasitoid eggs were removed from the leaf surface with a fine brush. To obtain parasitized aphids, plants with aphid colonies surrounded by *Endaphis* eggs were kept in a 100 × 60 × 60 cm wood and screen cage in the greenhouse. After 3–4 days, parasitized aphids showed

a characteristic trait of *Endaphis* parasitism: black irregular spots visible on the dorsal and lateral surface of the thorax and abdomen (Kirkpatrick, 1954). To obtain adults of *Endaphis*, parasitized aphids were placed on a piece of Taro stem (*Colocasia esculenta*) in a Petri dish ($d = 15$ cm) filled with 250 ml of sand and covered with a 25 cm plastic cup. These pupation boxes were incubated in the laboratory under controlled conditions (24.1 ± 0.7; 61% rh; 10L:14D). Adults were sexed under a microscope based on the morphology of the two distal flagellomeres which are clearly binodal in males and uni-nodal in females (Figs. 1B and 4F).

2.2. Taxonomic processing

Immature stages and adults of *Endaphis* were preserved in 70% ethanol. Specimens were mounted in Canada balsam on microscope slides using the method reported in Gagné (1989). Terminology for adult morphology generally follows usage in Gagné (1981b) and McAlpine (1981), and or larval morphology that in Gagné (1989).

2.3. Experiments on life-history traits

3.1.1. Pattern of oviposition in the field

Ten red ginger plants infested with 70 s instar aphids each were exposed for 24 h in a banana patch (at KARC, Kauai). After exposure, the plants were observed under the microscope and *Endaphis* eggs were counted. Pictures of eggs and aphids were taken and the distance between each egg and the nearest aphid was measured using ImageJ (Research Services Branch, National Institute of Mental Health, Bethesda, MD, USA). If eggs of predators were found on the exposed plants, they were incubated in Petri dishes ($d = 5.5$ cm; laboratory conditions given above) and reared to the adult stage for identification. Aphids from these exposed plants were transferred with a fine brush to a new ginger leaf and observed for 10 days in order to check for the presence of any hymenopteran parasitoids. Any aphid mummy of aphidiine or aphelinid wasps was isolated in a small plastic tube until emergence of the adult.

3.1.2. Pupation substrate

In order to test the effect of substrate type on pupation success, pupation boxes were filled with various substrates: potting soil (Sunshine Mix #4 Aggregate Plus, Sun Gro Horticulture Canada Ltd.); fine sand (collected from Kekaha region, Kauai and sifted and washed with fresh water to remove all salt); or coarse sand (“play sand”, ACE Hardware, Kauai). A pupation box was composed of a Petri dish ($d = 15$ cm) containing a layer of pupation substrate and covered with a 25 cm plastic cup. Each substrate was mixed with purified water (25 ml of water for 250 ml of substrate) before it was poured in the pupation box. Thirty parasitized aphids were enclosed in each pupation box. Twice daily, we collected the emerged cecidomyiid adults. The experiment was repeated three times for each substrate type. Emergence rates were analyzed using Fisher’s exact test (Prism 5 for MacOSX, GraphPad Software Inc.).

Table 1

List of the known species of *Endaphis* and their hosts.

<i>Endaphis</i> species	Origin	Aphid host	Host plant	Reference
<i>E. perfidus</i>	Europe	<i>Drepanosiphum platanoides</i>	<i>Acer platanoides</i>	Kieffer (1896)
<i>E. compitalis</i>	Uzbekistan	Unknown	Unknown	Mamaev (1973)
<i>E. gregaria</i>	Canada	<i>Euceraphis gillettei</i>	<i>Alnus rubra</i>	Gagné (1981a,b)
<i>E. aphidimyza</i>	India	<i>Dactynotus sonchi</i>	<i>Carthamus tinctorius</i>	Grover and Kashyap (1988)
<i>E. maculans</i>	Florida, Trinidad	<i>Aphis spiraeicola</i>	<i>Viburnum odoratissimum</i>	Tang et al. (1994)
<i>E. fugitiva</i>	Hawaii	<i>Pentalonia nigronevosa</i> , <i>Aphis gossypii</i>	<i>Musa</i> , <i>Alpinia purpurata</i>	This study

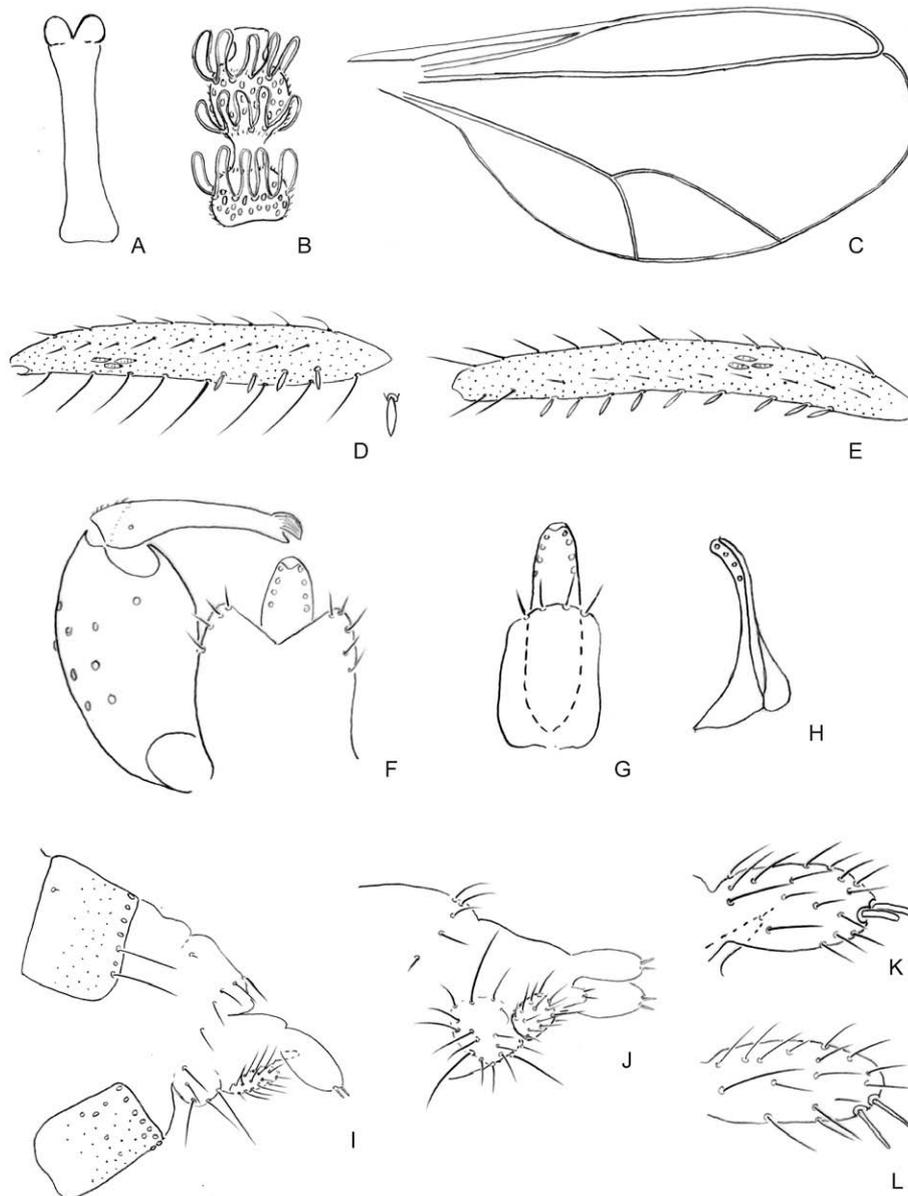


Fig. 1. (A–K) *Endaphis fugitiva*. (A) Third instar spatula, (B) male third flagellomere (ventral), (C) wing, (D) forefemur (mesal) with detail of one stout seta, (E) hindfemur (mesal), (F) gonopod, cerci and aedeagus (dorsal), (G) hypoproct and aedeagus (dorsal), (H) aedeagus (ventrolateral), (I) female abdomen, seventh segment to end (lateral), (J) same, eighth segment to end (ventrolateral), (K) detail of female cercus and hypoproct (lateral), (L) *Endaphis maculans*, female cercus (lateral).

In a second experiment, fine sand was used to evaluate the effect of water content in a sand substrate on pupation success. Pupation boxes were filled with 250 ml of the sand mixed with purified water (0, 15, 25, 35, 45, or 55 ml of water per 250 ml of sand). Thirty parasitized aphids were enclosed in each pupation box. Twice daily, newly emerged cecidomyiid adults were removed from the pupation boxes and sexed. The experiment was repeated three to six times for each level of water content. Time to emerge was analyzed using a χ^2 test on the number of emerged individuals per day (Prism 5 for MacOSX, GraphPad Software Inc.).

3.1.3. Longevity of adult *Endaphis*

In order to evaluate longevity of adult flies, newly emerged adults were sexed under a microscope and isolated in plastic vials with a piece of filter paper wetted with either 150 μ l of purified water or 150 μ l of a honey solution (3 g honey/30 ml purified water). Filter papers were renewed every other day. The number

of survivors was recorded daily. Twenty males and 20 females were followed for each treatment. Longevity of the adults was tested using survival analysis (LIFETEST, SAS v8, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Description of *E. fugitiva* Gagné and Muratori, new species (Figs. 1 and 2)

3.1.1. Diagnosis

Adults are distinct among other *Endaphis* spp. for the loss of the anterior pair of trichoid sensilla on the abdominal sternites. Males are unique in the genus for their apically recurved aedeagus (Fig. 1H) and females for the blunt-tipped pair of peg-like setae on the cerci (Fig. 1K). Other *Endaphis* species have a straight

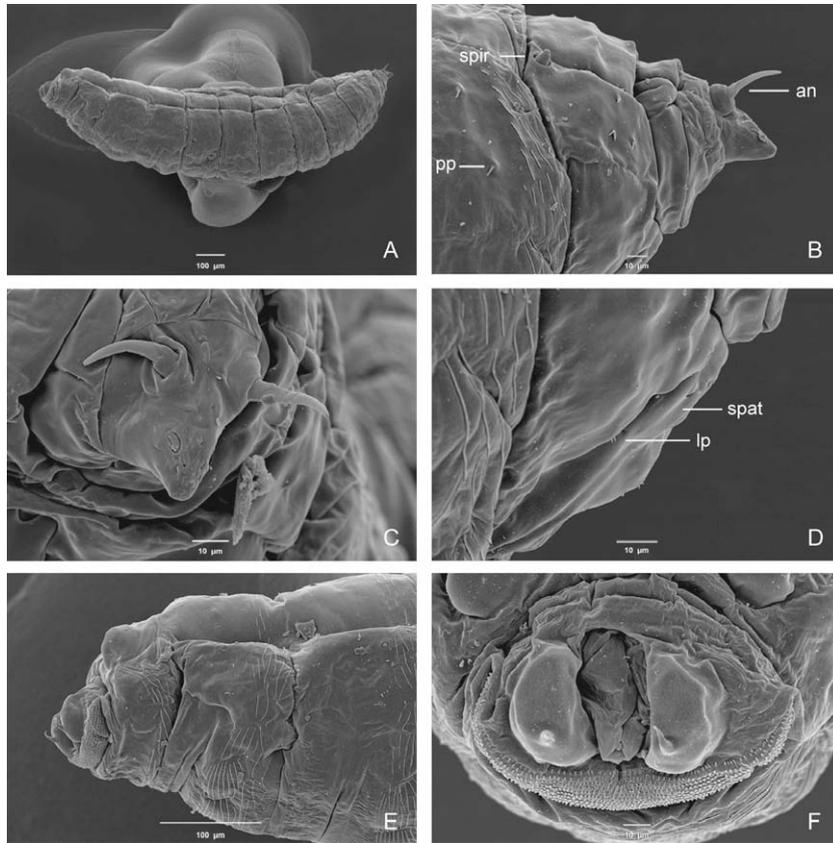


Fig. 2. (A–F) *Endaphis fugitiva*, third larval instar. (A) Whole body (ventrolateral), (B) anterior thoracic segments and head (ventrolateral), (C) head (anterior), (D) Venter of prothorax, (E) posterior abdominal segments (ventrolateral), (F) detail of terminal abdominal segment (posterior). an, Antennae; lp, lateral papillae; pp, pleural papillae; spat, spatula; spir, prothoracic spiracle.

aedeagus and apically tapered peg-like setae of the female cerci (Fig. 1L). Larvae of the new species are unique in the genus for the lack of dorsal papillae.

3.1.2. Adult

3.1.2.1. Head. Eyes connate, 8–9 facets long at vertex; facets circular, abutting one another except near lateral part of eye where facets separated by as much as $\frac{1}{2}$ facet diameter. Occiput with short dorsal protuberance bearing two prominent setae. Frons with 8–12 setae per side. Labella hemispherical, pointed apically, each with 6–8 lateral setae. Palpus 4-segmented. Male antennal flagellomeres (Fig. 1B) each with unequal nodes, with one circumflum on the basal nodes, two on the distal, the circumflar loops subequal in length; setulae covering nodes. Female flagellomeres with circumflar loops mostly appressed to nodes; necks short, about $\frac{1}{4}$ length of nodes.

3.1.2.2. Thorax. Wing (Fig. 1C) length 0.8–1.0 mm in male (range of eight specimens), 0.9–1.0 mm in female (range of six specimens); R5 nearly straight with a slight S-bend, joining C anterior to wing apex; membrane not noticeably mottled. Femora with several definite rows of setae running lengthwise among uniform covering of scales. Prothoracic femur (Fig. 1D) with one mesal, one lateral and one ventral row of setae, the last row with longer setae than other rows, and, mesoventrally on basal half, a row of 4–5 short, thick, spindleform setae. Mesothoracic femur with one mesal, one dorsal, one lateral and one ventral row of setae, setae of ventral row noticeably longest. Metathoracic femur (Fig. 1E) slightly bowed, with one mesal, one dorsal and one lateral row of setae of generally similar length, and ventral row of short, thick, spindleform setae

dispersed along entire length. Tarsal claws untoothed, curved beyond midlength; empodia attaining bend in claws; pulvillae about $\frac{1}{4}$ length of empodia.

3.1.2.3. Male abdomen. First through seventh tergites entire, rectangular, with a single, posterior row of setae, no lateral setae, covered with scales, and with two anterior trichoid sensilla; eighth tergite without vestiture except for two anterior trichoid sensilla. Second through seventh sternites rectangular with complete, mostly single row of setae along posterior margin, a horizontal row of smaller setae near midlength, and otherwise covered with scales. Eighth sternite trapezoidal with complete, single row of posterior setae and several scales; sternites without anterior pair of trichoid sensilla. Genitalia (Fig. 1F–H): cerci triangular, shallowly separated, with several apicolateral setae; hypoproct slightly concave apically, with 4 posterior setae; aedeagus broad, parallel-sided in dorsal view, rounded apically, narrow in lateral view, dorsally recurved; gonocoxite cylindrical, unlobed; gonostylus elongate, setulose on basal third, striate beyond.

3.1.2.4. Female abdomen (Fig. 1I–K). First through seventh tergites as for male; eighth tergite unsclerotized, with a few posterolateral setae; 10th tergum without setae; second through seventh sternites as for male; eighth sternite unpigmented, without vestiture; venter of ninth segment with two lateral, slightly swollen areas, each arrayed with setae of various lengths, some very long; 10th sternum about as wide as long, covered with setae; cercus large, ovoid, bilaterally flattened, setulose, sparsely covered with setae, and with two thick, apically blunt, peg-like setae apicoventrally (Fig. 1K); hypoproct about as wide as long, with two setae apically.

3.1.2.5. Third instar (Figs. 1A and Fig. 2A–F). Integument generally smooth except for narrow horizontal ridges on anterior portions of third thoracic and first through eighth abdominal segments. Head (Fig. 2B and C) conical, antenna five times as long as basal width, apodemes approximately as long as head capsule. Venter without pseudopods. Spiracles present dorsolaterally on prothorax and first through eighth abdominal segments, the hindmost pair diminutive. Spatula (Fig. 1A) elongate, narrow, bilobed anteriorly. Lateral papillae in two groups of three on each side of spatula, two of each group setose, the remaining one without seta, its base larger than the other two. Dorsal papillae apparent only as small convexities without openings and without setae. Pleural papillae (Fig. 2B) with short setae. Terminal segment (Fig. 2E and F) mesally divided, the resulting halves each rounded with an acuminate, dorsally recurved hook; venter with many rows of conspicuous spicules; papillae not apparent unless the hooks are modified papillae. Anus subdorsal.

3.1.2.6. Holotype. Male, associated with *P. nigronevosa*, Hawaii, Kauai Agricultural Research Center, deposited in the National Museum of Natural History in Washington, DC (USNM). Label data: “*E. fugitiva*, ex *P. nigronevosa*, Hawaii: Kauai Agr. Res. Stn, X-10-2007, F. Muratori, Can. balsam”.

3.1.2.7. Paratypes. Seven ♂, 5 ♀, same data as holotype; 4 ♂, 2 ♀, 19 larvae, same pertinent data except X-27-2007; 3 ♂, 1 ♀, associated with *P. nigronevosa*, Hawaii, Kauai, Wailua, IX-5-2000, R. Messing; all specimens in USNM.

3.1.2.8. Etymology. The name *fugitiva* is Latin for “fugitive” with reference to the habit of the mature larvae in escaping from the host when the aphid is attacked by predators.

3.2. Life-history traits

Adults of *Endaphis* are typical free-living flies. Females are slightly larger in size than males (tibia length: 0.33 ± 0.07 mm for females, 0.30 ± 0.06 mm for males, $M \pm SE$, $t = 4.653$, $df = 43$, $p < 0.0001$). When food (honey solution) is provided, adults can live more than 1 week. Fig. 3 shows the effect of food and sex on longevity of the adults. Females fed with a mixture of honey and water lived longer than females provided only water (Log Rank test, $\chi^2 = 24.53$, $p < 0.001$). Longevity of males was not influenced by the presence of honey in the diet (Log Rank test, $\chi^2 = 2.01$, $p = 0.157$). When the flies were fed with honey and water, females lived slightly longer than males, but this difference is only marginally significant (Log Rank test, $\chi^2 = 3.81$, $p = 0.051$). When food is provided, mean longevity is 9.7 ± 0.3 days for females and 7.5 ± 0.6 days for males. The proportion of males in the population sampled from a local banana field was 0.51 ± 0.01 ($n = 6$ samples of 30 *Endaphis*).

Endaphis fugitiva females lay their eggs on leaves near an aphid colony. In our samples, the mean distance between eggs and the nearest aphid was 2.5 ± 0.35 mm ($M \pm SE$, $n = 69$, $min = 0.29$ mm, $max = 13.3$ mm). The eggs are 241.4 ± 1.3 μ m long and 75.2 ± 0.7 μ m wide ($M \pm SE$, $n = 42$, Fig. 3A). Eggs hatched after 2 days (at 24 °C).

The newly hatched larvae (length = 261.0 ± 10.4 μ m, width = 69.4 ± 1.4 μ m, $M \pm SE$, $n = 10$) actively search for hosts, crawling on the leaf surface. At irregular intervals, the larva bends its body and lifts its head in the air in a distinctive behavior. The first instars bear two long straight antennae. The first larval instar climbs onto the abdomen of the aphid by crawling either up the legs or directly on the ventral side of the abdomen and then remains stationary for 5.5 ± 0.7 min ($n = 7$). Then, it enters inside the aphid between the base of a coxa and the thorax (Fig. 4B).

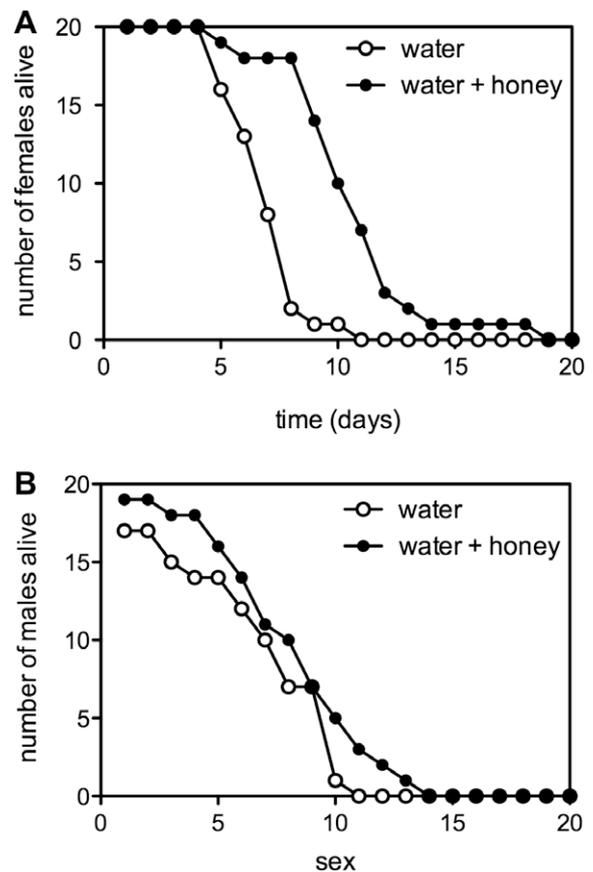


Fig. 3. Longevity of females (A) and males (B) of *Endaphis fugitiva* fed with either honey solution (●) or water alone (○).

The larva develops as an endoparasitoid for a period of 4 days (at 24 °C) from larval entrance until emergence.

The mature larva (total length = 1.48 ± 0.38 mm, $M \pm SE$, $n = 15$) emerges from the aphid (Fig. 4C) and is able to perform jumps of several cm in height. The mature larva spins a cocoon (Fig. 4D) and pupates in the soil. The emergence of adults occurs 13 days after larval emergence from the aphid (at 24 °C).

Pupation substrate type and moisture both influence the emergence of adults. Coarse sand is less suitable for pupation than soil (Fisher's exact test: $p < 0.0001$) or fine sand (Fisher's exact test: $p < 0.0001$) (Fig. 5). Dry sand is highly unfavorable to pupation success, but all other water content treatments tested gave adult/mature larvae ratios ranging from 0.5 to 0.95 (Fig. 6). High water content in the sand did not seem to affect pupation of the parasitoid. The emergence pattern of the adults showed that males emerged 0.42 days before the females ($\chi^2 = 34.45$, $df = 3$, $p < 0.0001$) (Fig. 7).

3.3. Host range and competitors

Endaphis fugitiva has been found in the field on the banana aphid, *P. nigronevosa*, on banana plants and on red ginger flowers. The parasitoid has also been found in the field on the melon aphid, *Aphis gossypii* (Messing and Klungness 2002), and has been successfully reared, from egg to adult, on *A. gossypii* in the lab. In the present study, the conditions described above allowed the complete cycle of *E. fugitiva* inside the laboratory on *P. nigronevosa* on red ginger for two generations.

Among competitors of *E. fugitiva* found in the experimental banana field (KARC, Kauai), we found the parasitoids, *Aphidius colemani* and *Aphelinus* sp. (Hymenoptera: Aphelinidae); and the

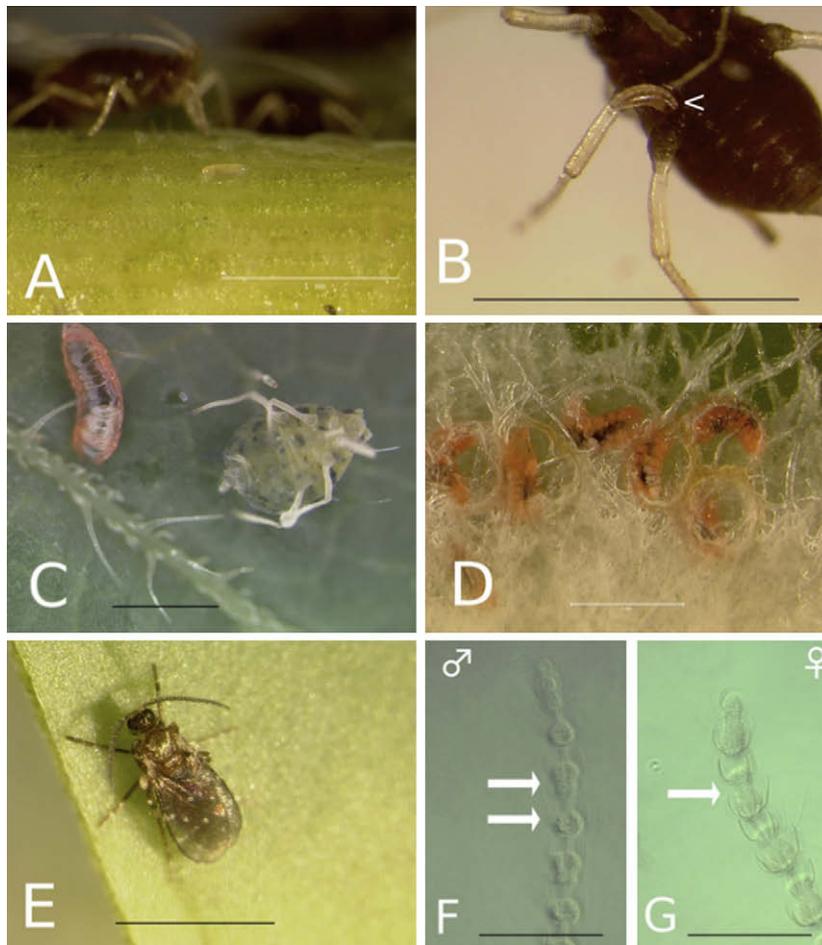


Fig. 4. Major steps of *Endaphis fugitiva* life cycle. (A) Egg on red ginger leaf, (B) first larval instar entering the host, (C) mature larva emerging from the host, (D) pupae inside cocoon, (E) adult female, (F) male antennae, two nodes for one flagellomere. (G) Female antennae. Scale bar = 1 mm.

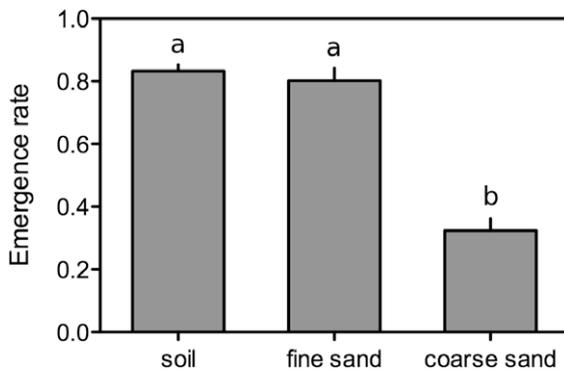


Fig. 5. Effect of substrate on the pupation success of *Endaphis fugitiva*. Bars with same letter are not significantly different ($\alpha = 0.05$).

predators, *Micromus timidus* Hagen (Neuroptera: Hemerobiidae) and unidentified syrphids (Diptera: Syrphidae). No larval hyperparasitoids of *E. fugitiva* were found.

4. Discussion

Parasitoids are among the most important biological control agents of aphids, both under natural conditions and in aphid pest management programs (Hughes, 1989). For most of the research

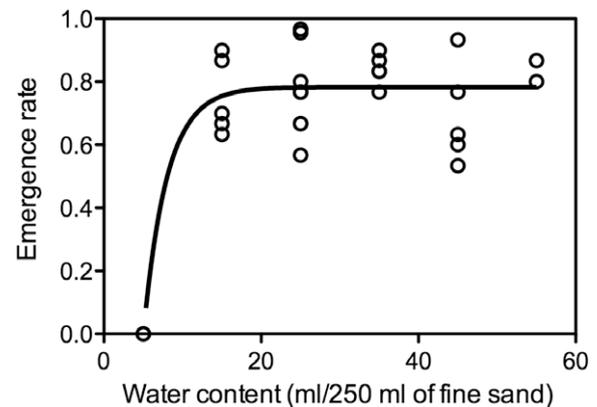


Fig. 6. Effect of water content of the substrate on the pupation success of *Endaphis fugitiva*. Line indicates the exponential model ($R^2 = 0.73$) given by $[ER = 0.78 (1 - 5.2 \exp(-0.33 \cdot WC))]$ where ER, emergence rate; WC, water content.

community, as well as for biological control practitioners, aphid parasitoids are usually thought of as small wasps belonging to the Braconidae or Aphelinidae. This paper describes a new species of aphid endoparasitoid, *E. fugitiva*, a cecidomyiid fly. The study of the life-history traits of this species offers interesting perspectives for both fundamental (phylogeny and behavioral ecology) and applied points of view (biological control).

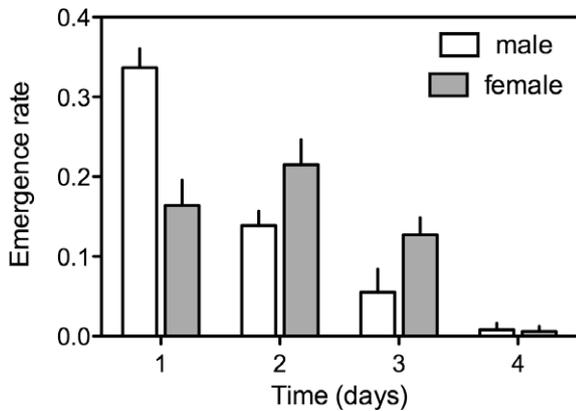


Fig. 7. Emergence pattern of adult male and female *Endaphis fugitiva* from the pupal stage in the laboratory at 24 °C.

4.1. Phylogeny

The genus *Endaphis* was first described by Kieffer with the description of *Endaphis perfidus* reared from *Aphis platanoides* Schrank in north-eastern France (Kieffer 1896, 1913). Including the new species herein, six species have been described, with all but one species being known as aphid endoparasitoids (the other has unknown biology) (Table 1). Among these, *Endaphis gregaria* Gagné (Mackauer and Footitt, 1979) is the only known gregarious aphid parasitoid.

Endaphis belongs to the tribe Lestodiplosini, all members of which are predators or parasitoids of insects and mites (Gagné 2004). Within the tribe, the closest relative to *Endaphis* is considered to be *Endopsylla* Meijere (Tang et al., 1994), with two known endoparasitic species, one from a psyllid, the other from a tingid which belong to the order Hemiptera (suborder Heteroptera: Tingidae) (Gagné 2004). Studies are needed on the phylogeny of the Cecidomyiidae family in order to address evolutionary questions such as the transition from a predatory to parasitoid life history.

4.2. Life-history traits

Adult females of *E. fugitiva* lived slightly longer than males when food was provided, but this difference was not significant. Moreover, honey provision increased longevity in females but not in males. In Cecidomyiidae, adult longevity is highly variable, ranging from less than one day for the ephemeral *Rhopalomyia californica* Felt, a gall maker on *Baccharis* (Rosenheim et al., 2007), 9.8 ± 0.52 days for *Feltiella acarisuga* Vallot, a gall midge predaceous on mites (Gillespie et al., 2000) to up to 19 days for *Aphidoletes aphidimyza* (Havelka and Zemek, 1999).

Compared to Aphidiine wasps, eggs of *E. fugitiva* are large (250 μ m long) since they must contain enough resources for the first instar larvae that need energy to search on the leaf surface and locate suitable hosts. Eggs are laid near the aphid colony (maximum 1 cm). In the laboratory, the first instar larva is able to crawl up to 10 cm from its hatching site. This gives the larva the ability to encounter several hosts, and thus host discrimination by the larva is likely to occur in this species. The stationary behavior of the first instar larva on the abdomen of the aphid can be given two hypothetical functions: (1) host evaluation, for determining either host species suitability or previous parasitism, (2) host feeding on hemolymph, for energy intake for the newly hatched larva.

The pupation success is dependent on the substrate type: while fine sand and soil are suitable for up to 80% of individuals, coarse sand leads to low adult emergence. Studies on other diptera species that pupate in the soil give inconsistent results, with signifi-

cant effect of soil type on pupation of *Ceratitis capitata* (Diptera: Tephritidae) (Ali Ahmed et al., 2007) and *Bactrocera tryoni* Froggatt (Diptera: Tephritidae) (Hulthen and Clarke, 2006), while no effect was found on *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae) (Chen and Shelton, 2007). Our results also show that *Endaphis* mature larvae need a moist substrate to pupate successfully. Therefore, they might be well adapted to the high humidity of the banana agro-ecosystem and to the high rainfalls of banana growing areas in the Hawaiian Islands. As we have no knowledge of the area of origin of this new species, we cannot speculate on the selective pressures that may have give rise to the life-history traits that are discussed here.

Extensive research has been conducted on the behavior of various species of hymenopteran aphid parasitoids, from proximate (Outreman and Pierre, 2005; Henry et al., 2006; Muratori et al., 2006) to evolutionary approaches (van Alphen et al., 2003; Muratori et al., 2008a,b). With species in the genus *Endaphis*, we have appropriate models to address questions using comparative behavioral ecology that might lead us to greater understanding of convergence in the evolution of host exploitation (Eggleton and Belshaw, 1992; Boivin and van Baaren, 2000; Brodeur and Boivin, 2004). In the system presented here, several parasitoid species from different families and even different orders exploit the same host: we found *P. nigronevosa* to be parasitized in the field by *Aphidius colemani* (Braconidae: Hymenoptera), *Aphelinus* sp. (Aphelinidae: Hymenoptera) and *E. fugitiva* (Cecidomyiidae: Diptera). The co-existence of these species raises questions of host discrimination and larval competition at the inter-specific (and even inter-order) level, which would have consequences on the efficiency of biological control programs when these species co-occur.

4.3. Potential for biological control

Several factors make *E. fugitiva* a good candidate for classical biological control of *P. nigronevosa*.

First, host species known for *E. fugitiva* include *P. nigronevosa* and *A. gossypii*, which are economically important pests of banana, taro, and many other crops in Hawaii (Messing and Klungness, 2002). To control these aphid species, several parasitoids and predators have been deliberately introduced to Hawaii without a total control of the aphid population. In the Hawaiian islands, *E. fugitiva* is reported as a fortuitous biological control agent (Messing and Klungness, 2002) but this species might be interesting for classical biological control in other areas.

Second, we were able to maintain the species inside the laboratory for two generations. Thus, scaling-up of the rearing to larger production should be achieved without major problems and allow field trials of augmentative aphid biocontrol.

Third, since the area of origin of *E. fugitiva* is unknown, nothing is known about its original host range, competitors or predators. In Hawaiian ecosystems, no hyperparasitoids of *Endaphis* larvae have been found. Limiting factors of *Endaphis* population dynamics may be abiotic factors that lead to mortality of the first instar larvae, or below ground predation on pupae.

Fourth, like many subtropical areas, some areas of Hawaii are known to receive ample rainfall. In the Hanalei district of Kauai where banana and taro are grown, annual rainfall is estimated to be 3000 mm (USDA NASS, 2006). Our results showed that wet conditions do not have a detrimental effect on the pupation of *Endaphis* larvae in the soil. This might lead to a good tolerance of the parasitoid to high moisture levels common in banana plantations.

Fifth, the fact that *Endaphis* larvae actively search for hosts might be advantageous for biocontrol. Hymenopteran parasitoids of aphids have shown limited efficiency at finding aphids that feed inside rolled leaves of bananas. In a comparative study, Stadler and Volkl (1991) showed that *Lysiphlebus testaceipes* spends most of its

time searching on open leaves, while *Aphidius colemani* partitioned its time on the plant between open and concealed areas. During our experiments, we noted that *Endaphis* first larval instar is able to crawl through tiny spaces, such as parafilm sealed Petri dishes. This would enhance the parasitism of aphids within rolled leaves. We recognize that the efficiency of the adult *Endaphis* female to locate aphid colonies will greatly influence the importance of host location ability by the larvae; here additional research is clearly needed.

The use of biological control agents to reduce the spread of plant viruses by aphids depends mainly on their ability to prevent alate production in an aphid colony, which is partially due to crowding conditions brought on by high population densities (Mueller et al., 2001). Very few studies have addressed the ability of natural enemies to influence the degree of virus spread in a crop (Wellings, 1991), although there is anecdotal evidence of the parasitoid *Aphidius salicis* Haliday reducing the spread of Carrot Mottle Dwarf Virus by the aphid *Cavariella aegopodii* Scopoli (Huffaker and Messenger 1976). Further studies are needed to assess the ability of *Endaphis* to locate hosts at low densities, and to maintain aphid populations under the threshold at which alates are produced.

5. Conclusions

This paper sheds light on life-history traits of a new species of aphid parasitoid, but it also raises ecological questions. In our opinion, the genus *Endaphis* has received less attention than it deserves. In order to make biological control safer, more efficacious and more predictable, we need additional data on the life-history traits of the biological agents used. The combination of several parasitoids with different life cycles might be the best approach for bio-control of *P. nigronevosa*, and we hope that this paper will stimulate further research on these interactions.

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