DESCRIPTION OF THE LARVAL INSTARS OF *LIXADMONTIA FRANKI* (DIPTERA: TACHINIDAE)

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ABSTRACT

We describe the larval stages of *Lixadmontia franki* Wood & Cave (Diptera: Tachinidae). The fly is a specialist parasitoid of bromeliad-eating weevils and a potential biological control agent for controlling an invasive bromeliad-eating weevil, *Metamasius callizona* (Chevrolat) (Coleoptera: Curculionidae), in Florida. Morphological characteristics that can be used to distinguish the instars of *L. franki*, including the mouth hook and cephalopharyngeal skeleton, body size and color, spinulae patterns, and presence and structure of spiracles, are described, measured, and illustrated. Fly larvae live in the host's body cavity and attach their caudal end to the host's tracheal tubes. The first instar builds a respiratory funnel and the second instar remains attached to the same point and builds upon the funnel. The third instar does not build upon the respiratory funnel. The third instar disconnects from the respiratory funnel shortly before exiting the host. First and second instars are metapneustic, but the third instar is amphipneustic.

Key Words: Larval development, Cyclorrhapha, *Metamasius*, parasitoids

RESUMEN

Se describen los estadios larvales de *Lixadmontia franki* Wood y Cave (Diptera: Tachinidae). La mosca es un parasitoide especialista en picudos que atacan a las bromélias y un agente potencial de control biológico para controlar un picudo invasivo, *Metamasius callizona* (Chevrolat) (Coleoptera: Curculionidae), en Florida. Se describen, miden e ilustran las características morfológicas que se pueden usar para distinguir los estadios de *L. franki*, incluyendo el gancho bucal y el esqueleto cefalofaríngeo, el tamaño y color del cuerpo, el patrón de espinitas y la presencia y estructura de los espiráculos. Las larvas de la mosca viven en la cavidad corporal del hospedero y se sujetan su ápice caudal a los tubos traqueales del hospedero. El primer estadio construye un túbulo respiratorio y el segundo estadio se queda sujetada al mismo punto y aumenta el túbulo. El tercer estadio no aumenta el túbulo respiratorio. El tercer estadio desconecta del túbulo respiratorio poco antes de salir del hospedero. Los primeros dos estadios son metanéusticos, pero el tercer estadio es anfinéustico.

Palabras Clave: Desarrollo larval, Cyclorrhapha, *Metamasius*, parasitoides

This paper describes the larval instars of *Lixadmontia franki* Wood & Cave (Diptera: Tachinidae), a specialist parasitoid of bromeliad-eating weevils, whose adult was described by Wood & Cave (2006). *Lixadmontia franki* is native to montane cloud forests in Honduras and Guatemala, where its natural host is *Metamasius quadrilineatus* Champion (Coleoptera: Curculionidae), a bromeliad-eating weevil that consumes bromeliads in the genera, *Tillandsia, Vriesea*, and *Guzmania* spp. (Suazo et al. 2008). *Lixadmontia franki* is of interest because of its potential use as a biological control agent to control an invasive bromeliad-eating weevil, *Metamasius callizona* (Chevrolat), in Florida (Frank & Cave 2005). *Metamasius callizona* is native to Mexico, Guatemala, and Belize. The weevil was discovered established on native bromeliad populations in Florida in 1989, and since then the weevil has spread to nearly fill its new potential range and has caused great destruction to native bromeliad populations (Frank & Thomas 1994; Frank & Cave 2005). In the Enchanted Forest Sanctuary, Titusville, Florida, the weevil destroyed 87% of a large bromeliad population in 6 months and, at 27 months, 97% of the population was destroyed (Cooper et al. 2013). In the laboratory, *L. franki* was shown to parasitize *M. callizona* at least as readily as it parasitizes *M. quadrilineatus* (Frank & Cave 2005). In 2007, after a description of the fly was made and host-range testing showed the fly to be specific to bromeliad-eating weevils, permission was received to release the fly. Since then, we have made several releases in 8 weevil-infested

sites in Florida throughout the seasons. Releases were made from 29 June 2007 to 25 April 2013. Post-release monitoring has resulted in a single incidence of parasitism in the field (Cave 2008; Cooper et al. 2011).

Like many other tachinid species (Wood 1987; Foote 1991; Stireman et al. 2006; O'Hara 2008a), *L. franki* is ovolarviparous (Cooper 2009) and has first instars that actively search for hosts (Suazo et al. 2008). *Lixadmontia franki* female flies have modified common oviducts that function as brood chambers in which embryos develop to first instars (Suazo et al. 2008). *Lixadmontia franki* requires 8 days post-mating before neonate larvae become apparent in the brood chamber. A gravid *L. franki* can have up to 50 larvae and 100 eggs in her brood chamber at a time. The female deposits eggs containing completely developed embryos on weevil-infested bromeliads. The larvae hatch almost immediately, then move into the infested plant and search for weevil larvae. Upon finding a weevil larva, the fly larva uses its mouth hook to make a hole in the weevil larva's integument, usually on an intersegmental membrane, and then slips through the hole, into the host's body. The fly larva grows and develops inside its still-living host. The host dies just before the fly larva emerges from the host and pupates. A weevil host may support 1 to several fly larvae (Cave 2008).

Tachinid larvae respire by attaching posteriorly to the host's integument (usually at the entry wound) or tracheal tube, thus connecting the parasitoid's posterior spiracles with the ambient air (Thompson 1960; Foote 1991; Michalková et al. 2009). When the first instar enters a host, it may remain in the body cavity or migrate to a particular region in the host, such as the peritrophic membrane, salivary glands, ganglia, or muscles (Thompson 1960; Ichiki & Shima 2003; O'Hara 2008a; Michalková et al. 2009). Larvae that reside in host tissue other than the body cavity may pull tracheal tubes into that region and then attach to the tubes (Ichiki & Shima 2003). The point at which the parasitoid attaches itself may be fixed (Thompson 1960; Michalková et al. 2009) or the larvae may be able to change position within the host (Ichiki & Shima 2003). Some larvae may migrate from particular host tissues back to the host's body cavity (O'Hara 2008a). Larvae that reside in the host's body cavity must contend with the host's phagocytic immune response. Tachinids have done so by hi-jacking the phagocytes and using them to form a respiratory funnel at the point of attachment to the integument (Michalková et al. 2009) or tracheal tube (Thompson 1960; O'Hara 2008a). We wanted to know where in *M. callizona L. franki* larvae reside, whether they create respiratory funnels, and whether they remain attached to the same location or change points of attachment.

The mouth hook and cephalopharyngeal skeleton are derived characteristics of muscomorphous brachycerans that are composed of remnants of cranial sclerites and various mouthparts that have retracted into the thorax (Foote 1991). The mouth hook and cephalopharyngeal skeleton have been used for distinguishing muscomorphan instars of a species (Pettit 1990; Lawrence 1997; Ubero-Pascal et al. 2012) as well as from other species (James & Gassner 1947; Thompson 1960; O'Hara 2008b) and have played an important part in unraveling the phylogenetic relationships within this Division (Foote 1991; O'Hara 2008a). We observed and measured the mouth hook and cephalopharyngeal skeleton of each *L. franki* instar and used these observations to distinguish *L. franki* first, second, and third instars from each other and to compare the mouth hooks and cephalopharyngeal skeletons of *L. franki* with those of other tachinid larvae. We also observed body shape and color of *L. franki* as well as spinulae patterns and posterior and anterior spiracles.

Materials and Methods

Host and Parasitoid Sources

Metamasius callizona larvae and *L. franki* adults were taken from colonies reared at the Entomology and Nematology Department at the University of Florida in Gainesville, Florida, as well as from colonies that were kept at the Hayslip Biological Control Research and Containment Laboratory at the Indian River Research and Education Center in Ft. Pierce, Florida. The method used for rearing *L. franki* was based on a method designed by Suazo et al. (2006). For maintenance of the weevil and fly colonies, weevils were reared on pineapple tops. Pineapple tops infested with third instar weevils were exposed to flies for parasitism for 10 days then removed, and the weevil larvae were extracted from the plants. Weevil larvae were fed pineapple leaves and monitored daily for fly larvae exiting the weevil larvae and subsequently pupating. Fly puparia were kept in 15 mm × 140 mm Petri dishes with moist paper towel and emerging adult flies were either returned to the colony or used for experimentation. To get weevil larvae for artificial parasitism, we kept egg-laying *M. callizona* individually in 18.5-ml (5-dram) vials and gave each a fresh piece of pineapple leaf daily (about 5 cm of leaf cut from the base of the leaf). The weevils used the leaves for eating and oviposition. Eggs were harvested daily. When we fed the weevils, we checked the leaves that we removed from the vials for eggs. Those leaves containing an egg were collected and set individually in 20 mm × 60 mm Petri dishes with a piece of moist paper towel. When the weevil larvae hatched, they were placed individually in 35-ml cups filled with pineapple mash. The

mash was pineapple stems and leaves chopped up in a food processor and pressed into the cup. The larvae in these cups were used for artificial larviposition.

Artificial Larviposition and Instar Descriptions

As adult flies emerged from their puparia, they were sexed and placed in 0.6 × 0.6 m metal-framed cages with nylon screen, where they were allowed to mate. The cages were misted several times a day to maintain proper humidity and to provide water. A pollen/honey mixture was provided for the flies. Once mated, the females were separated from the males. Between 9 and 14 days after mating (by which time pharate first instar fly larvae would have developed), gravid females were dissected in tap water under a microscope, stunned but still alive. A fly was stunned by placing it into a vial filled with water then vigorously shaking the vial. The fly was removed from the vial and its abdomen was opened and the brood chamber removed. The neonate larvae were released by opening the brood chamber with sharp forceps, and then gently nudging the larvae out with the bristles of a small paint brush. Larvae seen moving were extracted with a dropper and squirted into 35-mL cups containing a third instar weevil on pineapple mash. Five fly larvae were deposited per cup. Thirty weevil larvae were artificially parasitized then kept at 25 °C. At 1-10 days after artificial larviposition, the weevil larvae were dissected and searched for *L. franki* larvae developing inside the host. Three weevil larvae were dissected each day.

Observations and measurements were made using a compound microscope with a drawing tube (Leica MZ16) and from images that were taken using scanning electron microscopy. Measurements of the body size (length and width), length of the mouth hook (from the anterior tip of the mouth hook to the base of the mandibular sclerite), length of cephalopharyngeal skeleton (from the anterior edge of the hypopharyngeal sclerite to the posterior tip of the dorsal wing), and width of the respiratory funnel (at the widest point of the sclerotized part of the funnel) were taken for each instar. Averages and confidence intervals were calculated for each trait and compared.

Other observations included: location of fly larvae in the host; body color; the presence or absence of spinulae and, if present, the shape and location of the spinulae; the method of respiration for each instar; and the type (fixed or mobile) and point (host integument or tracheal tube) of attachment. Drawings were made of the 3 instars' bodies and mouth hooks and cephalopharyngeal skeletons.

Lixadmontia franki is in the subfamily Exoristinae in the tribe Blondeliini (Wood & Cave 2006). The mouth hooks and cephalopharyngeal skeletons of *L. franki* instars were compared with those of other tachinids in the subfamily Exoristinae. These included *Lixophaga diatraeae* (Townsend) (tribe Blondeliini) (Thompson 1960); *Exorista larvarum* (Linnaeus) (tribe Exoristini) (Michalková et al. 2009); *Chetogena lophyri* (Townsend) (= *Phorocera hamata*) (tribe Exoristini) (Baldwin & Coppel 1949); and *Smidtia* (= *Omotoma*) *fumiferanae* (Tothill) (tribe Winthemiini) (Coppel & Smith 1957) (ITIS 1997).

Results

One hundred fifty fly larvae were artificially larviposited on 30 weevil larvae. Thirty-3 fly larvae were found in 17 of the weevils. Twenty-six of the fly larvae were alive and 7 were dead. Of the 26 living larvae, 8 were first instars, 10 were second instars, and 8 were third instars. Of the dead larvae, 2 were first instars, 3 were second instars, and 2 were third instars. The number of fly larvae found in a host ranged from 0 to 5 (13 with 0; 7 with 1 fly larva; 7 with 2 larvae; 1 with 3 larvae; 1 with 4 larvae; and 1 with 5 larvae).

All 3 *L. franki* instars resided in the body cavity of the host. Two of the first instars were found living freely in the host, just under the integument; they were the first larvae dissected from the weevil larvae, on the second day after artificial larviposition. All other first instars were found already attached to the lateral, longitudinal trunk of the host's tracheal system, usually near the host's anterior or posterior spiracles. First instars formed respiratory funnels at the point of attachment. All second instars and 3 of the third instars were found attached to the respiratory funnel that was formed by the first instar. The other 5 third instars were detached and living freely inside of the host. First and second instar exuviae remained attached to the funnel after each molt.

For all measurements, $n = 8$ for first instars; 10 for second instars; and 8 for third instars, except for measurements of the respiratory funnels, where $n = 6$ for first instars; 10 for second instars; and 3 for third instars.

First Instar

Description. Body elongate, rounded anteriorly and pointed posteriorly (Fig. 1a). Cephalic segment spineless with a smooth edge and subtle features. Integument transparent. Anterior edges of thoracic and abdominal segments encircled by wide bands of spinulae. The widths of the bands of spinulae not consistent; spinulae that form the bands not in straight, parallel lines, but randomly and homogenously situated within the band. Spinulae on thoracic segments form wider bands (12 to 16 spinulae wide) than the spinulae that circle

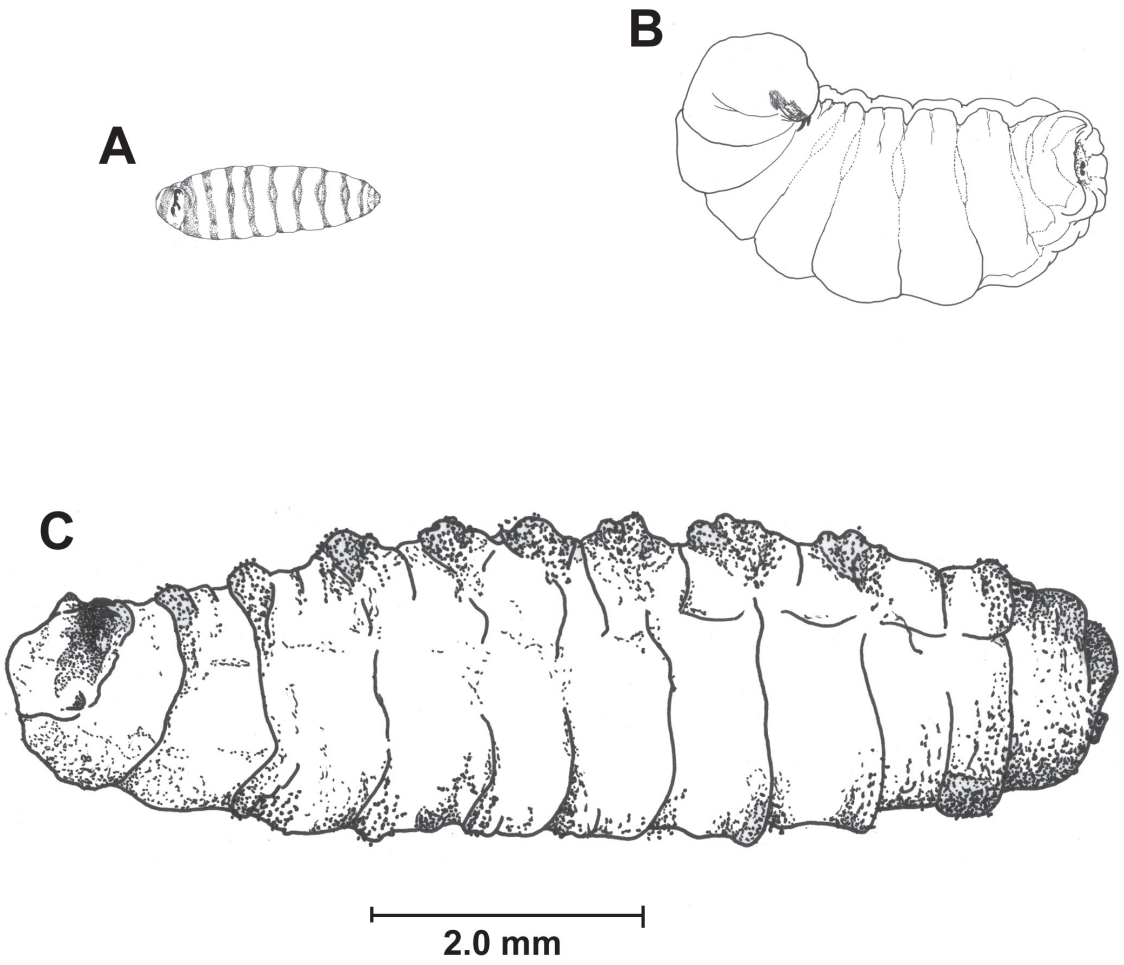

Fig. 1. Ventral view of *Lixadmontia franki* A) first instar; and lateral view of *L. franki* B) second instar; and C) third instar.

the abdominal segments (4 to 6 spinulae wide). Ventral side of abdomen with bands of spinulae split medial-ventrally with a bare area between them. No spinulae were noted on the caudal segment. Mean body length and width 1.31 mm and 0.48 mm, respectively (Table 1).

Cephalopharyngeal skeleton long and slender and unarticulated (Fig. 2a). Labrum hatchet-like. Dorsal edge of labrum straight; slight bump at antero-dorsal angle. Anterior and posterior edges of labrum smooth, slightly convex, and converging to form a blunt ventral tip. Labrum lightly sclerotized. Lateral sclerites long and thin anteriorly, widening posteriorly; lightly sclerotized. Dorsal edge of intermediate region with a bump at anterior ends of hypopharyngeal sclerites; otherwise,

TABLE 1. LISTED ARE THE MEANS AND CONFIDENCE INTERVALS FOR THE LENGTH AND WIDTH OF THE BODY, LENGTH OF THE MOUTH HOOK AND CEPHALOPHARYNGEAL SKELETON, AND THE WIDTH OF THE RESPIRATORY FUNNEL FOR *LIXADMONTIA FRANKI* INSTARS.

Instar	Body length (mm) ± CI	Body width (mm) ± CI	Mouth hook length (mm) ± CI	Cephalopharyngeal skeleton length (mm) ± CI	Width of the respiratory funnel (mm) ± CI
1	1.31 ± 0.27	0.48 ± 0.18	0.052 ± 0.013	0.10 ± 0.021	0.18 ± 0.014
2	2.91 ± 0.34	0.97 ± 0.086	0.11 ± 0.024	0.26 ± 0.026	0.85 ± 0.086
3	7.59 ± 0.65	2.30 ± 0.32	0.24 ± 0.027	0.58 ± 0.04	0.87 ± 0.13

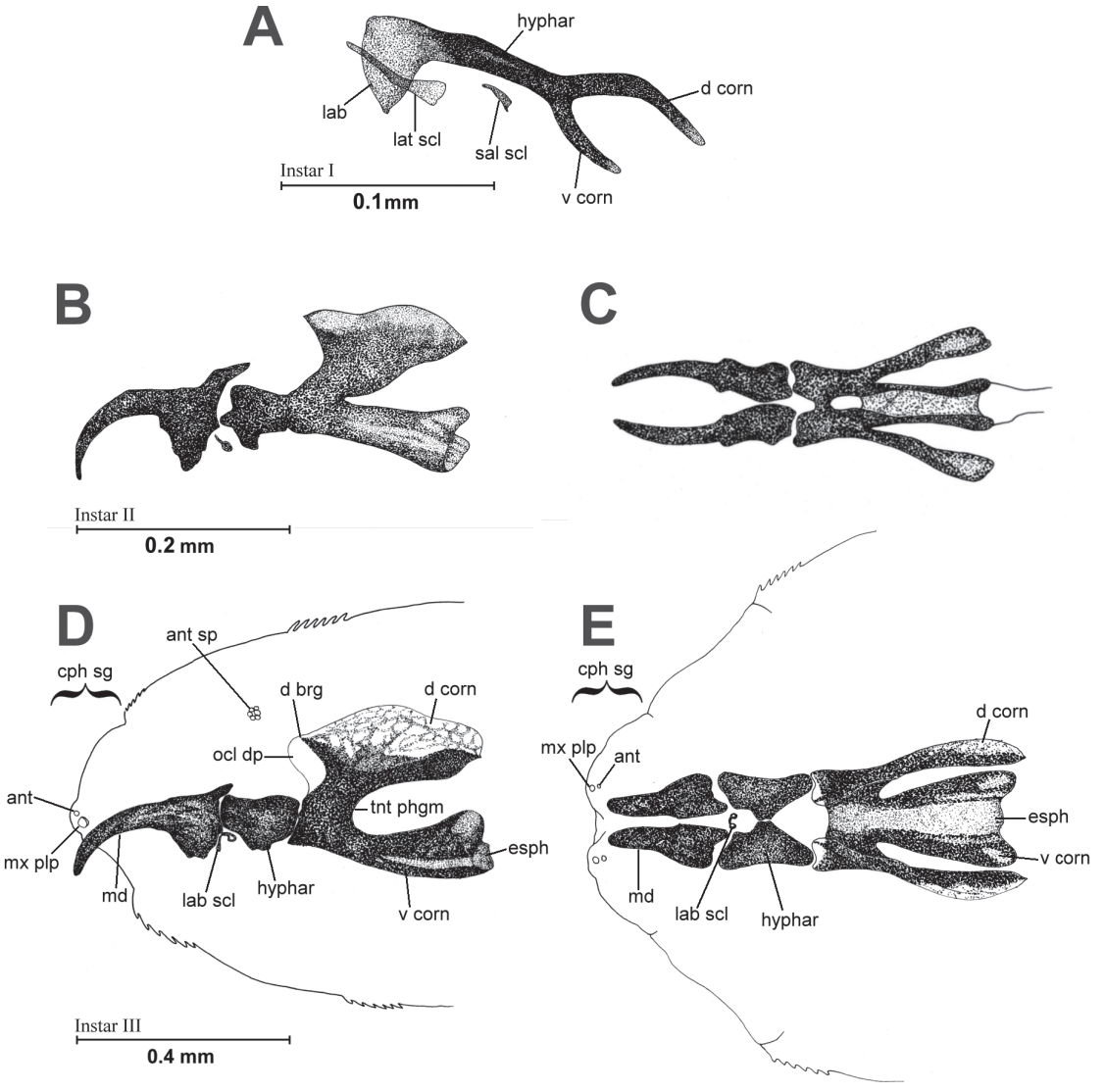

Fig. 2. Lateral view of *Lixadmontia franki* mouth hook and cephalopharyngeal skeleton for A) first instar, lateral view; B) second instar, lateral view; C) second instar, dorsal view; D) third instar, lateral view; and E) third instar, dorsal view. Abbreviations: ant = antenna; ant sp = anterior spiracle; cph sg = cephalic segment; db = dorsal bridge; dc = dorsal cornu; esph = esophagus; hyphar = hypopharyngeal sclerite; lab = labrum; lab scl = labial sclerite; lat scl = lateral sclerite; md = mandible; mx plp = maxillary palpus; ocl dp = ocular depression; sal scl = salivary duct sclerite; tnt phgm = tentorial phragma; v corn = ventral cornu.

dorsal and ventral edges are straight and move smoothly into dorsal and ventral cornua. Salivary duct sclerites long and thin anteriorly, widening posteriorly; moderately sclerotized; located below hypopharyngeal sclerites. Hypopharyngeal sclerites and dorsal and ventral cornua heavily sclerotized except posterior ends of cornua, which are lightly sclerotized. Dorsal cornua longer than ventral cornua but about the same width. Mean length of labrum 0.052 mm; mean cephalopharyngeal skeletal length 0.10 mm (Table 1).

Respiratory system metapneustic. Posterior spiracles slightly protruding from caudal end. No apparent spinulae around posterior spiracles. Respiratory funnel in the shape of a funnel with tip of funnel attached to the host's tracheal tube and then widening to encircle the posterior end of the maggot. Respiratory funnel small and delicate, lightly and uniformly sclerotized (Fig. 3a), sometimes with a membrane that enclosed the maggot. Mean respiratory funnel width 0.18 mm (Table 1).

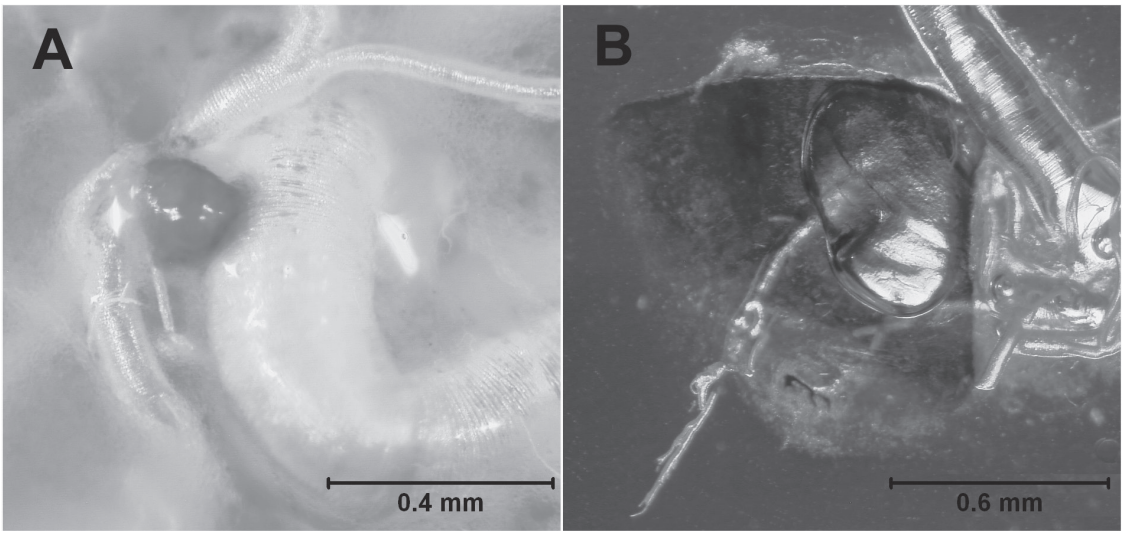

Fig. 3. Respiratory funnel for *Lixadmontia franki* A) first instar and B) second instar.

First instars were found 2-5 days after artificial larviposition.

Lixadmontia franki versus *L. diatraeae*. The mouth hook and cephalopharyngeal skeleton of first instar *L. franki* and first instar *L. diatraeae* are slender in form and have hatchet-like labrums and dorsal cornua that are longer than the ventral cornua. Differences between the mouth hooks and cephalopharyngeal skeletons of these 2 species are:

- *L. franki* labrum with blunt ventral tip; *L. diatraeae* labrum with rounded ventral apex.
- Anterior edge of *L. franki* labrum smooth, slightly convex; anterior edge of *L. diatraeae* feebly dentate along its upper three-fourths.
- *L. franki* lateral sclerites long and slender anteriorly but widening posteriorly; *L. diatraeae* lateral sclerites long and slender, not widening posteriorly.
- *L. franki* dorsal and ventral cornua of similar width; *L. diatraeae* ventral cornua wider than dorsal cornua.
- *L. franki* hypopharyngeal sclerites transition smoothly into dorsal and ventral cornua; *L. diatraeae* has smooth transitions from hypopharyngeal sclerites into dorsal cornua, but the ventral edges of the hypopharyngeal sclerites transitioning to the ventral cornua are interrupted by anterior edges of the ventral cornua that are almost perpendicular to the longitudinal axis then curving anteriorly into a point at antero-ventral angle.
- *L. franki* salivary duct sclerites relatively small, long and thin anteriorly and widening posteriorly; *L. diatraeae* salivary duct sclerites relatively large and anvil-shaped.

Lixadmontia franki versus *Exorista larvarum*. The mouth hooks of the first instars of these 2 species differ in that:

- *L. franki* labrum hatchet-like with smooth edges; *E. larvarum* labrum hook-like, curved ventrally with acute anterior point and with dorsal sawtooth edge.

Lixadmontia franki versus *Chetogena lophyri*. Differences between the mouth hooks and cephalopharyngeal skeletons of the first instars of these 2 species are:

- *L. franki* labrum hatchet-like and with smooth dorsal edge; *C. lophyri* labrum hook-like, curved ventrally with acute anterior point and with 10 prominent teeth on dorsal edge.
- *L. franki* dorsal and ventral cornua of similar width; *C. lophyri* dorsal cornua broader than ventral cornua.

Lixadmontia franki versus *Smidtia fumiferanae*. Differences between the mouth hooks and cephalopharyngeal skeletons of the first instars of these 2 species are:

- *L. franki* labrum hatchet-like, terminating in blunt tip; *S. fumiferanae* labrum hook-like, curved ventrally with acute anterior point and serrated dorsal edge.
- *L. franki* dorsal and ventral cornua of similar width; *S. fumiferanae* dorsal cornua broader than ventral cornua.

Second Instar

Description. Body elongate, rounded anteriorly and posteriorly (Fig. 1b). Cephalic segment spineless with antennae and maxillary palps on antennomaxillary lobes. Integument semi-opaque, white to cream-colored. Anterior and posterior margins of thoracic and abdominal segments encircled with spinulae bands with widths of 5 – 6 spinulae. Similar to first instars, the widths of the bands of spinulae not consistent; spinulae that form the bands not in straight, parallel lines, but randomly and homogenously situated within the band. Spinulae triangular, relatively much smaller and sparser than the spinulae on first and third instars and with much less body area covered by spinulae (Figs. 4a-b). Mean body length and width 2.91 mm and 0.97 mm, respectively (Table 1).

Mandibular sclerites heavily sclerotized (Figs. 2b-c). Mandibles curved ventrally and terminating in acute point. Base of mandibular sclerites with dorsal and ventral processes. Dorsal process slender, arising almost perpendicular to the longitudinal axis, then bending and pointing dorso-posteriorly; posterior end with smooth, rounded tip. Ventral process somewhat triangular with rounded ventral apex. Labial sclerites small with slender, elongate anterior portion and circular posterior portion. Hypopharyngeal sclerites heavily sclerotized; block-shaped when viewed laterally and H-shaped when viewed dorsally. Dorsal and ventral cornua not as heavily sclerotized as the hypopharyngeal and mandibular sclerites. Dorsal cornua with lighter sclerotization along dorsal edge. Dorsal bridge pointed anteriorly. Dorsal cornua broader but about the same length as ventral cornua. Dorsal and ventro-posterior edges of dorsal cornua wavy, converging and terminating in a point at the posterior end. Ventral cornua long and with consistent width, with a slight widening at dorso-posterior angle. Lightened sclerotization in dorso-posterior corner of ventral cornua and longitudinally along the midline of the lateral side. Mean length of mouth hook 0.11 mm; mean cephalopharyngeal skeletal length 0.26 mm (Table 1)

Respiratory system metapneustic. Anterior spiracles absent (Fig. 4a). Posterior spiracles asymmetrical, with 3 lobes, 2 with spiracular openings that radiate from the ecdysial scar (Fig. 5a). Distance between the posterior spiracles about twice the width of a spiracle. Caudal end lacking spinulae. Respiratory funnel heavily sclerotized from tip to about 2/3 of the way to distal edge then lightly sclerotized (Fig. 3b). Mean respiratory funnel width 0.85 mm (Table 1).

Second instars were found 2-8 days after artificial larviposition.

Lixadmontia franki versus *L. diatraeae*. The mouth hook and cephalopharyngeal skeleton of *L. franki* second instars are similar in shape and appearance with the mouth hook and cephalopharyngeal skeleton of second instar *L. diatraeae*. Both have mandibular sclerites strongly curved ventrally and terminating in an acute point and dorsal and ventral processes on the bases of the mandibular sclerites. Differences between the mouth hooks and cephalopharyngeal skeletons of the second instars of these 2 species are:

- *L. franki* mandibles not as strongly curved ventrally as *L. diatraeae* mandibles.
- *L. franki* dorsal process slender, bent at center, pointing dorso-posteriorly, terminating in smooth, rounded tip; *L. diatraeae* dorsal process not bent, terminating in bi-lobed apex.
- *L. franki* dorsal and ventral cornua elongate; *L. diatraeae* dorsal and ventral cornua very broad and compact appearance.

Lixadmontia franki versus *Chetogena lophyri*. Differences between the mouth hooks and cephalopharyngeal skeletons of the second instars of these 2 species are:

- *L. franki* mandibles more ventrally curved; *C. lophyri* mandibles directed ventrally without curvature.
- *L. franki* dorsal cornua not very broad and with wavy dorsal and ventro-posterior edges that terminate in posterior point; *C. lophyri* dorsal cornua oval shaped with smooth edges and no posterior point.

Lixadmontia franki versus *Smidtia fumiferanae*. Differences between the mouth hooks and cephalopharyngeal skeletons of the second instars of these 2 species are:

- *L. franki* mandibles relatively longer than *S. fumiferanae* mandibles.
- *L. franki* dorsal and ventral processes on bases of mandibular sclerites slender, not stocky, dorsal process with blunt, rounded tip; *S. fumiferanae* dorsal and ventral processes on bases of mandibular sclerites large and stocky, dorsal process spear-shaped.
- *L. franki* dorsal wing with wavy dorsal edge; *S. fumiferanae* dorsal wing with rectangular protrusion on dorsal edge of dorsal cornua.

Third Instar

Description. Body stout, rounded anteriorly and posteriorly (Fig. 1c). Cephalic segment spineless with antennae and maxillary palps on antennomaxillary lobes (Figs. 2d-e). Integument yellow to cream-colored. Anterior margins of thoracic and abdominal segments encircled with bands of spinulae (Fig. 4c). A few types of spinulae present: triangular, occurring singly (Fig. 6a) or in

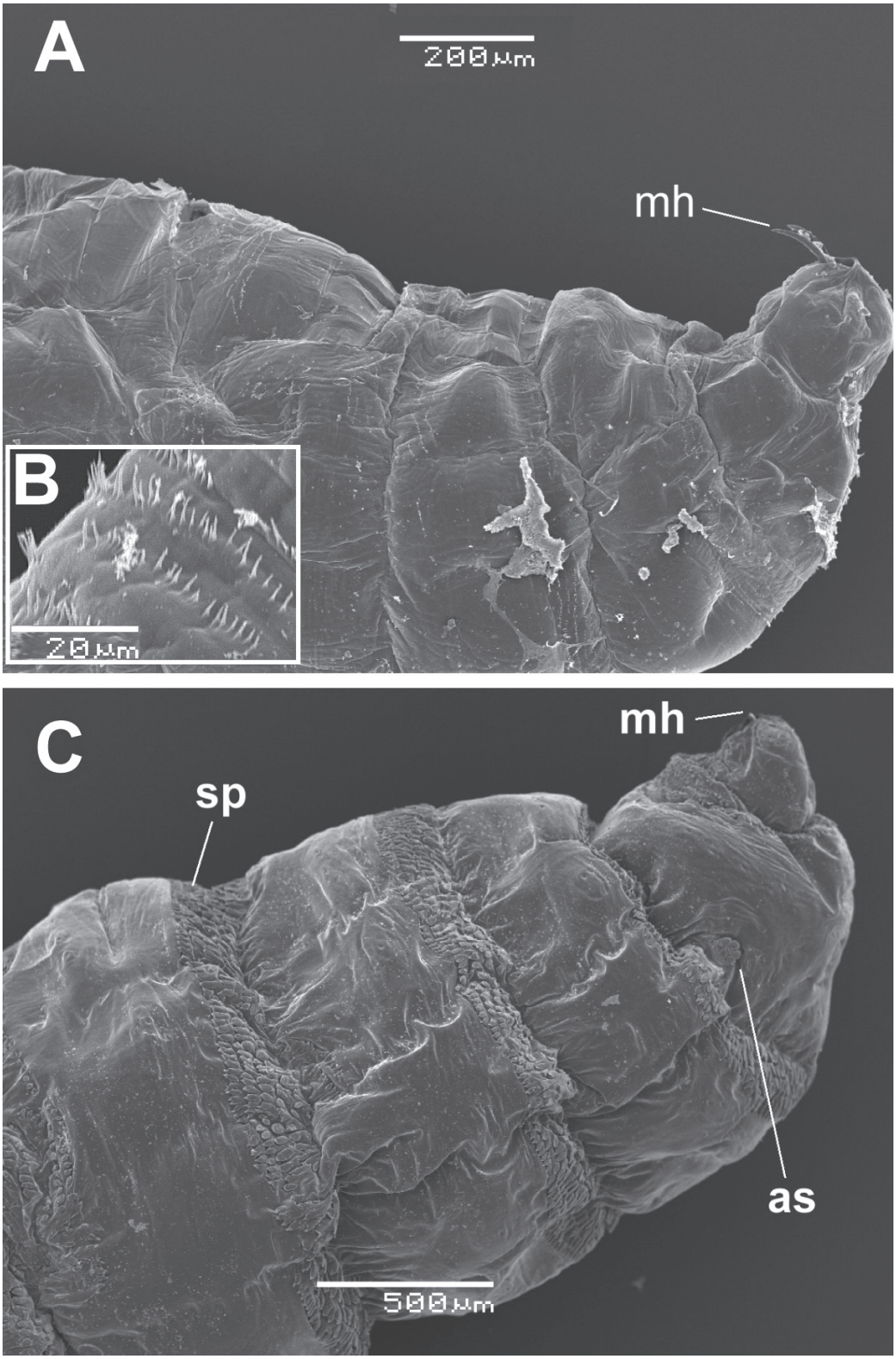

Fig. 4. Lateral view of the anterior body of *Lixadmontia franki* A) second instar; B) close up of second instar spinulae, and C) third instar. Abbreviations: as = anterior spiracle, mh = mouth hook, sp = spinulae.

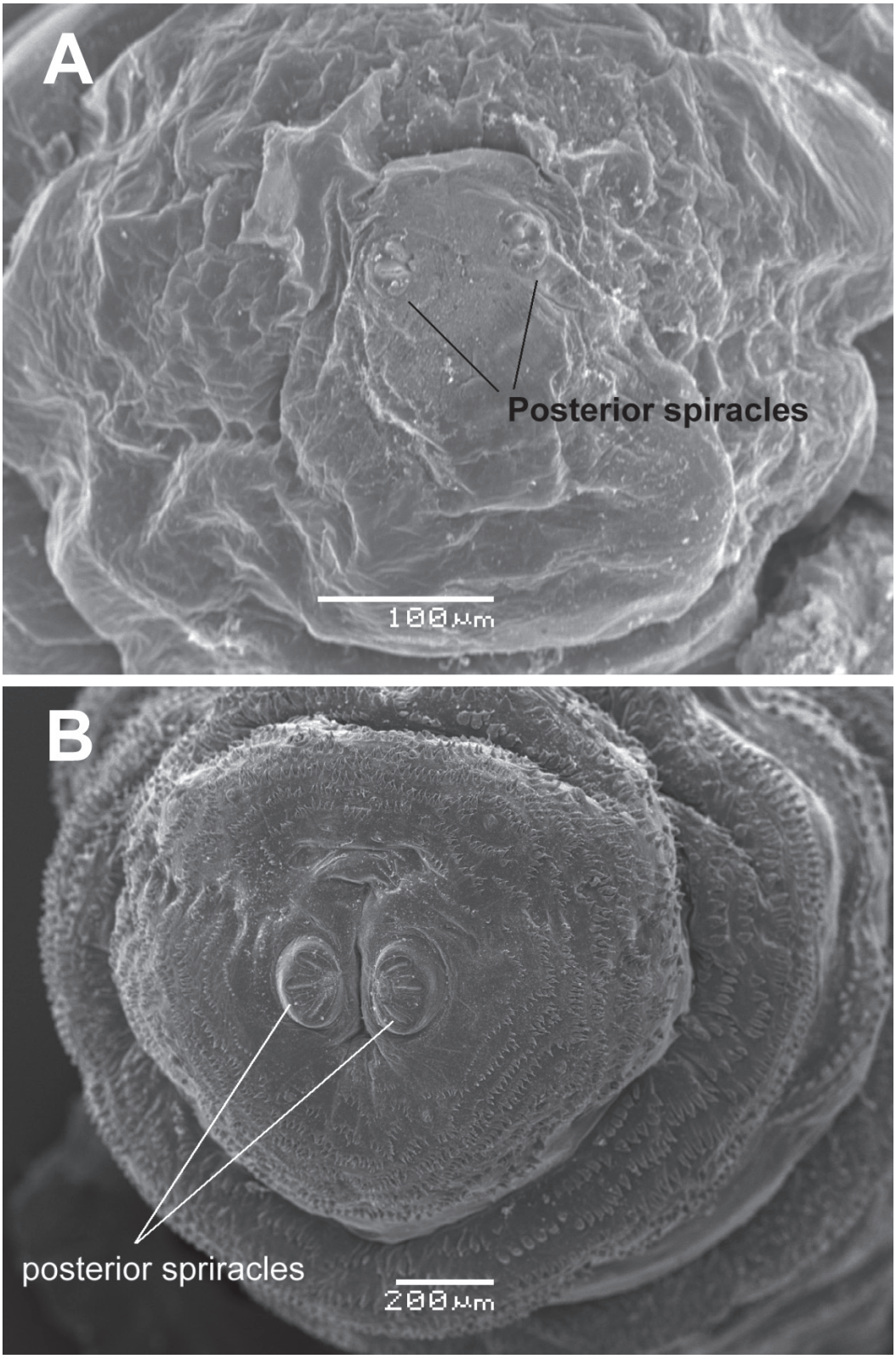

Fig. 5. Posterior end of *Lixadmontia franki* A) second instar; and B) third instar, showing posterior spiracles.

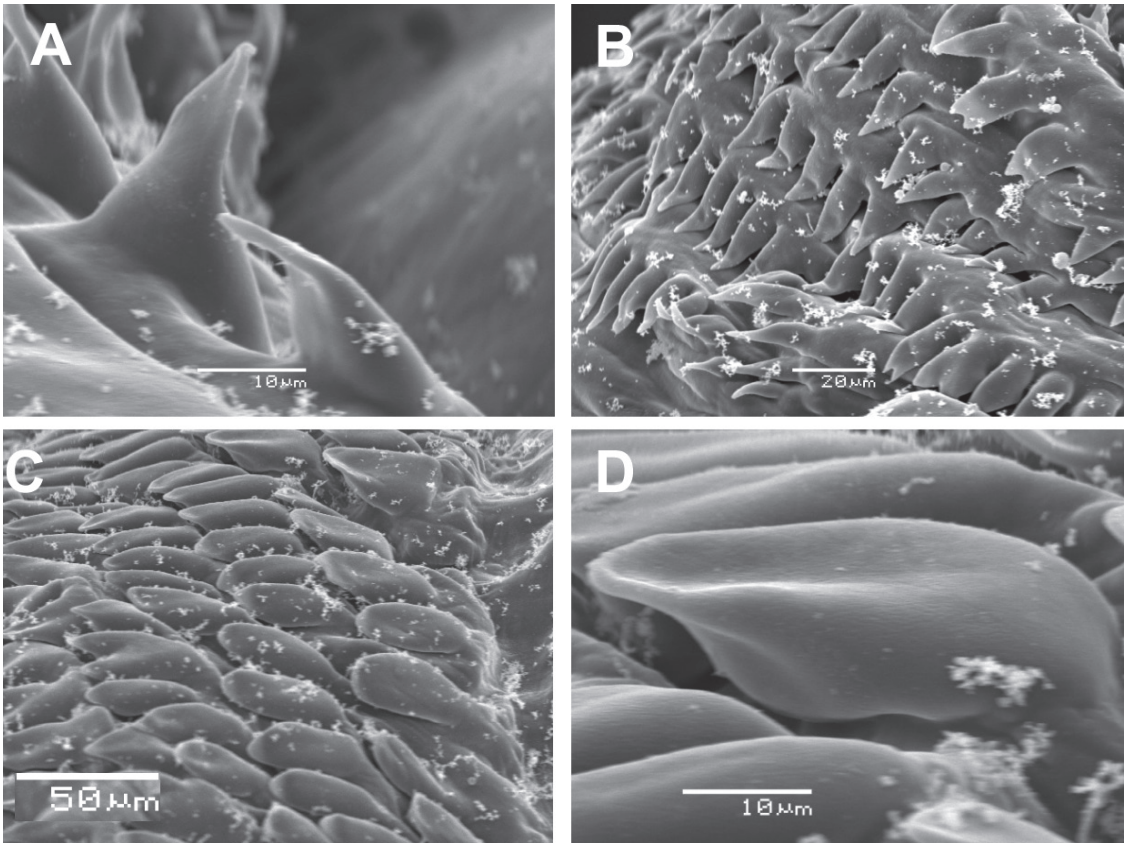

Fig. 6. Spinulae on *Lixadmontia franki* third instar body: A) triangular shaped; B) triangular shapes in overlapping sheets; C) ovoid shaped; and D) ovoid shaped with concave distal surface.

overlapping sheets (Fig. 6b); and ovoid (Fig. 6c), sometimes with concave distal surface (Fig. 6d). Location and shape of spinulae variable for a given specimen; in general, the overlapping sheets of triangular-shaped spinulae circled the first thoracic segment; singly occurring triangular shaped spinulae found on lateral and dorsal sides of body. Ovoid shaped spinulae found on dorsal and ventral surfaces of the body; those on the ventral side with well-defined concavity on distal surface. Mean body length and width 7.59 mm and 2.30 mm, respectively (Table 1).

Mandibular sclerites heavily sclerotized (Figs. 2d-e). Mandibles parallel to longitudinal axis then curving ventrally, terminating in an acute point. Dorsal and ventral processes on bases of mandibular sclerites. Dorsal process small and thin, terminating in blunt, rounded tip. Ventral process short and block-shaped. Labial sclerites with inverted V-shape; anterior portion long and slender, posterior portion circular with a hole in the center. Hypopharyngeal sclerites heavily sclerotized, somewhat block-shaped laterally, H-shaped dorsally. Dorsal cornua heavily sclerotized along posterior half up into dorsal bridge. Dorsal half of dorsal cornua transparent with variably sclerotized tissue rising up from the heavily sclerotized posterior portion, creating a pattern of lines and swirls. The patterns varied with specimens. Part of dorsal cornua anterior to dorsal bridge transparent. Dorsal cornua similar to second instar, with wavy dorsal and ventro-posterior edges that converge and terminate in a point at the posterior end. Ventral cornua similar to second instar, long and with consistent width, then flaring at the dorso-posterior end, and with areas of lightened sclerotization in dorso-posterior corner of ventral cornua and longitudinally along the mid-line of the lateral side. On the third instar, these areas of lightened sclerotization are more pronounced because of the relatively heavier sclerotization (compared with the second instar) that surrounds the areas. Mean length of mouth hook 0.24 mm; mean cephalopharyngeal skeletal length 0.58 mm (Table 1).

Respiratory system amphipneustic. Anterior spiracles located posterolaterally on prothorax (Fig. 4c). Posterior spiracles protruding and ringed by triangular spinulae (Fig. 5b); peritreme heavily pigmented; asymmetrical; about 2.5

times wider than distance between them; with 4 lobes, 3 of which with a spiracular slit; lobes and slits radiating from ecdysial scar. Respiratory funnel similar to second instar respiratory funnel in sclerotization and size (mean respiratory funnel width 0.87 mm; Table 1).

Third instars were found 7-10 days after artificial larviposition.

Lixadmontia franki versus *L. diatraeae*. The mouth hook and cephalopharyngeal skeleton of third instar *L. franki* are similar in shape and appearance to the mouth hook and cephalopharyngeal skeleton of third instar *L. diatraeae*. Both have mandibular sclerites that are less ventrally curved than their respective second instars; and dorsal and ventral processes on the bases of the mandibular sclerites that are similar in shape and size. Differences between the mouth hooks and cephalopharyngeal skeletons of these 2 species are:

- *L. franki* dorsal and ventral cornua are about the same length; *L. diatraeae* dorsal cornua are longer than ventral cornua.
- *L. franki* dorsal and ventral cornua not broad; *L. diatraeae* dorsal and ventral cornua broad.
- *L. franki* dorsal cornua with wavy dorsal and ventro-posterior edges that with posterior point; *L. diatraeae* dorsal cornua with smooth edges, slightly pointed posteriorly.

Lixadmontia franki versus *Chetogena lophyri*. Differences between the mouth hooks and cephalopharyngeal skeletons of the third instars of these 2 species are:

- *L. franki* mandibles ventrally curved; *C. lophyri* mandibles short and directed anteroventrally.
- *L. franki* dorsal cornua with wavy dorsal and ventro-posterior edges that terminate in posterior point; *C. lophyri* dorsal cornua with smooth edges and no posterior point.

Lixadmontia franki versus *Smidtia fumiferanae*. Differences between the mouth hooks and cephalopharyngeal skeletons of the third instars of these 2 species are:

- *L. franki* dorsal wing triangular shaped with wavy dorsal edge and posteriorly pointed; *S. fumiferanae* dorsal wing rectangular shaped and without wavy edge.
- *L. franki* ventral cornua truncated at posterior end; posterior end of *S. fumiferanae* ventral cornua scooped.

Distinguishing *L. franki* First, Second, and Third Instars

First instar *L. franki* can be easily distinguished from second and third instars by first instar single mouth hook and lateral sclerites, fused skeleton, and slender dorsal and ventral cornua.

Second and third *L. franki* instars can be distinguished by the following characteristics:

- Second instar has more ventrally curved mandibles than third instar.
- Dorsal and ventral processes on the bases of third instar mandibular sclerites are smaller than the dorsal and ventral processes on the bases of the second instar mandibular sclerites.
- Second instar dorsal processes on bases of mandibular sclerites bend about midway; third instar dorsal processes do not bend.
- Third instar has more heavily pigmented dorsal and ventral cornua.
- Third instar dorsal half of dorsal cornua have variable sclerotization that forms patterns; second instar has lightened sclerotization on dorsal half of dorsal cornua, but no patterns are formed.

Discussion

Lixadmontia franki and *L. diatraeae* belong to a clade within the tribe Blondeliini in which adults are characterized by females with globose abdomens (to support an enlarged common oviduct that serves as a brood chamber for developing eggs and larvae) and ovolarviposition (Wood 1987; Wood & Cave 2006). Of the tachinids with which *L. franki* was compared, the mouth hook and cephalopharyngeal skeleton of *L. franki* first instars are most similar in appearance to those of first instar *L. diatraeae*, especially the hatchet-like labrums and lateral sclerites. The hatchet-like labrums are in contrast with the labrums of *E. larvarum*, *C. lophyri*, and *S. fumiferanae*, which are hook-like, with an acute terminal point and sawtooth dorsal edge. *E. larvarum* and *C. lophyri* are in the tribe Exoristini and *S. fumiferanae* is in the tribe Winthemiini, closely related tribes that are considered basal to the subfamily Exoristinae (ITIS 1997; Stireman 2002). Exoristini and Winthemiini females lay macro-type eggs on hosts, where the eggs develop and the larvae hatch and immediately cut their way into their host (Wood 1987).

The hook-like labrum and sawtooth dorsal edge may be ideal for *E. larvarum*, *C. lophyri*, and *S. fumiferanae* to pierce and cut through a host's integument, but for what purpose would the hatchet-like labrum serve *L. franki* and *L. diatraeae*? Might it aid in navigation through complex habitat? Teskey (1981) mentions that some brachycerous larvae use their mouth hook as an anchor to facilitate locomotion; perhaps a

hatchet-like labrum would provide a better anchor than a hook-like labrum in decaying, chewed up plant matter and frass. Do all members of Blondeliini have hatchet-like labrums? *C. concinnata* is a blondeliine, but of a different clade than *L. franki* and *L. diatraeae*. Adult females in the clade to which *C. concinnata* belongs have a modified abdominal sternum which is used to ovolarviposit directly into the host's body cavity (Ichiki & Shima 2003). It would be interesting to note the shape and similarity of labrums for members of this clade and to view it in relation to their habit of direct ovolarviposition. Unfortunately, there are not enough described specimens available for comparison and, of those that are available, not enough of their life histories is understood to be able to deduce patterns of the mouth hook and cephalopharyngeal skeleton relative to the life histories of first instar Exoristinae or of the tribe Blondeliini. However, there is evidence that patterns may exist. This was successfully accomplished by O'Hara (1988b), who examined the mouth hooks and cephalopharyngeal skeletons of several members in the tribe Siphonini (subfamily Tachininae) and was able to use that information to state several phylogenetic hypotheses.

L. franki second and third instar mouth hooks and cephalopharyngeal skeletons, like many other tachinids, are similar to each other while dissimilar to their associated first instar. First instar tachinids are elongate and slender and have fused cephalopharyngeal skeletons and a labrum for the mouth hook (Wood 1987). Second and third tachinid instars have robust cephalopharyngeal skeletons and a pair of hook-like mandibles with acute terminal points. There was more variability between *L. franki* first instars and the first instars of the other species to which they were compared than there was between *L. franki* second and third instars and the second and third instars to which they were compared, a condition of Tachinidae that has been noted (Foote 1991; O'Hara 2008a). However, there is enough variation that second and third instar *L. franki* can be distinguished from each other and from the second and third instars of other tachinids.

Second *L. franki* instars had larger respiratory funnels than first instars but third instars had respiratory funnels that were similar to second instars (based on the calculated confidence intervals), indicating that the respiratory funnel was built upon during the first and second stadia but not the third. This is reasonable because the third instar, similarly to other tachinids (Foote 1991; O'Hara 2008a), rapidly consumes what remains of the host, killing the host and halting the immune response. Respiratory funnel size can be used to distinguish between first and second *L. franki* instars but not between second and third instars. Because each *L. franki* larva has only 1 respiratory funnel, the number of respiratory funnels counted in a host can be used as a reliable count of the number of larvae that parasitized a host.

First and third *L. franki* instars are active and exposed for parts of their stadia (the first instar must search for a host to parasitize and the third instar must exit the host and search for a place to pupate) while the second instar remains inside the host. *Lixadmontia franki* first and second instars are metapneustic and the third instar is amphipneustic. The first instars are able to function on one set of spiracles because they are much smaller than third instars. *Lixadmontia franki* third instars are almost 6 times larger than first instars (body length and width and lengths of mouth hooks and cephalopharyngeal skeletons) and the extra set of spiracles on the third instar would provide a greater amount of oxygen for the third instar's much larger body. Also, the larger, denser spinulae of the first and third instars (compared with the second instar) may protect the first and third instars and/or aid with locomotion (O'Hara 2008a). The ovoid spinulae with concave distal surfaces on the third instar located on the ventral side of the abdomen likely aid in locomotion.

The 3 instars were found across a wide range of days (first instar, 2-5 days after fly larvae were deposited on the pineapple mash; second instars, 2-8 days; and third instars 7-10 days). Suazo et al. (2008) showed that development time of *L. franki* from penetration of the host to pupation ranged from 13 to 21 days in *M. quadrilineatus* at 21 °C, suggesting there may be high variability in the growth rate of *L. franki* larvae. More study is necessary to determine the average duration for each instar's development.

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