

Life history and immature stages of *Allograpta zumbadoi* THOMPSON, a phytophagous flower fly (Diptera: Syrphidae: Syrphinae)

[Lebenszyklus und Jugendstadien von *Allograpta zumbadoi* THOMPSON,
einer phytophagen Schwebfliege (Diptera: Syrphidae: Syrphinae)]

by

Menno P. VAN ZUIJEN and Kenji NISHIDA

Wageningen (The Netherlands)

San José (Costa Rica)

Abstract

The life history and the immature stages of *Allograpta zumbadoi* THOMPSON, 2000 (Syrphidae: Syrphinae) are described, illustrated, and compared with those of *A. centropogonis* NISHIDA, 2003. These two species are the only leaf-mining syrphines known. The larva of *A. zumbadoi* shows several similarities with those of *A. centropogonis*, e. g. presence of papillae with antennomaxillary organs surrounding the mouth, the serrated labrum and labium, an identical pattern of segmental sensilla, and spiracular openings that extend radially. Major differences between the mature larvae of the two species were found in the body shape and setation, the number of teeth on the labrum and labium, the robustness of the head skeleton, and the shape of the posterior respiratory process. The immature stages of *A. zumbadoi* were found on three different species of *Centropogon*, a genus of the bellflower family (Campanulaceae). The larvae in all instars are solitary. The first and early second instar larvae are leaf miners, the mid-second instar larvae and subsequent stages are stem borers. Pupation takes place on the host plant near the stem base, which has been observed both in the laboratory and in the field.

Key words

Syrphidae, *Allograpta*, Neotropical Region, Costa Rica, larval biology, larval morphology, Campanulaceae, *Centropogon*, *Burmeistera*

Zusammenfassung

Der Lebenszyklus und die Jugendstadien von *Allograpta zumbadoi* THOMPSON, 2000 (Syrphidae: Syrphinae) werden beschrieben, abgebildet und mit denen von *A. centropogonis* NISHIDA, 2003 verglichen. Diese beiden Arten sind die einzigen bekannten blattminierenden Syrphinae. Die Larve von *A. zumbadoi* zeigt mehrere Ähnlichkeiten mit *A. centropogonis*, z. B. das Vorhandensein von den Mund umgebenden Papillen mit Antennomaxillarorganen, ein gesägtes Labrum und Labium, ein übereinstimmendes Muster von Segmentalsensillen und ringförmig erweiterte Atemöffnungen. Wichtige Unterschiede zwischen den erwachsenen Larven beider Arten liegen in der Körperform und Beborstung, der Anzahl der Haken auf dem Labrum und Labium, der Stärke des Kopfskeletts und der Form der Hinterstigmen. Die Jugendstadien von *A. zumbadoi* wurden auf drei verschiedenen Arten von *Centropogon*, einer Gattung der Glockenblumengewächse (Campanulaceae), gefunden. Das erste und das frühe zweite Larvenstadium sind ausschließlich Blattminierer, das mittlere zweite und alle nachfolgenden Larvenstadien sind Stengelbohrer. Die Verpuppung findet am Stengelgrund der Wirtspflanze statt, was sowohl im Labor als auch im Freiland beobachtet werden konnte.

Stichwörter

Syrphidae, *Allograpta*, neotropische Region, Costa Rica, Larvalbiologie, Larvalmorphologie, Campanulaceae, *Centropogon*, *Burmeistera*

Introduction

In Costa Rica, approximately twenty species of *Allograpta* have been recorded (INBIO 1997–2006) and there still remain a few undescribed species (F. C. THOMPSON, pers. comm. 2006; MENGUAL et al. 2009). Prior to the study by NISHIDA et al. (2003), phytophagous species were

unknown in the genus *Allograpta*, i. e. the larvae of the genus were known to be predators of aphids and other small hemipterans (THOMPSON et al. 2000, ROJO et al. 2003). The larva of *A. centropogonis* is a gregarious, social leaf miner, on several species of *Centropogon* (Campanulaceae) in high elevations of Costa Rica (NISHIDA et al. 2003, COSTA 2006). The discovery of the phytophagy in the genus enlightened further studies of the genus *Allograpta* (MENGUAL et al. 2008, 2009; WENG & ROTHERAY 2009).

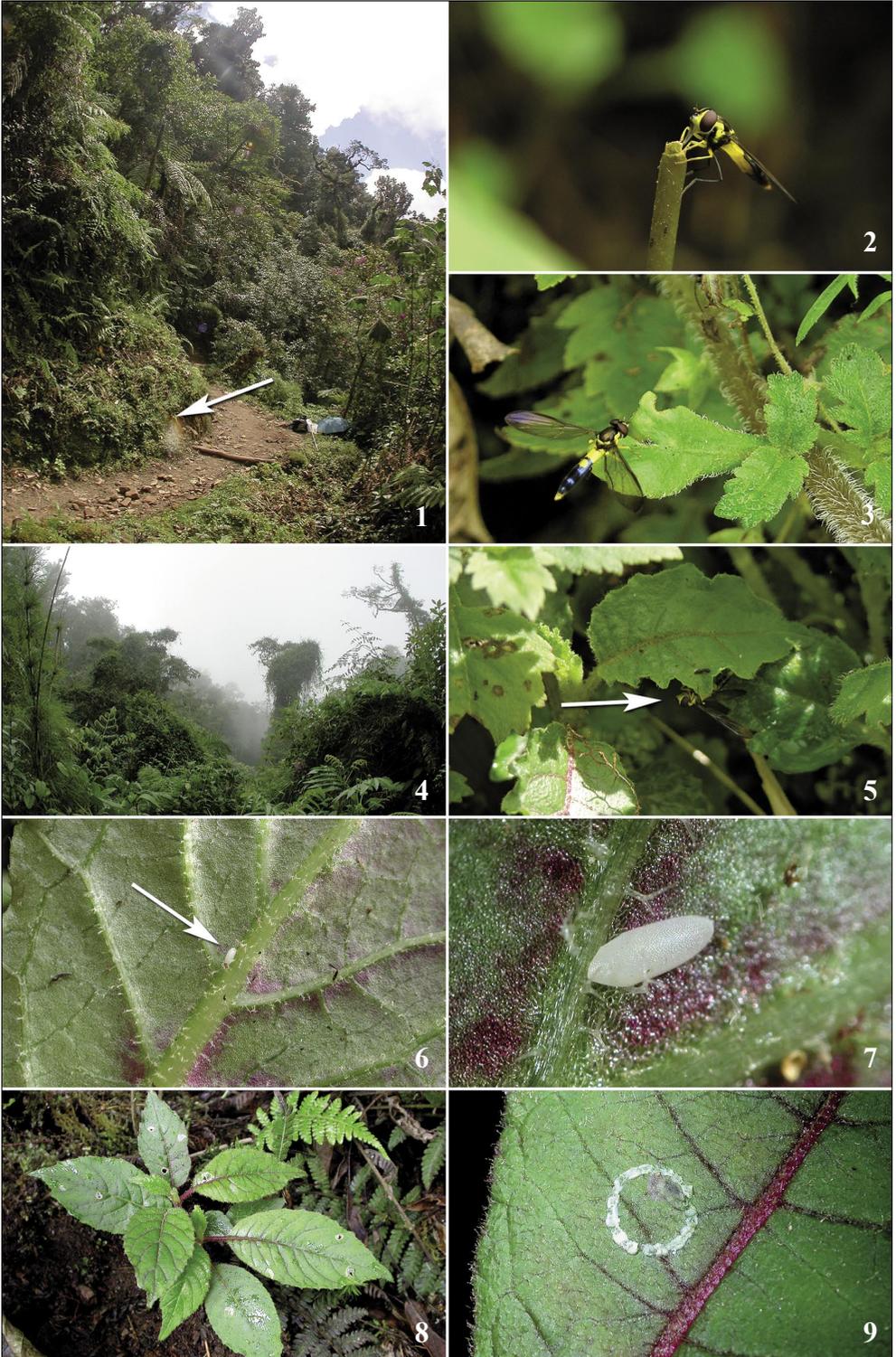
There are some records of plant-feeding larvae in the subfamily Syrphinae. For example the larvae of *Toxomerus politus* (SAY, 1823) feed on the pollen and surface tissues of corn, *Zea mays* L. (Poaceae) (RILEY & HOWARD 1888, RICHARDSON 1915, HEISS 1938, MARIN 1969), and larvae of *Toxomerus apegensis* (HARBACH, 1974) feed on pollen of *Olyra obliquifolia* STEUDEL (Poaceae) (REEMER & ROTHERAY 2009). Recently, another non-predacious *Allograpta*, *A. (Fazia) micrura* (OSTEN SACKEN, 1877), has also been found in Costa Rica; its larvae exclusively feed on pollen in *Castilleja* (Scrophulariaceae) flowers (WENG & ROTHERAY 2009). Other phytophagous syrphid larvae are known in *Cheilosia*, *Eumerus*, and *Merodon* in the subfamily Eristalinae. Most of these eristalines are stem borers in asteraceous plants or feed in bulbs of Liliaceae (ROTHERAY 1988, 1990, 1993; STUKE 2000). A recent study (MENGUAL et al. 2008) concluded, on the basis of molecular analysis, that phytophagy was most likely acquired secondarily and at least twice in the history of genus *Allograpta*.

We here report rearing and field observations of a second *Allograpta* species with phytophagous larvae, *Allograpta zumbadoi* THOMPSON, 2000. The morphological characters of adult *A. zumbadoi* are unique and easily distinguished from all other described *Allograpta* species; the species is currently placed in the subgenus *Costarica* (MENGUAL et al. 2009; see discussion below). Adult specimens have been collected in high elevation cloud forests above 2,500 m in the Talamanca and Volcán Barva regions in Costa Rica. The species is possibly endemic to this region (THOMPSON et al. 2000). In this study the life history and immature stages of *A. zumbadoi* are described, illustrated, and compared to those of *A. centropogonis* and a possible third phytophagous '*Allograpta*' species.

Materials and methods

Studied host plant. *Centropogon ferrugineus* (L. f.) GLEASON (Campanulaceae) is one of sixteen species of *Centropogon* found in Costa Rica. It occurs on both the Pacific and Caribbean slopes in the Barva and Talamanca regions, from approximately 2,000 to 3,000 m above sea level (INBio 1997–2006). More than 230 species have been described in the genus *Centropogon*. Its centre of diversity is in the cloud forest habitats of western South America (GENTRY 1996). The plant is well known from studies of pollination and hummingbirds in Costa Rica (COLWELL et al. 1974, KOPTUR et al. 1990). One of the main characters of the family Campanulaceae is the exudation of latex which is produced in the phloem and is known to deter many insects and other herbivorous animals from consuming the leaves, stems, and other parts of the plants (SPEER 1994–2006).

Figs 1–9: On the life history of *Allograpta zumbadoi* THOMPSON on *Centropogon ferrugineus*. – 1: Habitat along the trail near the Llano Bonito refuge with mid-day to afternoon sun, arrow pointing to the area where young host plants, immature stages, and ovipositions were observed; – 2: Female perched on a non-host plant near *C. ferrugineus* (latero-frontal view); – 3: Female perched on a non-host plant approaching host plants (dorsal view); note the metallic blue colour reflection on the abdomen; – 4: Habitat, showing steep hill open area seen from the trail; – 5: Female (arrow) on the underside of the host plant leaf in oviposition; – 6: Egg (arrow); – 7: Egg (close-up dorsal view); – 8: Circular mines made by early instar larvae on leaves seen as white scars; – 9: Ring with a coagulated milky sap of the plant on upper side of the leaf that exuded along the cut made by the first instar larva (under captive conditions).



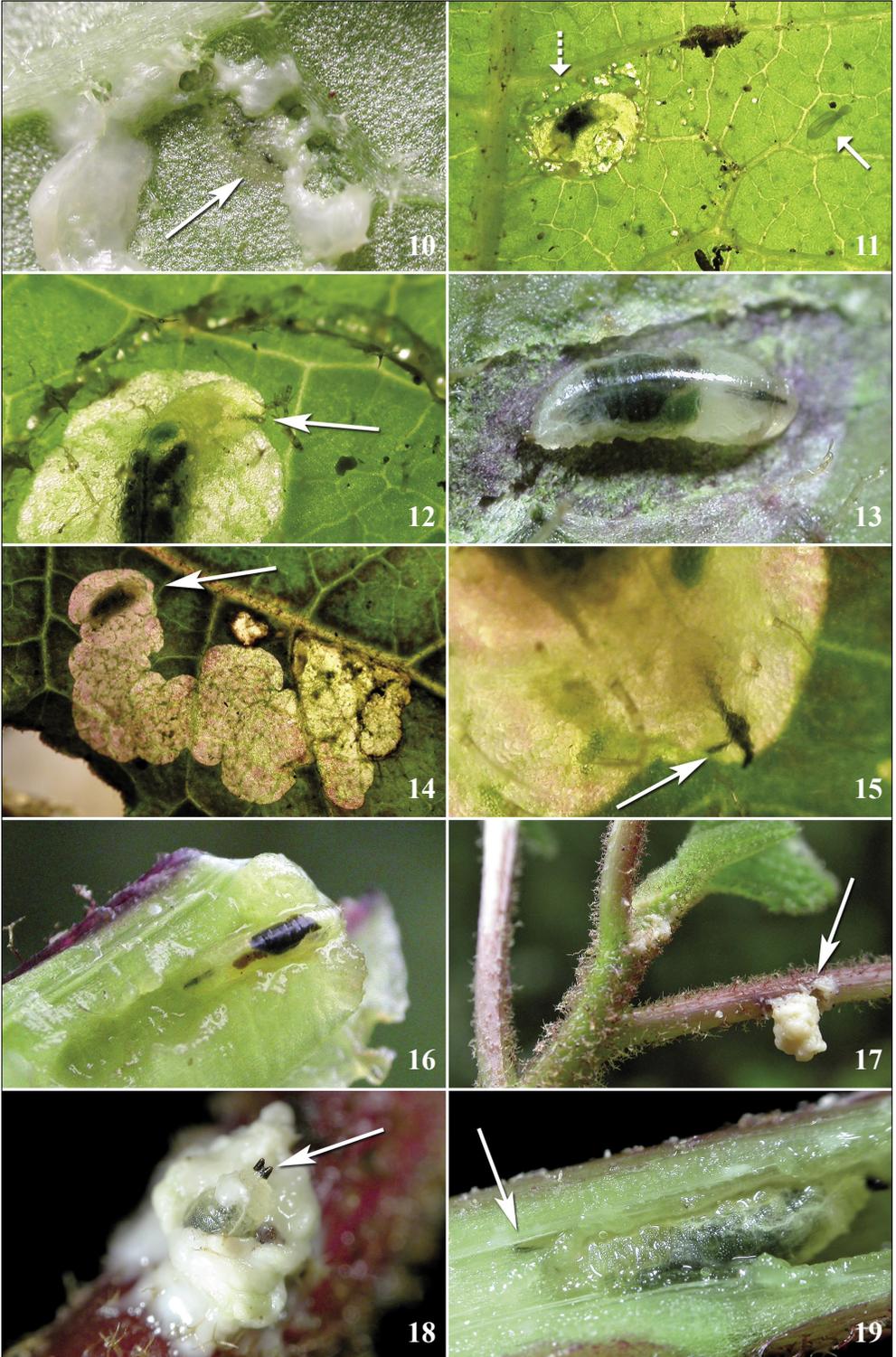
The plants growing along the trail of the Chirripó study site appear to get trimmed (cut by trail maintenance crews) occasionally and thus young re-grown shoots were prevalent.

Biology. Most of the observations in the field were conducted in April 2003, February 2005, and June 2006, near Llano Bonito in a cloud forest of Chirripó National Park, San José Province, Costa Rica. The site (Figs 1, 4) is located in an oak forest along the trail approximately 1.0 km above from the Llano Bonito refuge (2,550 m elevation, 09°27'08"N 83°32'20"W). One part of the trail is on a steep hill where there is a relatively open area (Fig. 4). When not covered by clouds, the mid-day to afternoon sun shines strongly along the trail and on the host plants. A few additional observations were made between January 2000 and August 2001 in Villa Mills, Cerro de la Muerte, a high elevation oak forest (NISHIDA et al. 2003). The eggs and larvae were collected along with entire host plants, including the epiphytes growing on them, and brought back to San José (1,235 m elevation, 09°55'49.1"N 84°02'32.9"W) for rearing. They were placed in a refrigerator (7.0 to 8.0 °C) with no lights during the day, and at night they were placed outside at an ambient temperature of ca. 20 °C, to approximate the natural habitat temperature of Llano Bonito. The general external morphology, head skeleton, and posterior respiratory process (PRP) of immature stages were compared with those of *A. centropogonis*. Also, the stem-boring larvae collected in the Villa Mills area in *Centropogon valerii* STANDL., and *C. talamancensis* WILBUR (NISHIDA et al. 2003) were compared to those of *A. zumbadoi* from Llano Bonito. Some eggs, larvae, pupae, and parasitoids were preserved in 80 % ethanol (ROTHERAY 1993). The specimens obtained during this study were deposited in Instituto Nacional de Biodiversidad (INBIO), Museo de Zoología (UCR), National Museums of Scotland, and the private collection of Menno P. VAN ZUIJEN (Wageningen, Netherlands). High resolution (4.0–8.0 mega pixels) digital still images (color photographs) were taken by Nikon Coolpix cameras and were modified with Adobe Photoshop®. Terminology for the immature stages follows ROTHERAY (1993).

Results

Life history. One to three ♀♀ were observed at the Llano Bonito site during two days in February 2005. On the first day, one ♀ was observed in the early afternoon at 1 : 30 pm when it arrived during a period of weak sunlight. The ♀ perched on a non-host plant (Fig. 2) near *Centropogon ferrugineus*; however the sunlight soon faded and the ♀ flew away without approaching the host plants. On the second day, one of the other two ♀ was seen flying and perching on plants on the slope in strong direct sunlight and windless conditions, around 12 : 30 pm, but it flew away from the slope as soon as hikers approached the site. Approximately 30 minutes later, the third ♀ (Fig. 3) appeared in a similar manner as the second ♀ (possibly same individual). The ♀ perched on a non-host plant for several seconds, and then flew closer to the host plant landed and walked briefly on several plants in its near vicinity, before and finally landing on the host plant.

Figs 10–19: On the life history of *Allograpta zumbadoi* THOMPSON on *Centropogon ferrugineus*. – **10:** First instar larva (arrowed) starting to mine inside the ring (underside view); note the coagulated latex; – **11:** First instar larva mining leaf; note the egg shell (arrowed) and dotted holes (dotted arrow) in circular form; – **12:** First instar larva feeding inside the ring; arrow pointing at the head skeleton; note the dotted holes along the cut; – **13:** First instar larva inside the circular mine (dorsal view; upper epidermis removed); – **14:** Blotch mine of late second instar larva (arrow) in captivity; – **15:** Second instar larva mining leaf; note the widely opened labium (arrowed); – **16:** Early second instar larva boring in the centre of a stem (dorsal view); – **17:** Entrance hole (arrow) on petiole with coagulated latex made by the second instar larva; – **18:** Second instar larva in the midst of exuded latex that is starting to bore into the stem; note the pair of cone-shaped PRP (arrow); – **19:** Second instar larva boring and feeding on centre portion of the stem tissue, arrow pointing at the head skeleton.



When it was on the host plant, it went to the underside of the leaf (Fig. 5), walked for a while apparently searching for a spot to oviposit, paused, and laid an egg. Then it either continued to walk on the plants apparently looking for other adequate leaves on which to oviposit or came up on top of the leaf and flew to other plants and 'rested' for some ten seconds. This walking and flying from plant to plant was done approximately 5–20 cm above the ground. The ♀ oviposited three to four eggs on two plants, i. e. one egg per leaf and one to two eggs per plant, before it tried to fly away from the site (It was captured as a voucher specimen.).

Several relatively young but fully grown *C. ferrugineus* were infested with both *A. zumbadoi* and *A. centropogonis* larvae. Nevertheless, no eggs of the two species were seen on the same leaf.

Most of the immature stages of *Allograpta zumbadoi* were found in an open sunny area on young plants or young re-grown shoots of *C. ferrugineus* which were less than 30 cm tall ($n > 30$). Eggs (Figs 6, 7) were laid singly on the undersides of relatively young, small and thin leaves, mostly one per leaf ($n = 30$); on four leaves there were two or three eggs. The smallest leaf with an egg was ca. 20 mm long x 12 mm wide and was located in the apical area of a small plant. Of 39 eggs found, approximately 50 % of them were laid adjacent to the primary vein and 25 % along secondary veins near the primary vein; others were found in the outer part of the leaf. A single plant (shoot) contained up to five eggs (12.5 %); others had four eggs (6.3 %), three eggs (18.8 %), two eggs (37.5 %), and one egg (25 %) ($n = 16$ plants).

The first instar larva, after hatching from the egg, made a circular cut or ring (3.8 ± 1.2 mm; $n = 19$) in diameter on the lower epidermis and vascular tissue from outside near its egg shell. The circular cut also consisted of more or less evenly spaced small holes which reached the upper epidermis (Figs 11, 12), apparently made by the larva feeding on the mesophyll. The larva then fed on the leaf tissue inside the circular cut by mining the mesophyll tissue (Figs 10–12). A milky sap made by the plant emerged from the cut and coagulated (Figs 9, 10). The cut and holes were apparently made by scissors-like movement of the larval labrum. The rings and circular mines were easily seen on the upper epidermis as white scars (Fig. 8). Usually one to three per leaf was observed. After consuming the tissue inside the ring, the larva made another ring on the same leaf and then fed inside it. However, no ring was observed on the third mine, which was more irregular, made on the same leaf; the larva probably mined the leaf without making a ring. No faecal deposits were observed either in these mines or on the surface of the leaf. The moult to the second instar larva apparently took place in the mine. The early second instar larva was also found mining the leaf without making a circular cut. The middle to late second instar larva bored into the stem or leaf petiole (Fig. 17) in the upper portion of the plant and started to feed in the central portion of the stem while tunneling downward (Figs 16, 19). The holes where the larvae entered were usually completely covered with coagulated milky sap (Figs 17, 18, 20) and thus the infested plants were easily noticed. Old entrance holes (Fig. 21) were exposed, the coagulated milky sap apparently being washed off by rainfall. Usually either a second or third instar larva was found feeding in each tunnel (Figs 16, 19, 22–25). In some cases, two to three tunnels occurred in a single stem, e. g. one or two recently started tunnels on the upper portion of the stem and a large, long tunnel on the lower portion. Presumably the younger larvae would not have had enough to eat when they moved to the lower portion in these cases. The larvae fed on the stem tissue by opening and closing the labium while moving the head skeleton ventrally, bringing it closer to the body (Figs 22, 23, 25). The larvae also moved the head skeleton sideways (Fig. 24) by twisting its prothoracic area as in *A. centropogonis* (NISHIDA et al. 2003). The larva seemed to exit from the entrance hole in the stem since no hole was found in the lower portion of the stems in vacated tunnels. In none of the plants which

had larvae tunneling did the stems show any external differences with respect to uninhabited plants, except for the white circular scars on leaves and coagulated milky sap on the side of stems. Both in the field and in the lab, larvae were capable of moving to other stems, and of feeding on more than one stem. In a few cases under rearing conditions late second and early third instar larvae mined leaves and made blotch mines after boring stems (Figs 14, 15). On one occasion, a third instar larva was observed protruding its posterior respiratory process out of the entrance hole after a heavy rainfall filled the tunnel with water (Fig. 26).

Under laboratory conditions, mature third instar larva exited the tunnel and pupated on the host plant near the base of the stem (near ground level), which was usually covered with epiphytes (Figs 28, 29). In June 2006 six puparia (one per stem) were found in this position in the field and two puparia were found in a cluster of liverworts which surrounded the stem base. To pupate on the surface of the host stem, the mature larva dug a shallow groove in the stem tissue where it attached itself (Fig. 27). This attachment was apparently assisted by the milky sap of the host plant – all of the puparia found in the field or reared in laboratory were embedded in this groove and adhered tightly to the plant in this manner, and some of these puparia were thickly covered with milky sap (Figs 28, 29). Of the eight puparia collected in the field, one was brown and empty (hollow); one was somewhat greenish and contained an unformed *A. zumbadoi* pupa; two had a circular hole in the anterior part of the dorsum and were empty – apparently a parasitoid had emerged from them; and five were parasitized by *Woldstedtius* spec. (Ichneumonidae: Diplazontinae), possibly *W. eduardoi* GAULD & HANSON, 1997, one per puparium. None of the ten mature larvae that were brought to the laboratory produced any parasitoids.

On the host plants in the field, white flies (Aleyrodidae), leafhopper nymphs, and adults (Cicadellidae) were more or less frequently observed infesting the undersides of leaves. On or near the base of the stem and on roots, spittlebug nymphs were commonly observed. However, *A. zumbadoi* larvae were never observed preying on these small hemipterans.

Eggs and larvae of all stages were more constantly observed in April 2003 (n = ca. 20) and February 2005 (n = ca. 15), but in June 2006 most of the immature stages found were either in young second instar larva or puparia (n = ca. 15). Examination of larvae collected in the Cerro de la Muerte region revealed that *A. zumbadoi* also feeds on *Centropogon talamancensis* and *C. valerii*. Two small plants of an undetermined species of Campanulaceae, possibly *Burmeistera*, were also found infested by the early stages of *A. zumbadoi* at the Chirripó site. The circular mines on leaves were somewhat similar to those of *A. zumbadoi* recorded on *Burmeistera* cf. *parviflora* E. WIMM. ex STANDL., at Vara Blanca site (10°10'51"N 84°06'20"W; 2,000 m) on Volcán Barva.

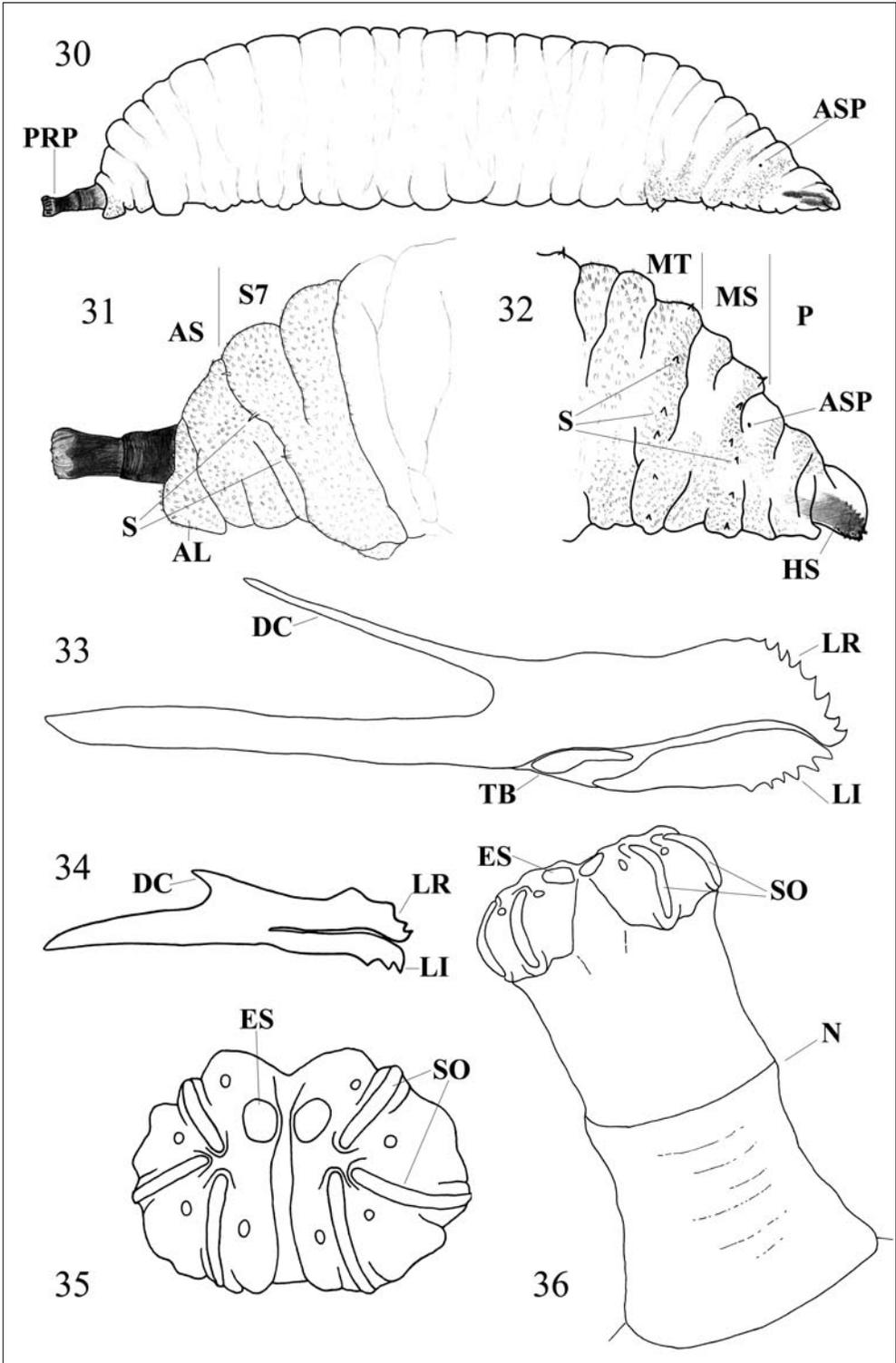
Material examined: Two eggs, two first instar, three second instar, fifteen third instar larvae, and six puparia preserved in 75 % ethanol, a few dry-preserved egg shells, and digital images taken under natural and laboratory conditions; COSTA RICA: Chirripó National Park, 2,550 m, April 2003, February 2005, June 2006, in San José Province, larvae ex. leaf mines and bored stems on *Centropogon ferrugineus*; Villa Mills, Cerro de la Muerte, 3,000 m, October 2000, in San José Province, larvae ex. bored stems on *C. valerii* and *C. talamancensis*.

Description of the immature stages. **Egg** (Figs 6, 7): ca. 1.2 mm long, white, elongate ovoid, ventrally flattened. The anterior end (where head develops) is slightly wider and more sharply pointed than posterior end; chorion with scale-like patterns seen under 32 x or higher magnifications (n = 4). **First instar larva** (Figs 10–13, 34): up to ca. 2.2 mm long, dorso-ventrally flat; head skeleton 0.5 mm long, with two rows of three to four teeth each (first pair at the apex fused; Fig. 34); with a pair of PRP which are pale brown, conical, not fused (n = 3). **Second instar larva** (Figs 16, 18, 19): up to ca. 5.5 mm long, cylindrical; head skeleton 0.9 mm long, similar to third instar larva; PRP brown to black, not fused (n = 4).

General appearance of third instar larva (Figs 22, 25, 30, 35, 36, 38, 39): sub-cylindrical to cylindrical, semi-translucent to pale yellowish-brown (some greenish when feeding on greenish-colored stem tissue) with white fat body covering hind gut, tapering anteriorly, truncate posteriorly; locomotory prominence with shallow grooves, lacking prolegs and crochets; apex of head skeleton with serrated dorsal and ventral margins (Fig. 38); most of the body covered with colorless to white setae (setae dark brown in some preserved larvae; Fig. 39); PRP long, dark brown to black with a nodule in middle portion. **Third instar larva** (Figs 22–26, 30–33, 35, 36): 9.0–12.0 mm long, 1.5–2.0 mm wide, sub-cylindrical to cylindrical in cross section, tapering anteriorly, truncate posteriorly; dorsal and ventral sensilla accompanied by single seta each; sensilla of prothorax, ventral surface of meso- and metathorax, abdominal segments, and anal segment lacking setae; pattern of segmental sensilla as in other syrphine larvae (ROTHERAY & GILBERT 1989, 1999). **Head** (Fig. 38): antennomaxillary organs mounted on laterally flattened-papillae (basal width 0.13 mm) which cover sides of mouth; head skeleton (Fig. 33); 1.35–1.5 mm long, labrum 0.2 mm thick (side of toothed area), labrum and labium 0.1 mm wide (scooping surface); arrangement similar to other syrphines (HARTLEY 1963, ROBERTS 1970), i. e. labrum and labium elongate and more or less equally developed at tip; tip of labrum and labium not sharply pointed as in other syrphines; dorsal surface of labrum and ventral surface of labium near apex scoop-shaped (concave) and each margin bearing eight teeth and five to six teeth each respectively, with first tooth at apex composed of a fusion of the two rows (margins); teeth symmetrically arranged (left and right side) and larger toward apex (Figs 33, 38); mandibles apparently reduced or lost, dorsal cornu narrow and spike-shaped (Fig. 33) as in *A. centropogonis* (NISHIDA et al. 2003). **Thorax and abdomen** (Fig. 32): mouth covered with fleshy lobes (width 0.08 mm) dorsally and ventrally which are extensions of prothoracic margins; entire prothorax, lateral margins of thorax to first abdominal segment and along transverse folds on dorsal surface of anterior margin of meso- and metathorax covered with pale brown to brown sclerotised vestiture composed of ca. four to six micro-spicules in form of a small band; elsewhere surface of integument with short, colorless to white evenly scattered stout setae (Fig. 39), except ventral surface of mesothorax to abdominal segment 6 (matt); anterior spiracles present on posterior fold of prothorax; abdominal segments 1–7 with three flattened transverse dorsal folds; paired locomotory prominences on abdominal segments 1–7 bearing shallow grooves and sensilla 9–10, apex matt and bearing setae; anal segment dorsally and ventrally equally developed, with two dorsal and three ventral folds, 1.2 mm high, lower than abdominal segment 7 (ca. 2.1 mm), surface matt with semi-translucent vestiture, truncate at apex, bearing a pair of lobes (ca. 0.12 mm long by 0.45 mm wide); anal opening transverse. **Posterior respiratory process** (PRP) (Figs 26, 30, 31, 35, 36; Table 1): ca. twice as long as broad, ca. 1.0 mm long, basal width 0.4–0.45 mm, apical width 0.4–0.45 mm, smooth, entirely sclerotised (both basal and distal half sclerotised), dark brown to black, distal half slightly nar-

Figs 20–29: On the life history of *Allograpta zumbadoi* THOMPSON on *Centropogon ferrugineus*. – **20:** Entrance holes with coagulated latex (arrowed) made by third instar larva; – **21:** Old entrance hole exposed after the latex washed off; – **22:** Young third instar larva feeding on stem tissue (dorsal view); – **23:** Anterior portion of the body of a third instarlarva; note head skeleton and prothorax bending ventrally; – **24:** Close-up dorsal view of the thorax of third instar larva, twisting thorax and feeding on the stem tissue; arrow pointing at head skeleton; – **25:** Mature third instar larva feeding (dorsal view); note the greenish colour and white fat body; – **26:** PRP of third instar larva; note the nodule in the middle portion (dorso-lateral to posterior view); – **27:** Young puparium on lower portion of the host plant stem surface (dorso-lateral view); arrow shows the shallow groove on stem dug prior to pupation; – **28:** Position of puparium (arrowed), on the side of stem near ground level; note coagulated latex and epiphylls covering the puparium; – **29:** Close-up view of the puparium of the Fig. 28; note darker coloration than the puparium in Fig. 27.





Tab. 1: Distinguishing characters between the third instar larvae of *Allograpta zumbadoi* THOMPSON and *A. centropogonis* NISHIDA.

Character		<i>A. zumbadoi</i> THOMPSON	<i>A. centropogonis</i> NISHIDA
Larval biology		solitary ring leaf miner in early stage, solitary stem borer in late stage	gregarious external leaf feeder in early stage, gregarious leaf miner in middle stage, solitary leaf miner in late stage
Body in general	Body shape	cylindrical to sub-cylindrical	sub-cylindrical to flattened
	Setae	short and stout all over body	lacking setae on ventral surface and anal segment
	Number of abdominal transverse dosal folds per segment	three	four
Posterior respiratory process (PRP)		long (1.0 mm incl. base), nodule in middle part, apex and base sclerotised	short (0.25 mm incl. base), apex sclerotised and base fleshy
Head skeleton	Length/shape	1.35–1.5 mm, robust	1.1 mm, slender
	Number of teeth per row on dorsal surface of labrum	eight	four to five
Antennomaxillary organs		two separate rings	no clear separation between the two rings

rower than basal half, basally and apically broadened with a nodule in middle portion, 0.4 mm wide (Figs 26, 36), width in between nodule and base/apex narrower (0.35 mm); spiracular plates each with three pairs of spiracular openings which extend over side of sclerotised apex, four pairs of interspiracular nodules (Figs 35, 36), often lacking setae. **Puparium** (Figs 27–29, Table 1): ca. 6.0–7.0 mm long, brown to dark brown and covered with brown setae (more visible than in third instar larva), dorsal surface inflated and arched, pupal spiracles absent, anal segment narrow, posterior spiracle present.

Remarks. The third instar larva of *A. zumbadoi* is distinguished from that of *A. centropogonis* by the following characters (Table 1): body sub-cylindrical to cylindrical, body covered with short and stout setae, three transverse dorsal folds, long sclerotised PRP with nodule in middle portion, and head skeleton robust (ca. 1.3 x thicker) and the ‘scooped’ surface of labrum and labium ca. 1.5 x wider (*A. centropogonis* = 0.06–0.07 mm) with more dentations. Additionally, the shape of the antennomaxillary organs differ between the two species: *A. zumbadoi* has two separate rings (Fig. 38) and *A. centropogonis* has no clear separation between the two rings (Fig. 37). Also, the

Figs 30–36: Larval morphology of *Allograpta zumbadoi* THOMPSON. – **30:** Third instar (entire lateral view). Abbreviations: ASP = anterior spiracle, PRP = posterior respiratory process; – **31:** Anal end of the third instar (lateral view). Abbreviations: AL = apical lobe, AS = anal segment, S = sensilla, S7 = abdominal segment 7; – **32:** Head to thorax of the third instar. Abbreviations: ASP = anterior spiracle, HS = head skeleton, MS = mesothorax, MT = metathorax, P = prothorax, S = sensilla; – **33:** Head skeleton of the third instar. Abbreviations: DC = dorsal cornu, LI = labium, LR = labrum, TB = tentorial bar; – **34:** Head skeleton of the first instar. Abbreviations: DC = dorsal cornu, LI = labium, LR = labrum (tentorial bar not drawn); – **35:** Spiracular plates of PRP of the third instar (caudal view). Abbreviations: ES = ecdysial scar, SO = spiracular openings; – **36:** PRP of the third instar (dorsal view). Abbreviations: ES = ecdysial scar, N = nodule, SO = spiracular openings.

two rows of teeth at apex of the labrum of some specimens of *A. centropogonis* were alternating (left side and right side not symmetrical).

Approximately seven out of twenty third instar larvae found boring stems of *C. ferrugineus* in Chirripó and *C. valerii* and *C. talamancensis* in the Cerro de la Muerte, had a shorter PRP (ca. 0.4–0.5 mm, including a fleshy base) (Fig. 40). These larvae probably represent a third species of phytophagous ‘*Allograpta*’ [mentioned as the second species of phytophagous syrphid larvae that occurs on *Centropogon* in NISHIDA et al. (2003)]. Larvae of this third species share host plant species with *A. centropogonis* and *A. zumbadoi*. The body shape, number of teeth on the labium and labrum, and stem-boring biology of the third instar larvae are similar to those of *A. zumbadoi* (also found mining leaves under laboratory conditions); however, they can be separated by the absence of body setae, shorter head skeleton (ca. 0.95–1.0 mm long), two separated labia, four rows of teeth, and the shorter PRP. No adults have been reared from these larvae.

Discussion

Allograpta zumbadoi is the second species from the subfamily Syrphinae which has been confirmed as leaf tissue feeder in their larval stage. It is currently classified in the subgenus *Rhinoprosopa* (THOMPSON et al. 2000), while *A. centropogonis*, is classified in the subgenus *Fazia* (NISHIDA et al. 2003, MENGUAL et al. 2009). F. C. THOMPSON in MENGUAL et al. (2009) proposes a new subgenus with *A. zumbadoi* as the type species, and revises the classification of the genus *Allograpta*. These two species, plus the third stem-boring ‘*Allograpta*’ species mentioned above, all share same species of *Centropogon* as hosts and a similar geographic distribution, including that of *Centropogon* species in Costa Rica. Other species in Costa Rica with unknown immature stages but having very similar distributions to these three species include *A. fasciata* CURRAN, 1932 (INBIO 1997–2006), an undescribed species similar to *A. centropogonis* mentioned in NISHIDA et al. (2003), and an undescribed sibling species of *A. zumbadoi* (F. C. THOMPSON, pers. comm.). The pollen-feeding larva of *A. (Fazia) micrura* (OSTEN SACKEN, 1877) also occurs in middle to high elevations of Costa Rica (WENG & ROTHERAY 2009). These biological data are important since they will add meanings to the phylogenetic and biogeographical studies of Syrphinae and the genus *Allograpta* (MENGUAL et al. 2008).

With respect to the feeding behaviour of the larvae, the circular cut made by the first instar larva prior to mining seems to represent an attempt to minimize exposure to the latex. The cut caused the host plant to exude latex along the cut, thus cutting off the flow of latex into the inside of the circle where larva fed. The first and second rings also probably reduce the amount of latex on the leaf, thus the circular cut is absent on the third mine or the mine made by the early second instar larva. Moreover, first instar larvae of *A. centropogonis* feeding behaviour may also significantly reduce the amount of latex in the plant; the larvae feed externally on the apical shoot causing a substantial amount of latex exudation prior to when the larva starts to mine the leaves (NISHIDA et al. 2003). The toxicity of the latex of *Centropogon* may function as protection against some phytophagous insects (SPEER 1994–2006) and the feeding behaviour of the larvae in both species appears designed to reduce exposure to the latex.

The inner portion of the stem and petiole tissues of the host *Centropogon* plants, where middle to late instar *A. zumbadoi* larvae feed, did not exude latex when damaged, so the latex is apparently limited to only the outer portion where vascular bundles are located. Finally, the coagulated latex along the ring and at the entrance hole may function as a protective barrier against some natural enemies, but this possibility requires further study.

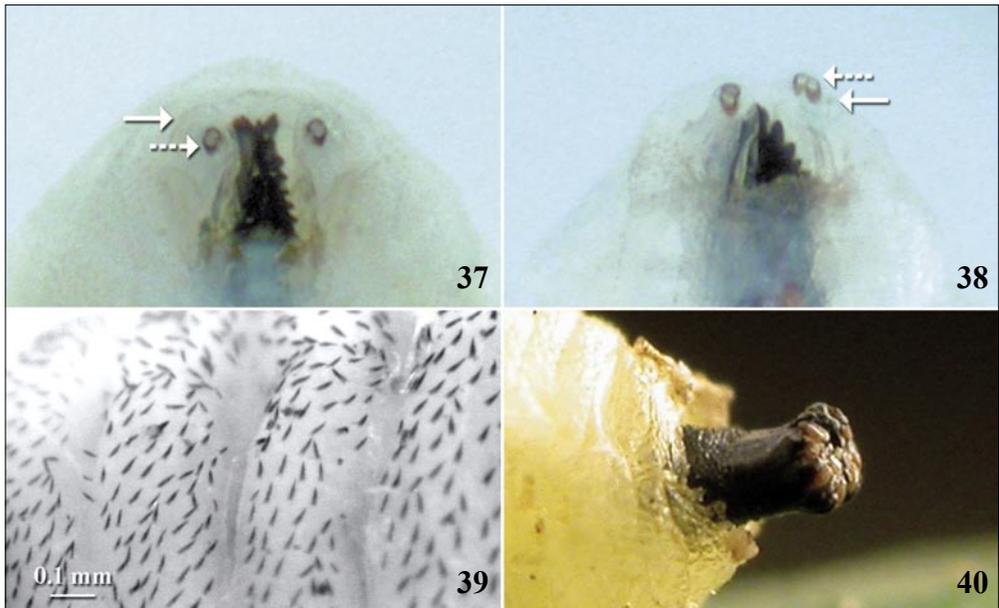


Fig. 37: Ventral view of prothorax of last instar larva of *Allograpta centropogonis* NISHIDA showing tips of the head skeleton and papillae (arrow); note the shape of antennomaxillary organs (dotted arrow). **Fig. 38:** Latero-ventral view of prothorax of last instar larva of *Allograpta zumbadoi* THOMPSON showing tips of the head skeleton and papillae (arrowed); note the shape of antennomaxillary organs (dotted arrow). **Fig. 39:** Setae in subdorsal area of abdominal segments (lateral view) of *Allograpta zumbadoi* THOMPSON (of ethanol preserved specimen). **Fig. 40:** Posterior respiratory process of the third phytophagous '*Allograpta*' species; note the short length and lack of a nodule in middle portion.

The length of the head skeleton of third instar *A. zumbadoi* larva is similar to that of *A. centropogonis*; however, the labrum and labium is ca. 1.5 times wider. In the third *Allograpta* species (the unknown stem borer) the width of the labium and labrum is similar to that of *A. zumbadoi*, although the head skeleton is shorter. The thickening of the labrum and labium in the stem-boring species appears to be an adaptation to feeding in stems, since stem tissue is probably harder than leaf tissue.

The *Woldstedtius* parasitoid found in the puparia of *A. zumbadoi* represents the first diplazontine wasp known to attack a non-predacious flower fly, and apparently this is one of the first cases that a diplazontine has been reared in Costa Rica (P. E. HANSON, pers. comm. 2006). Holarctic Diplazontinae, including *Woldstedtius*, are known to be endoparasitic koinobionts of eggs and larvae of syrphid flower flies that are predacious on small hemipterans (GAULD & HANSON 1997). Although there were some small hemipterans on the *Centropogon* leaves, the larvae were not directly associated with them. At least some Holarctic Diplazontinae that parasitize syrphid predators of hemipterans are attracted by honeydew (GAULD & HANSON 1997), which seems unlikely in the *Woldstedtius* found in the present study. It is unknown in which immature stage of *A. zumbadoi* this parasitoid species oviposits; specimens reared from field-collected second and third instar larvae never produced any parasitoids [see also NISHIDA et al. (2003)] and thus it is possible that the larvae are attacked during the period between when they leave the tunnel and pupate.

Resumen (in Spanish)

Se ilustra y describe la morfología de los estadios inmaduros y la historia natural de la segunda especie de Syrphinae no predatoria, *Allograpta zumbadoi* THOMPSON, 2000. Se compara esta especie con *A. centropogonis* NISHIDA, 2003. Estas son las únicas dos especies conocidas de Syrphinae minadoras foliares. La larva muestra algunas similitudes con la de *A. centropogonis*, como por ejemplo: órganos antenomaxilares rodeando la boca, labro y labio serrados, patrón de sensilas en segmento, y aberturas espiraculares extendidas radialmente. Las diferencias más significativas entre las dos especies se encuentran en la forma y setas del cuerpo, el número de dientes en el labro y el labio, la robustez del esqueleto de la cabeza y la forma de los procesos respiratorios posteriores. Se han encontrado los estadios inmaduros en tres especies de *Centropogon* (Campanulaceae). Las larvas en todos los estadios son solitarias. Las larvas son minadoras de hojas durante el primer estadio y la etapa temprana del segundo estadio, convirtiéndose posteriormente en barrenadoras de tallos. Tanto en el campo como bajo condiciones de laboratorio, el desarrollo de la pupa se dio en la parte de la base del tallo, cerca de las raíces de la planta hospedadora.

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Authors' addresses

Menno P. VAN ZUIJEN
Kolkakkerweg 21-2
6706 GK Wageningen
The Netherlands
E-mail: mennopepijn@gmail.com

Kenji NISHIDA
Escuela de Biología
Universidad de Costa Rica
2060 San José
Costa Rica
E-mail: kenji.nishida@gmail.com

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