SYMBIOSIS (2008) 45, 135–141 ISSN 0334-5114

A new species of high mountain Andean fig wasp (Hymenoptera: Agaonidae) with a detailed description of its life cycle

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(Received February 20, 2007; Accepted May 17, 2007)

Abstract
The interaction between plants of the gènus *Ficus* (Moraceae) and wasps of the family Agaonidae (Hymenoptera) has been well studied for a long time. However, there are few studies concerned with the basic biology of the wasps in general and even fewer studies in the Neotropics. This paper describes a new species of fig wasp, *Pegoscapus bacataensis*, pollinator of the Andean fig tree, *Ficus andicola*; as well as provides the first detailed description of the fig wasp life cycle. Five larval instars were detected, with the last two having sclerotized mouthparts. The pupa is exarate. Adults emerge about 130 days after eclosion, a time span longer than that commonly reported for pollinating fig wasps.

Keywords: Agaonidae, *Pegoscapus bacataensis*, life cycle, larva, pupa, fig-wasp

1. Introduction

The members of the pollinating fig wasps, family Agaonidae (Hymenoptera: Chalcidoidea) (Rasplus et al., 1998), have an obligate mutualistic relationship with fig trees (*Ficus*, Moraceae) being greatly dependent on its fruits (syconia) where they complete their entire development (Galil and Eisikowitch, 1968; Ramirez, 1974; Janzen, 1979).

The New World fig wasp fauna is composed of about 101 described species; with an estimated total number, including non-pollinating species, that may reach 850 species (Rasplus and Soldati, 2006). The Agaonidae, containing little more than fifty described species, are distributed between two genera, *Tetrapus* and *Pegoscapus*. The number of species and the delimitation of the species is difficult and not well known for the latter genus (Bouček, 1993; Wiebes, 1995).

There are several studies on life cycles and immature stages or instars of entomophagous families in the Chalcidoidea (Clausen, 1964; Stehr, 1987), as well as a large number of papers studying the figs-wasps interaction (see for example Weiblen, 2002). However, very few have focused on the basic characterization of the fig wasps life cycle. The brief information available describes a life cycle of no more than five weeks but no details about larval growth rates or instar intervals have been published (Galil and Eisikowitch, 1968; Joseph and Abduhariman, 1981). This is surprising given that the closest wasp-plant interaction occurs while the immature wasps are feeding on the plant tissue, as has been widely demonstrated in other insect-plant systems (Ehrlich and Raven, 1964; Schoonhoven et al., 2005).

Ficus andicola is a monoecious fig species belonging to subgenus *Urostigma* section *Americana*. Unpublished works on *Ficus andicola* in Colombia have reported information on pollinating as well as non-pollinating wasps (Ramos, 1985; Cardona, 2005), nevertheless these works do not describe the wasp species. The present study describes a new species of pollinating fig wasp, *Pegoscapus bacataensis*, hosted by *Ficus andicola*, and provides a detailed description of the wasp life cycle.

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2. Materials and Methods

The study was carried out between April 12th and August 16th 2006 at the campus of the Universidad Nacional de Colombia, Bogotá (4°38'N, 74°4'W; altitude: 2620 m) and figs were collected from a single tree of *Ficus andicola*. The weather during this period was a monthly precipitation of 151 mm, a mean temperature of 14.1°C, and an average of 4.6 hours of sunshine per day.

Ten branches of the selected tree of *Ficus andicola* were marked, and checked every 48 hours between 8th and 12th of April, for occupied syconia. Occupation was determined by looking for wing remnants left at the ostiole by the female when passing through it. Figs were marked according to the date of occupation. By April 12th the number of occupied figs was large enough for ten syconia to be sampled every three-four days. The fruits were collected and preserved in 90% ethanol.

Two ovaries from each syconium were dissected and a total of 20 ovaries were dissected per sampling. The larvae present were examined. For each dissected individual we recorded: the relative position of the larvae inside the ovary, a detailed description of the larvae, and the size of larvae. The presence of a colonizing female was corroborated by the observation of body remains inside the lumen of the dissected fig.

Due to the poor morphological differentiation of the larvae, each instar or stage was determined using body dimensions which change significantly. To measure body length of the curved larvae, short straight parts along the midline of the body were measured and added up to compute the total body length. Measurements were taken with a WILD M8 Stereo microscope (Wild Heerbrugg, Wetzlar).

In order to collect specimens of the pollinating wasp, *Pegoscapus bacataensis,* ripening fruits were collected when the wasps were about to emerge. Each fruit was put into a separate vial and sealed with a layer of cotton. Once emerged, the wasps were preserved in vials with 90% ethanol. For microscope examination adults were cleared with a 10% KOH solution. The body parts were dissected and mounted in Canada balsam on glass slides. Larval specimens were mounted and preserved on slides in a 50:50 v/v polyvinyl alcohol-glycerin solution. Voucher specimens were deposited at the entomological collection of the Instituto de Ciencias Naturales of the Universidad Nacional de Colombia, Bogotá, Colombia. For electron microscopy (SEM), specimens treated by critical point drying, were dissected and mounted for metal coating. Morphological descriptions follow Bouček (1993) and Wiebes (1995) for body parts and Copland (1973) for female genitalia.

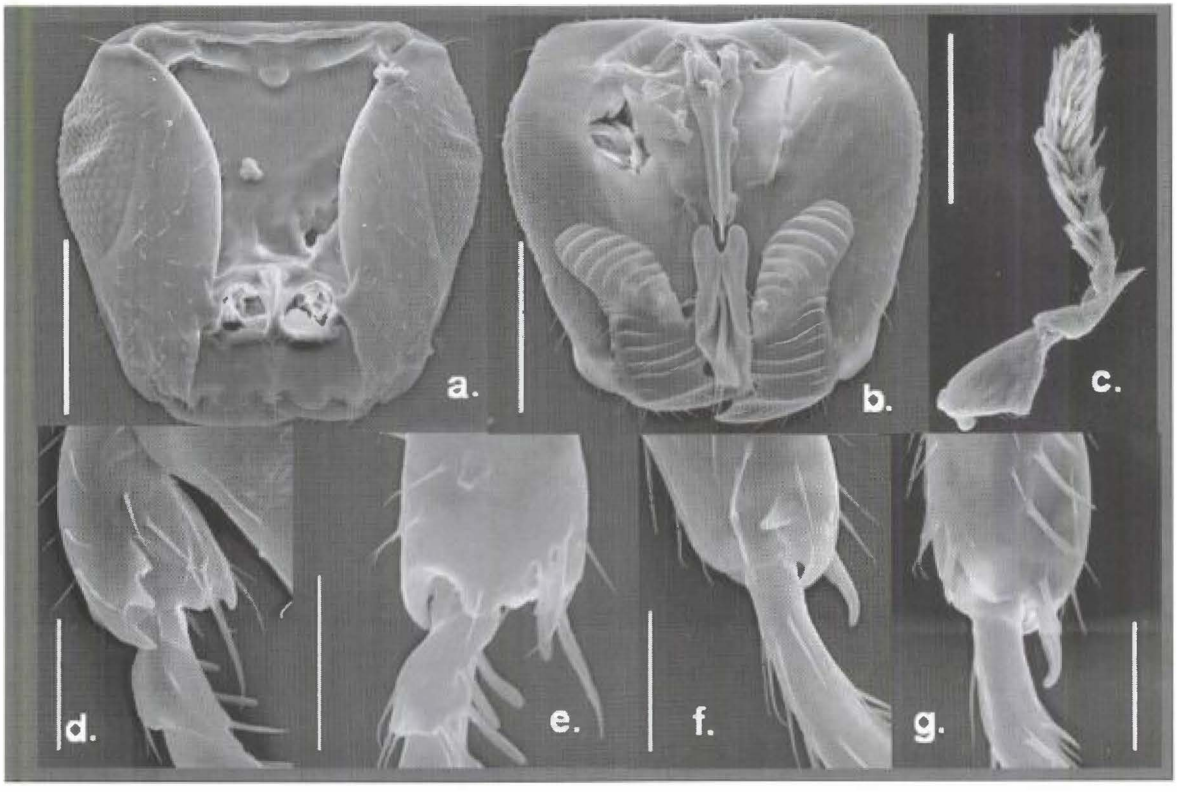

Figure 1. Female structures: a. head dorsal view (median head area collapsed due to partial dehydration); b. head ventral view (Scale bar = 200 µm); c. antennae (Scale bar = 200 µm); d. fore tibia external view; e. fore tibia internal view (Scale bar = 50 µm); f. hind tibia external view; g. hind tibia internal view (Scale bar = 50 µm).

3. Results

Pegoscapus bacataensis *nov. sp. description*

Female

Coloration and sculpturation: Head, thorax, abdomen, coxae, and femora dark brown, tibiae light brown, tarsi yellowish. Body not sculptured, rather with lustrous appearance. Setae sparsely arranged all over the body.

Head: Almost square, 1.1× as wide as long across compound eyes (Fig. 1a); eyes 1.4× as long as high; gena very narrow; two postero-lateral ocelli with a third one included in the membranous area across the face (Fig. 1a); antennal scape 1.5× as long as wide, thickened in the middle, in lateral view with an anterior median blunt protuberance (Fig. 1c); two condyle articulations between pedicel and scape; third antennal segment projecting distally into an acute point that reaches the distal part of the fourth antennal segment (Fig. 1c); antennal segments 1 through 4 without sensillae (Fig. 1c); the number of sensillae on segments 5–11 as follows: 7-11-12-11-12-12-9. Clypeal margin sinuate (Fig. 1a); mandibles with a middle dorsal tooth and 8–9 ventral lamellae which are less pronounced on their sides than in the middle; mandibular appendages with 7–8 lamellae, the first one appears as a blunt pointed denticle (Fig. 1b). Maxilla bearing one or two lateral setae; gula pointed, reaching posterior space between maxillae (Fig. 1b).

Mesosoma: Mesosternal pollen pockets, diagonal and external to middle coxae (Fig. 2b); center of prosternum with several short setae; pronotum with a lateral line of 6–7 setae (Fig. 2b); propodeal spiracle rounded; legs with sparsely and evenly distributed hairs, femorae and tibiae slightly flattened laterally; fore basitarsus without spines (Fig. 1d); fore tibia with one external tricuspidate tooth (Fig. 1d) and one internal monocuspidate tooth (Fig. 1e); hind tibia with an anteroexternal tricuspidate tooth (Fig. 1f), and with an apicointernal spine that does not reach the first half of tarsomere 1, two pre-apical spines half the length the apical one, and a median longitudinal row of four pre-apical setae progressively larger distally (Fig. 1g); wings clearly setose; with a row of dense hairs on the posterior margin of the wing, this row starts at the same height of the stigmal vein and reach the wing apex. Post marginal vein very reduced or absent. HW with three hamuli. Rectangular areola above the HW insertion.

Metasoma: Apex of metasomal mucro with eight long setae; spiracle of eighth tergite semi-ovate; terga with basal and distal margins straight; ovipositor sheaths as long as metasoma. The pygostyles bear four long sensory setae, the longest apical one almost doubles the pygostyle length; ovipositor ramus with 28 denticles on its outer margin.

Measurements: Body 1.5–1.3 mm. head length = 0.34 mm. width = 0.38 mm; eye length = 0.15 mm; gena w = 0.02 mm; Post-ocellar distance = 0.27 mm; ocelus-ocular distance = 0.14 mm; antennal scape: l = 0.14 mm, w = 0.1 mm; fore wing sub-marginal = 0.6 mm, marginal = 0.1 mm, stigmal = 0.15 mm.

Male

Coloration and sculpturation: Head light shiny brown, mesosoma testaceous, metasoma light yellow. Legs testaceous; body not sculptured, rather with lustrous appearance. Head with setae sparsely distributed.

Head: Almost square, 1.1× as wide as long, laterally rounded (Figs. 3a, 3d); temple width 2.4× eye width; head 4.4 times as long as the antennal groove. Septum weakly defined. Antenna with three short anulli, club composed of two segments.

Mesosoma: Tergites of pronotum-mesoscutum and these of metanotum-propodeum fused (Fig. 2c); mesoscutum as long as wide. Mesopleural plaques with a row of 6–7 little setae on its anterior inner margin. Mesosternum three times wider posteriorly than anteriorly, anterior margin sinuate (Fig. 2d). Fore and hind legs robust; posterioexternal margin of fore tibia with three teeth (Fig. 3c) and one posteriointernal apical tooth (Fig. 3b); these

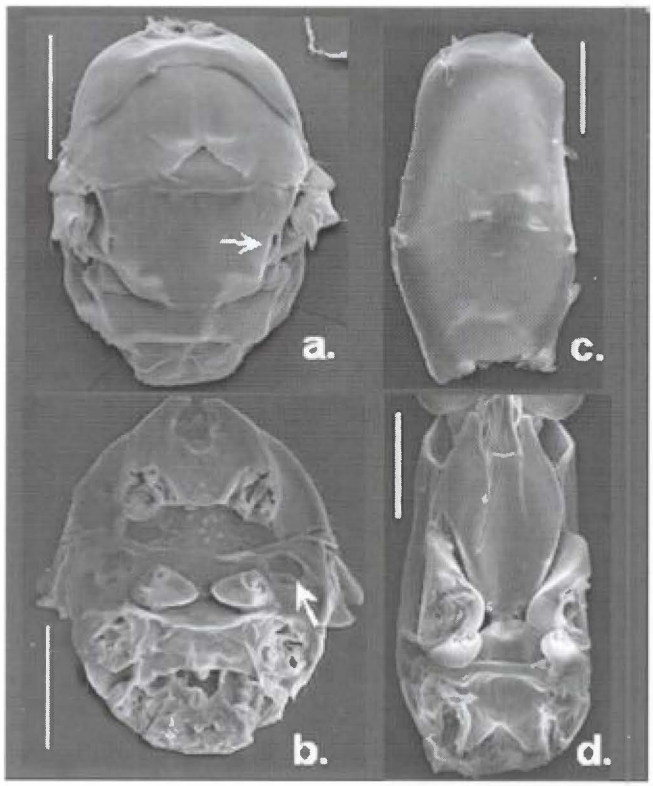

Figure 2. Female and male mesosoma. a. Female mesosoma dorsal view, rectangular areola indicated (arrow); b. female mesosoma ventral view, pollen pockets indicated (arrow) (Scale bar = 200 μm); c. male mesosoma dorsal view; d. male mesosoma ventral view (Scale bar = 200 μm).

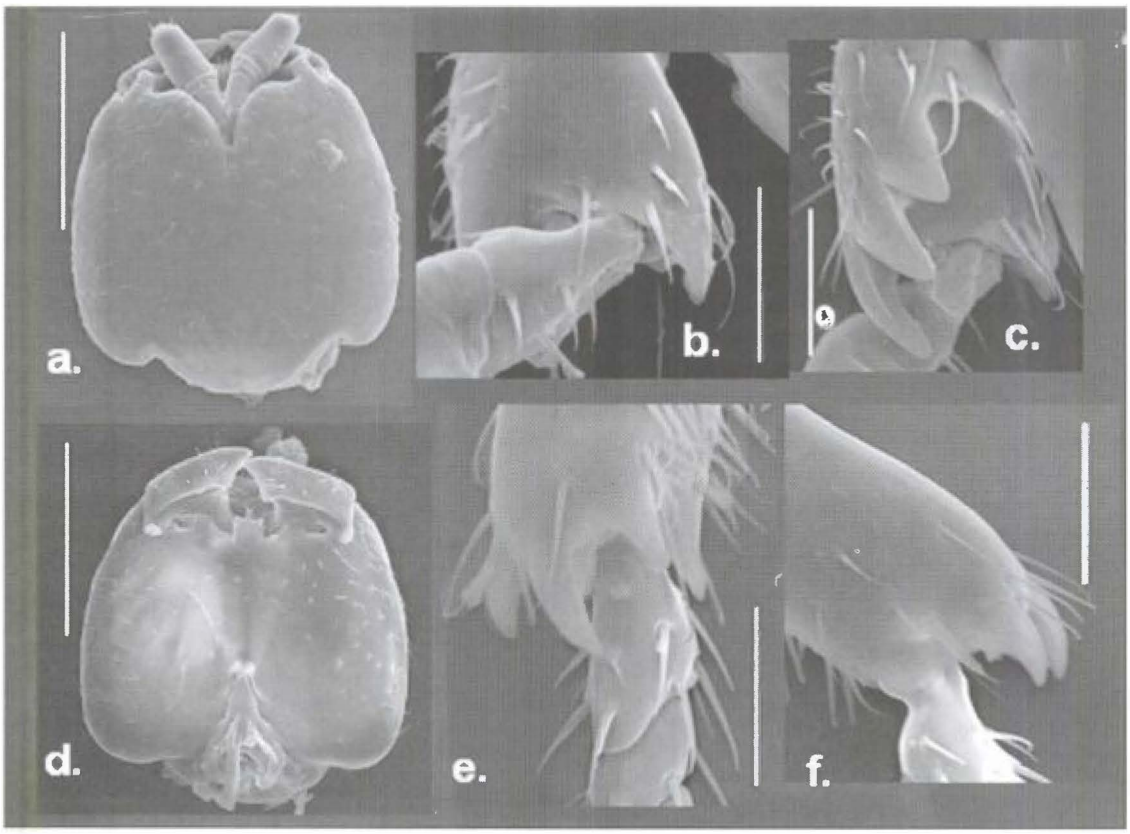

Figure 3. Male structures. a. head dorsal view; b. head ventral view (Scale bar = 20 µm); c. fore leg tibia internal view; d. fore leg tibia external view (Scale bar = 50 µm); e. hind tibia internal view; f. external view (Scale bar = 50 µm).

teeth conforms a concave area; hind leg with an external bicuspidate tooth (Fig. 3e) and one internal bicuspidate tooth, the anteroexternal cuspid with a small denticle arising medially (Fig. 3f).

Measurements: Body: 1.00–1.4 mm, head: l = 0.31 mm, w = 0.34 mm; eye: l = 0.07 mm; temple: w = 0.12 mm; antennal groove: l = 0.08 mm; pronotum: l = 0.05 mm, w = 0.3 mm; mesoscutum: l = 0.33 mm, w = 0.33 mm.

Etymology

The specific name of the species refers to the type locality. The current name of Colombia's capital city, Bogotá, is actually a derivation of the native Muiscan word Bacatá, one of the main localities where Muiscan pre-Hispanic civilization settled.

Diagnosis

Females of *Pegoscapus bacataensis* can be distinguished from other species by the following combination of characters: head almost square; antennal segments bear one row of sensillae; spiracular peritremata small; fore tibia bears three teeth in the dorso-apical comb; antiaxial tooth of hind tibia tri-cuspidate, last antennal segments setose; number of lamellae (7–8) on the mandibular appendage.

Distribution

Pegoscapus bacataensis is only known from Bogotá, at an altitude of 2620 m. Nevertheless, since the geographical range of *Ficus andicola* seems to be very wide across the Andean Cordilleras of Colombia, its pollinator is likely to have a wide geographical range.

Material examined

HOLOTYPE: Female. Deposited at the ICN-MHN. COLOMBIA, BOGOTA: Ciudad Universitaria, ex. *F. andicola*, 21.Ago.2006 (S. Jansen-G.). ICN-MHN 4054.

PARATYPES: 5 females and 3 males: 1 female deposited at the ICN-MHN, COLOMBIA. BOGOTA: Ciudad Universitaria, ex. *F. andicola*, 24. Feb. 2006 (S. Jansen-G.). 3 females and 2 males deposited at the ICN-MHN (ICN-MHN 4055-4060), same data as holotype. 1 female and 1 male deposited at the Alexander von Humboldt Collection, same data as holotype.

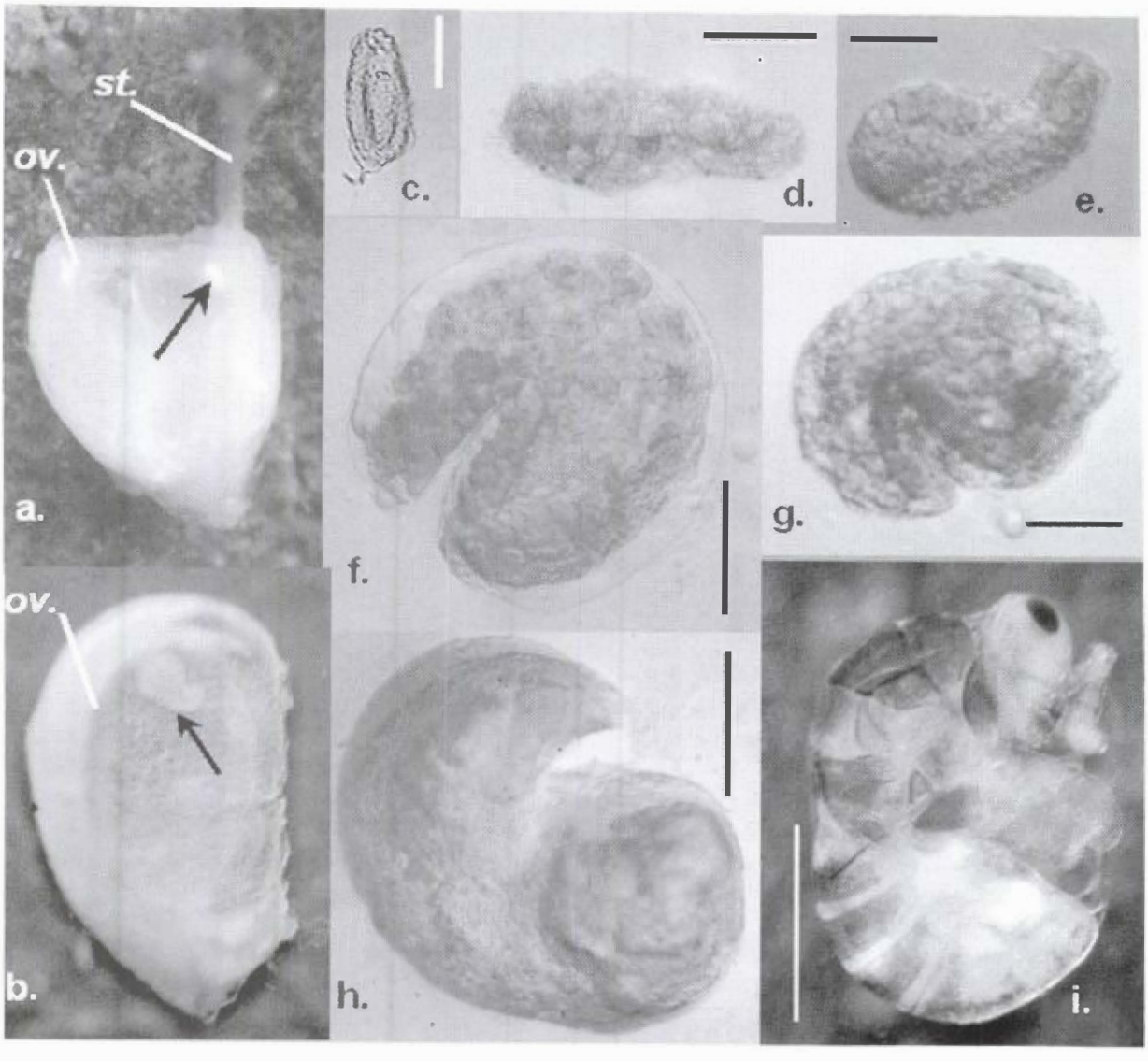

Figure 4. Immature instars of *Pegoscapus bacataensis*. a. egg (*arrow*) at the entry of the style-ovary channel; b. egg with remnants of peduncle (Scale bar = 0.05 mm). c. first larval instar (Scale bar = 0.05 mm); d. second larval instar (Scale bar = 0.10 mm); e. second larval instar (arrow) on ovary; f. third larval instar (Scale bar = 0.10 mm); g. fourth larval instar (Scale bar = 0.20 mm); h. fifth larval instar (Scale bar = 0.20 mm); i. Pupa: antennae, wing and ovipositor primordia are seen (Scale bar = 0.50 mm). *ov* = ovary; *st* = style.

Life cycle

Egg: The egg is ovate, laterally flattened (max. length 0.0925 mm; max. width 0.045 mm), with a peduncle of variable length that anchors it to the seed inner layers (Figs. 4a. 4c). The egg is always located at the entry of the style channel of the ovary.

Larva 1: This instar hatches 23 days from laying. It is white-translucent, elongate with little differentiation at body extremes (max. length 0.1035 mm; max. width 0.054 mm). No internal differentiation was observed (Fig. 4d).

Larva 2: Second instar was identified about 34 days from oviposition. Larva is sacciform (Fig. 4e) (max. length 0.192; max. width 0.095 mm) although some individuals with the comma-shape of hymenopteran larvae are observed (Clausen, 1962); one extreme of the larva is half the width of the other. The comma-shaped body is observed in the following instars. Larvae instars 1 and 2 commonly localize at the upper pole of the seed, attached to an inner layer (Fig. 4b).

Larva 3: This instar was observed from 48 days after oviposition (max. length 0.528; max. width 0.222 mm). It is white-translucent and it bends more pronouncedly than the previous instars (Fig. 4f). It tends to be at the center of the ovary. A dark brown tube along the midline of the body was observed, that could be the digestive tract.

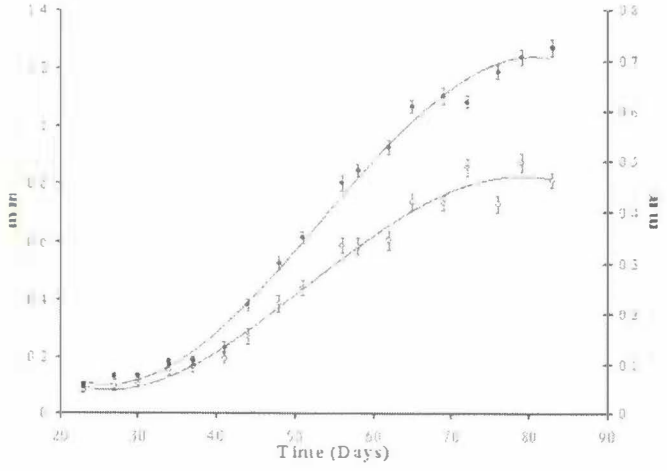

Figure 5. Growth curve of *Pegoscapus bacataensis* larvae. Body length (-●-) and maximum body width (-○-). Median values are presented. Line bars represent standard error of the mean. Data from 1st through 5th instars (23–84 days from oviposition).

Larva 4: The fourth larval instar shows up 56 days after oviposition (max. length 0.801 mm; max. width 0.336 mm). Clear cephalization is observed (Fig. 4g). Sclerotized light brown mouthparts are evident at the apex of the head. These extremely minute structures are composed of mandibles and a ring that surround these; the ring is formed by both labial and labral sclerites. This mouth region resembles that of a pteromalid wasp larva in the third instar (Stehr, 1987).

Larva 5: This instar is observed 73 days after oviposition. Cephalization becomes more evident; body growth slows down (max. length 1.13 mm; max. width 0.442 mm); the larva occupies most of the ovary space (Fig. 4h). During the final days of this instar fat droplets were observed inside the larvae.

Pupa: The pupa was found 87 days after oviposition; it is white and exarate (Fig. 4i) (max. length 0.850 mm; max. width 0.588 mm). During this instar the head is localized at the upper part of the ovary; the body is completely bowed and occupying the remnant space of the ovary.

Adult: Adults eclosed 130 days after oviposition.

Other observations

During our study we also found associated with *Ficus andicola,* species of the genera *Aepocerus* (Otitesellinae), *Anidarnes* (Sycophaginae), *Idarnes* (Sycophaginae), and a species of *Physothorax* (Torymidae). Their description and biology are the topic of future research.

4. Discussion

The generation time indicated in the literature for pollinating fig wasps is around four-five weeks. Nevertheless, longer life cycles have been reported from temperate regions during the colder seasons (Kjellberg et al., 1987; Compton, 1993) where generation times can be up to six months long. In subtropical regions were temperature changes are less pronounced during the year, lower temperatures are still sufficient to increase developmental time considerably (Bronstein and Patel, 1992). Since seasonality in the tropics does not involve major temperature changes during the year, it is likely that the low temperature in our high mountain areas is the reason for the 130 days life cycle of *Pegoscapus bacataensis*.

The S-shape of the growth curve of *Pegoscapus bacataensis* larvae reveals three phases (Fig. 5). The first one presents a very slow growth rate which may be due to the processes of organ and tissue formation (Hutchinson et al., 1997); in the second phase the larva exhibits exponential growth until the fifth larval instar. The last phase shows a slow growth rate. This slowing may be due to diminished resource availability and to the preparation for pupation (D'amico et al., 2001). Critical size and therefore critical weight could become restricted by ovary capacity at this stage. Janzen (1979) argues that besides ostiole and other syconial traits, seed size could be another reinforcer for fig wasp specificity as it is likely that galled seed size determines final body size. Future experiments in this area may resolve this question.

Growth curves with an S-shape pattern are common forms of insect larval development. For example, growth curves of the parasitoid wasps *Cotesia rubecula* and *Microplitis demolitor* (Hymenoptera: Braconidae) also have the same S-pattern when body dry weight is plotted against time (Harvey et al., 1999; Harvey et al., 2004). This pattern has also been observed in other chalcid wasps such as *Eupelmus orientalis* (Eupelmidae) and *Dinarmus basalis* (Pteromalidae) (Damiens et al., 2001).

This is the first time that the immature stages of a pollinating fig wasp have been thoroughly characterized. For *Pegoscapus bacataensis* there are five larval instars, the last two with sclerotized mouthparts. Since crucial features of insect-plant relationships occur at this stage of insect development, further study on pollinating and non-pollinating larval instars would open new perspectives for the understanding insect-plant relationship as well as larva-parasitoid interactions.

Acknowledgments

We thank professor Jean-Yves Rasplus for help with taxonomy and valuable comments. We also thank the Universidad Nacional de Colombia, Instituto de Ciencias Naturales (ICN) at Bogotá. Special thanks the staff of the Laboratorio de Biología Comparada de Insectos, at the ICN.

We are indebted with the anonymous reviewers who provide insightful comments to the paper.

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