SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Morphology of Immature Stages in the Neotropical Nonfrugivorous Tephritinae Fruit Fly Species *Rachiptera limbata* Bigot (Diptera: Tephritidae) on *Baccharis linearis* (R. et Pav.) (Asteraceae)

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Morfologia dos Estados Imaturos da Espécie de Mosca das Frutas Neotropical não Frugívora *Rachiptera limbata* Bigot (Diptera: Tephritidae) em *Baccharis linearis* (R. et. Pav) (Asteraceae)

RESUMO - *Rachiptera limbata* Bigot desenvolve-se em *Baccharis linearis* (R. et Pav) na região de Santiago, Chile. As larvas alimentam-se dos caules e secretam um líquido, que em contato com o ar, forma uma câmara larval, onde ocorre a passagem para o estádio de pupa. Os estádios larvais das espécies neotropicais de Tephritinae são pouco conhecidos. O presente estudo descreve a morfologia dos estádios larvais de *R. limbata*, comparando-os com outras espécies de Tephritinae, em uma abordagem filogenética. Foram analisados, em microsopia de luz e de varredura, o complexo antenomaxilar, as dobras da cutícula, as margens da abertura oral, o esqueleto cefalofaríngeo, os espiráculos anterior e posterior e os lóbulos anais das larvas de primeiro, segundo, terceiro estádios e da pupa. O esqueleto cefalofaríngeo é altamente esclerotizado e apresenta um orifício ou abertura na cornua ventral, característica essa que parece ser plesiomórfica em *R. limbata* e outras espécies neotropicais. Os espiráculos anteriores estão ausentes nas larvas de primeiro estádio. Nas larvas de segundo e terceiro estádios são desenvolvida formando cinco túbulos curtos dispostos em fileira única. Nas larvas de primeiro e segundo estádios, as aberturas dos espiráculos têm cerdas únicas; nas larvas de terceiro estádio não há cerdas. Essa última característica parece decorrer de uma atraso no desenvolvimento (neotenia) da larva de *R. limbata*, quando comparada a outras espécies neotropicais e neárticas.

PALAVRAS-CHAVE: Adaptação, neotenia, caráter plesiomórfico, apomórfico

ABSTRACT - *Rachiptera limbata* Bigot develops on *Baccharis linearis* (R. et Pav.) in the areas around Santiago, Chile. The larvae feed on stem tissues and secrete a liquid that hardens to form a protective feeding and pupation chamber. The immature stages of Neotropical species of Tephritinae are poorly known. In this paper, the morphology of the immature stages of *R. limbata* are described and compared, in a phylogenetic context, with other Tephritinae species. Antennomaxillary complex, pads, oral ridge, cephalopharyngeal skeleton, anterior and posterior spiracles and anal lobes of first-, second-, third-instar larvae and pupae were studied with optical and scanning electron microscopy. The cephalopharyngeal skeleton is darkly sclerotized and shows an opening or window in the ventral cornua. This trait seems to be plesiomorphic in *R. limbata* and in other Neotropical species. First-instar larvae anterior spiracles are absent; whereas in second and third instars spiracles are developed as a row of five short tubules. In first- and second-instar larvae, the posterior spiracular slit has only a single hair per bundle; whereas third-instar larvae lack hairs. This last trait seems to be consequence of a larval development delay and an apomorphic trait in *R. limbata,* compared to other Neotropical and Neartical species.

KEY WORDS: Adaptation, neoteny, plesiomorphic, apomorphic traits

The genus *Rachiptera* Bigot belongs to the subfamily Tephritinae. The species of Tephritinae are the most specialized of Tephritidae. The larvae of these non frugivorous species predominantly infest flower heads as well as stem buds, leaves or roots of Asteraceae the largest, most advanced, and widespread family of Angiosperms. Nevertheless, several species infest a few other families such as: Acanthaceae, Goodeniaceae, Lamiaceae, and Vervenaceae (Headrick & Goeden 1998, Norrbom *et al*. 1998, Han & McPheron 2000). The Tephitinae is a large and diverse subfamily with more than one hundred genera. However, it is believed to be a monophyletic group, which shares derived characters (synapomorphies) that are evidence of exclusive common ancestry (Han & McPheron 2000, Korneyev 2000).

The genus *Rachiptera* belongs to the tribe Eutretini Munro and includes four exclusive Neotropical species: *R. biarcuata* Hendel and *R. percnoptera* Hendel, distributed only in Chile; *R. virginales* Hering, an endemic species from Brazil; and *R. limbata* Bigot distributed in Argentina and Chile. The larvae of *Rachiptera* and *Strobelia* Rondani are the only species of Tephritidae that form a globular protective structure outside their gall (Aljaro *et al.*1984, Norrbom *et al*. 1998). *R. limbata* is a monophagous and bivoltine species that attacks the endemic *Baccharis linearis* (R. et Pav.) from Chile.

Females lay their eggs into branch apical meristems of *B. linearis*. The larvae feed from the stem inner tissues and secrete a liquid that is expelled throughout the damaged areas of the stem. This secretion is clear, but in contact with air forms a spongy material with high water absorption capacity. Pupation takes place within the larval chamber, approximately twenty days after oviposition. The chamber is non-dehiscent and adults must use an escape route made by the larva. Adult populations emerge during spring and summer (Porter 1929, Aljaro *et al*. 1984). Neotropical Tephritinae morphology of immature stages, with only a few exceptions, are poorly known (Frías 1985, Gandolfo & Hernández 1999, Steck & Wharton 1986). This paper describes the morphology of three instar larvae and pupae of *R. limbata*, compared other Tephritinae and Trypetidae morphology, discussed in an evolutionary biology framework.

Material and Methods

The research was conducted during spring and summer of 2003-2005, near Pirque, 33º lat.S, 71º long.W, 28 km southeast from Santiago City 650 m elevation. Immature stages of *R. limbata* were reared from field collected feeding chambers of *B. linearis*. The eggs were collected from apical meristems of *B. linearis* and first-instar larvae from small chambers (ca. 0.2 cm diam) located in the apical meristems of host plants. Second-instar larvae were collected from chambers ca. 0.5-1.0 cm of diam and third-instar larvae from chambers of ca. 1.5-2.5 cm of diam.

Twenty five larvae of each instar and twenty pupae were killed and fixed in boiling water for one minute and preserved in 70% ethanol. For optical studies larvae were treated overnight in 10% KOH following the methods of Steck & Wharton (1986); a process that allowed for unobstructed viewing of the cephalopharyngeal skeleton, antenomaxillary complex, anterior and posterior spiracular complexes. The treated larvae were dissected and the anterior and caudal segment were slide mounted in Canada Balsan.

Another ten larvae of each instar were prepared for scanning electron microscopy (SEM). The alcohol-stored specimens were cleaned in hot distilled water with a few drops of detergent, fixed in 3% glutaraldehyde in cacodylate buffer,then fixed in 1% osmium tetroxide, and dehydrated in a 50%, 70%, 95% and 100% acetone series. Specimens were prepared for SEM observations by using critical-point drying (Sovall Critical-Point Drying System) and coating with gold. The specimens were examined at an accelerating voltage of 30 KV on a JEOL.JSM-25-SII SEM. Terminology used follows that of Headrick & Goeden (1990), White & Elson-Harris (1992), White *et al*. (2000) and Frías *et al*.(2006).

Results

Egg. White, elongate, 0.79 to 0.98 mm long, and 0.37- 0.45 mm wide, with a broad short pedicel, 0.08- 0.15 mm long and 0.10- 0.14 cm wide. Ten eggs were measured in situ in apical meristem tissues (Fig. 1).

First-instar larva

Body. White, elongate-ellipsoidal, conical anteriorly, truncated posteriorly; length 0.7 - 0.9 mm; width 0.15-0.30 mm (n = 25) (Fig. 2)

Cephalic segment. Conical and sharp, with a longitudinal dorsal suture within the antennomaxillary sense organ, dorsally and laterally with six extended irregular, long smooth cuticular folds, oral ridges absent (Figs. 3-5); antennomaxillary sense organ is not sclerotized, antenna one segmented. Sensilla of maxillary palp, surrounded by a wide cuticular fold (Fig. 6). Cephalopharyngeal skeleton is darkly sclerotized, mandible black, mouthook tridentate (Figs. 4, 9), apical and preapical teeth brown, curved, and similar in length (Fig. 5), with a small third tooth (Fig. 9). Ventral apodeme of mandible well developed and broad, with a small sharp projection located posteriorly; dorsal apodeme curved, sharp, and well developed (Fig. 9). Hypopharyngeal sclerite, and bridge of hypopharymgeal sclerite, black, the latter located approximately in the middle of hypopharyngeal sclerite. Labial sclerite black, adjacent to hypopharyngeal sclerite. Parastomal bar brown, adjacent to hypopharyngeal sclerite; pharyngeal sclerite black, dorsal bridge of pharyngeal sclerite dark brown and sclerotized. Dorsal cornu black and bifurcated, with some brown areas; ventral cornu black, with some brown bifurcated areas and two concentric sclerotized

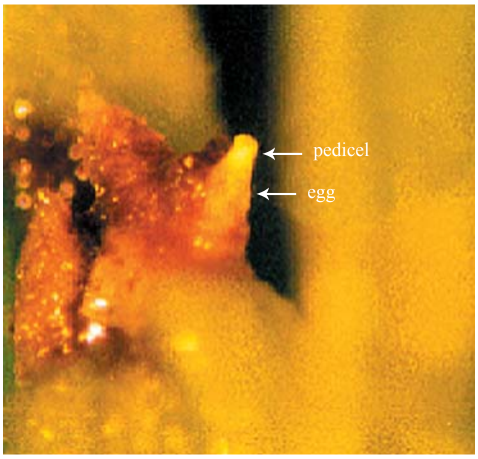


Fig. 1. Egg of *R. limbata* inserted in apical meristeme of *B. linearis*.

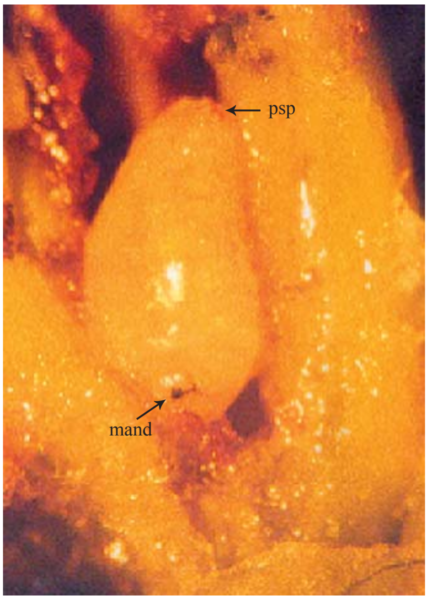


Fig. 2. First-instar larva of *R. limbata* feeding from stem inner tissues; posterior spiracles: psp, mandible: mand.

bars united posteriorly in a circular shape with a window located centrally; ventral bridge of pharyngeal sclerite black (Fig. 9).

Prothorax. Anterior area with a long cuticular fold; circunscribed by four rows of posteriorly directed, minute acanthae; dorsally with two round papilla sensilla (Figs. 3, 5); anterior spiracles absent.

Caudal segment. Circumscribed by minute acanthae (Fig. 7). Posterior spiracles *s*lightly sclerotized, peritreme yellow, with three spiracular slits nearly three times as long as wide; dorsal, ventral and lateral spiracular bundles with only one nonbranched hair (Fig. 8).

Second-instar larva

Body. Pale yellow, elongate-ellipsoidal, conical anteriorly, truncated posteriorly; 1.0 - 2.9 mm long and 0.8-1.5 mm wide.

Cephalic segment. Extended and distinctly pointed, with a longitudinal dorsal suture. Dorsum covered with irregular cuticular pads (Fig. 12), antennomaxillary sense organ with medial integumental petals and with lateral integumental petals (Fig. 13). Frontolateral view shows some irregular, extended pads, without oral ridges (Fig. 14). Antennomaxillary sense organ sclerotized, with a one-segmented antenna (Figs. 13, 15). Maxillary palps surrounded by a wide cuticular fold, with three papillate sensilla and two knob sensilla, one additional dorsolateral sensillum, equidistant from antenna and maxillary palp (Figs. 13-15). Cephalopharyngeal skeleton is darkly sclerotized, mandible black, mouthook tridentate (Figs. 10, 12); apical and preapical teeth brown, curved, and similar in length, third tooth is brown and small. Ventral apodeme of mandible well developed and broad, posteriorly with a small sharp projection, dorsal apodeme curved, sharp, and well developed. Hypopharyngeal sclerite and bridge of hypopharyngeal sclerite, black, this latter located approximately in middle of hypopharyngeal sclerite. Labial sclerite black, curved and separated from hypopharyngeal sclerite. Parastomal bar is brown, adjacent to hypopharyngeal sclerite; pharyngeal sclerite black, dorsal bridge of pharyngeal sclerite dark brown and heavily sclerotized. Dorsal cornu black and bifurcated, with some brown areas; ventral cornu is black with some brown areas, bifurcated, with two concentric bars darkly sclerotized, united posteriorly in a circular shape with an opening or window located centrally. Ventral bridge of pharyngeal sclerite is black (Fig. 10).

Prothorax. Smooth anterior area lacking rugose pads, circumscribed by four rows of posteriorly directed minute acanthae, posteriorly the dorsolateral area , covered by rugose pads (Fig. 12). Anterior spiracles slightly sclerotized with five short tubules (Figs. 12, 16).

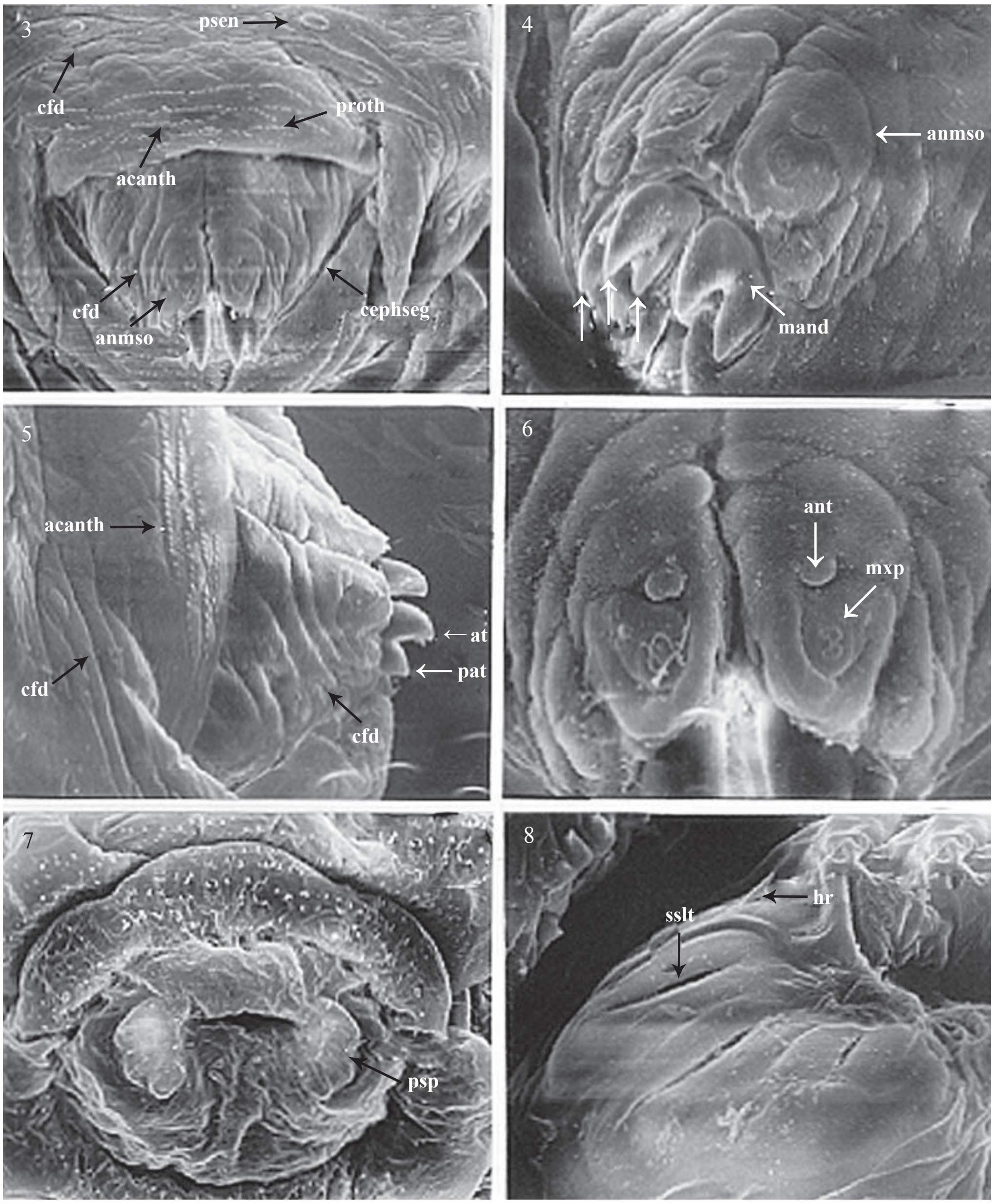
Caudal segment. Posterior spiracles, moderately sclerotized, peritreme yellow, with three spiracular slits nearly two and a half times as long as wide; with only one dorsal, ventral, and lateral nonbranched hair in spiracular bundles (Fig. 17).

Third-instar larva

Body. Pale yellow; length 3.50-4.88 mm, width 1.80-3.10 mm; elongate-ellipsoidal, conical anteriorly, truncated posteriorly, distinctly segmented, located inside of *B. linearis* larval chamber apical meristemes (Figs. 18, 19, 20).

Cephalic segment. Anteriorly extended to sharp apex, with a longitudinal dorsal suture. Dorsum covered with round cuticular pads (Figs. 21, 22). Lateral view shows extended pads, without oral ridges (Fig. 23). Medial integumental petals within the antennomaxillary sense organ and two long lateral integumental petals (Fig. 24). Antennomaxillary sense organ heavily sclerotized, with a one-segmented antenna. Maxillary palps surrounded by a wide cuticular fold, with three papillate sensilla and two knob sensilla, one additional dorsolateral sensillum, equidistant from antenna and maxillary palp (Fig. 24). Cephalopharyngeal skeleton darkly sclerotized, mandibles black, mouthook tridentate (Fig. 22), apical and preapical tooth curved and subequal in length (Fig. 21), third tooth shorter than apical and preapical tooth (Fig. 11). Ventral apodeme of mandible well developed and broad, posteriorly with a sharp projection; dorsal apodeme is rounded and well developed. Hypopharyngeal sclerite is black, hypopharyngeal bridge located approximately in middle of hypopharyngeal sclerite. Labial sclerite broad, black, curved, and separated from hypopharyngeal sclerite. Parastomal bar is brown, adjacent to hypopharyngeal sclerite; pharyngeal sclerite, black, dorsal bridge of pharyngeal sclerite, brown, and slightly sclerotized. Dorsal cornua black and bifurcated, with some brown areas; ventral cornu is black and bifurcated, with two heavily sclerotized concentric bars, united posteriorly in a circular shape with a window located centrally. A brown triangularly shaped ventral projection is located at the intersection of the two concentric bars. Ventral bridge of pharyngeal sclerite is black (Fig. 11).

Prothorax. Anteriorly smooth lacking rugose pads, posteriorly, the dorsolateral area covered by rugose pads (Fig. 21). Anterior spiracles slightly sclerotized with five



Figs. 3-8. First-instar larva of *R. limbata*. Cephalic segment and prothorax, anterior view (3); acanthae: acanth, antennomaxillary sense organ: anmso, cephalic segment: cephseg, cuticle fold: cfd, prothoxax: proth, papilla sensillum: psen. Cephalic segment, anterior view (4); antennomaxillary sense organ: anmso, mandible: mand, the arrows show the three teeth of mandible. Cephalic segment and prothorax, lateral view (5); acanthae: acanth, apical tooth: at, cuticle fold: cfd, preapical tooth: pat. Antennomaxillary sense organ, anterior view (6); antenna: ant, maxillary palp: mxp. Caudal segment, posterior view (7); posterior spiracles: psp. Posterior spiracles (8); spiracular slit: sslt, hair: hr.

short tubules in a single row (Figs. 21, 23).

Caudal segment. Intermediate area is smooth, lacking minute acanthae, dorsal ventral and lateral area with minute acanthae, anal lobe is unilobed and flat (Fig. 25). Posterior spiracles with heavily sclerotized peritreme and a moderately sclerotized, surrounded by a wide cuticular fold. Three spiracular slits, four times as long as broad, each with a wide and moderately sclerotized rima, and without dorsal, ventral, and lateral spiracular hairs (Fig. 26).

Pupa. Puparium black and barrel-shaped, ranging from

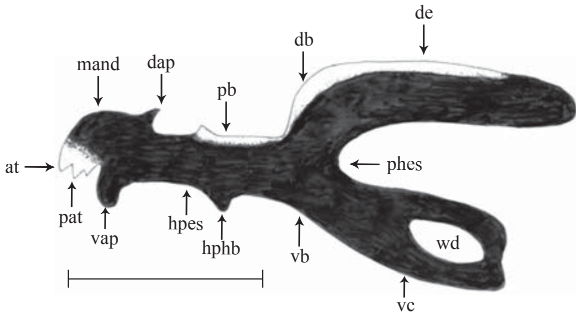


Fig. 9. Cephalopharyngeal skeleton of first-instar larva of *R. limbata*. Apical tooth: at; dorsal apodeme: dap; dorsal bridge: db; dorsal cornu: dc; hyphopharyngeal bridge: hphb; hyphopharyngeal sclerite: hphes; mandible: mand; preapical tooth: pat; pharyngeal sclerite: phes; ventral apodeme: vap; ventral bridge: vb; ventral cornu: vc; window: wd. Scale bar 10 µm.

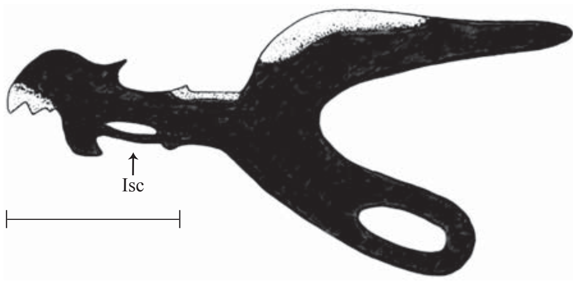


Fig. 10. Cephalopharyngeal skeleton of second-instar larva of *R. limbata*. Labial sclerite: lsc. Scale bar 10 µm.

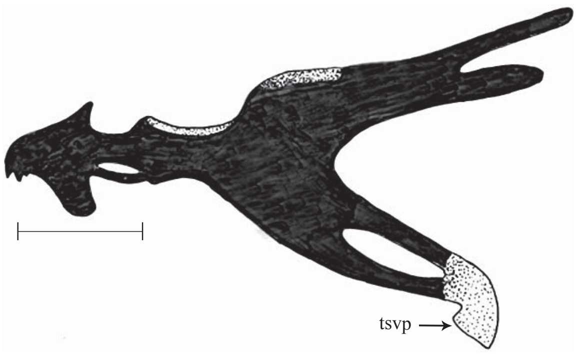


Fig. 11. Cephalopharyngeal skeleton of third-instar larva of *R. limbata*. Triangular shape ventral projection: tsvp. Scale bar 10 µm.

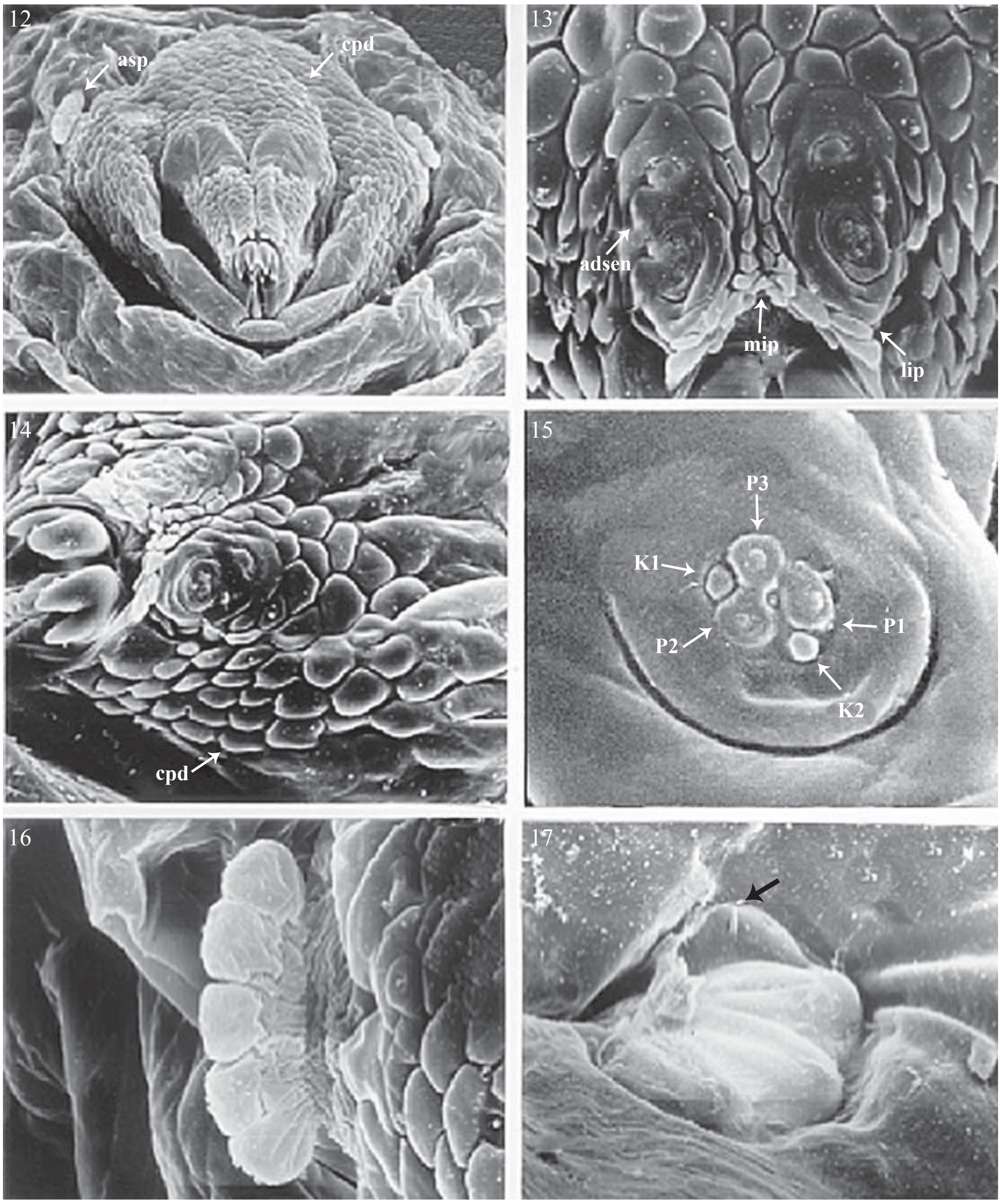
4.9-5.3 mm long to 2.3-3.2 mm wide. Pupation takes place in the larval feeding chamber, that measures ca.1.5-2.5 cm diam externally (Fig. 27). Anterior end of puparium oriented towards the apex of chamber. Third-instar larvae excavate the chamber wall to form a window at the apex of the chamber for the emerging adult to gain egress from the pupal chamber.

Discussion

Different larval development stages of *R. limbata* can be distinguished by size, morphology, and sclerotization of cephalopharyngeal skeleton, cephalic segment morphology, anterior and posterior spiracles, prothorax morphology, and features on the caudal segment. The labial sclerite of first-instar larvae is adjacent to the hypopharyngeal sclerite, and in second- and third-instar larvae the labial sclerites are curved and separated from the hypopharyngeal sclerite. In three instar larvae the ventral cornua is black, bifurcated, and with two sclerotized concentric bars united posteriorly in a circular shape with an opening or window located centrally. In the three instars larvae, at the intersection of the two bars is a brown and moderately sclerotized triangularly shaped ventral projection. This ventral cornua window has also been described in the Palearctic species *Xiphosia miliaria* Schrank, *Terellia ceratocera* Hendel, *Tephritis hyoscyami* L. (Persson 1963), in the Oriental species *Rhabdochaeta naevia* Ito and *R. asteria* Hendel (Sueyoshi 1999), in the Nearctic species *Eutreta diana* (Osten Sacken) and *Eutreta angusta* Banks (Steck & Wharton 1986) and in the Neotropical species *Trupanea foliosi* Frías, *T. footei* Frías, *T. chrysanthemifolii* Frías and *T. simpatrica* Frías (Frías 1985, Frías 2005), and *Pseudoeaspis biseta* Hendel (Gandolfo & Hernández 1999).

According to the phylogeny put forward by Korneyev (2000) on Tephritinae tribes and subtribe relationships, the species of the genera *Xiphosia* Robineau-Desvoidy and *Terellia* Robineau-Desvoidy belong to the “lower” and more primitive tribes of Tephritinae. The species of the genera *Rachiptera*, *Eutreta* Loew*, Rhabdocheta* Meijere*, Tephritis* Latreille*, Trupanea* Schrank*,* and *Pseudoeaspis* Hendel belong to the “higher” tribes: Eutretini (*Eutreta* and *Rachiptera*), Schisopterini Bezzi (*Rhabdocheta*),and Tephritini Newman (*Tephritis*, *Trupanea* and *Pseudoeaspis*). The wide distribution of these traits along different biotic regions and their presence in lower and higher tribes, appear as a plesiomorphic character in *R. limbata* and the other Neotropical species.

No anterior spiracles in first-instar larvae were detected in *R. limbata*. In second and third larval instars, the anterior spiracles appear as five short tubules in a single row, slightly sclerotized, located laterad of the posterior margin of the prothorax. First-instar larvae of *Rhagoletis pomonella* (Walsh) (Snodgrass 1924) and *Anastrepha ludens* Loew (Carroll & Wharton 1989) (Trypetinae) were described as having the anterior spiracles absent or with spiracles appearing as a minute pore only discernible with a scanning electron microscope as in *Bactrocera tryoni* Froggatt (White & Elson -Harris,1992). Second- and third-instar larvae have a variable number of tubules, between nine and 44 depending on the genera (White & Elson-Harris 1992, Frías *et al*. 2006). Similarly, first-instar larvae in the Tephritinae have anterior spiracles absent or that appear as flattened pores. Second- and third-instar larvae have a variable number, between one and six short tubules (Goeden 2001a, b; 2002). Nevertheless, in the first-instar larvae of *Procecidochares blanci* Goeden & Norrbom*, P. kristineae* Goeden, *P. lisae* Goeden*, Aciurina*



Figs. 12-17. Second-instar larva of *R. limbata*. Cephalic segment and prothorax, anterior view (12), anterior spiracles: asp, cuticle pad: cpd. Antennomaxillary complex, anterior view (13), additional dorsolateral sensillum: adsen, medial integumental petals: mip, lateral integumental petals: lip. Cephalic segment, dorsolateral view (14), cuticle pad: cpd. Maxillary palp (15), knob sensilla: k1, k2; papilla sensilla: p1, p2, p3. Anterior spiracles (16). Posterior spiracles (17), the arrow show the hair of spiracular slit.

idahonensis Steyskal, *A. michaeli* Goeden, and *A. ferruginea* (Doane)*,* anterior spiracles are also absent, but in second- and third-instar larvae these are flattened and recessed, and consist of two or five ovoid papillae (Goeden & Teerink, 1996a, Goeden & Teerink 1996c, Goeden & Norrbom 2001, Goeden & Teerink 1997a). The condition of having recessed anterior spiracles in second- and third-instar larvae could be a juvenile trait originated by hetrochronic larval development or neoteny that reduces the rate of morphological develop of these structures, such as has been postulated by De Beer (1951), Hardy (1954), and Gould (1977).

The cephalic segment of first-instar larvae of *R. limbata* have six elongated dorsolateral cuticular folds; and the prothorax anteriorly flattened and circumscribed by four rows of posteriorly directed minute acanthae. The prothorax, posteriorly, bears a smooth longitudinal cuticular fold.

Fig. 18. Chambers of *R. limbata* in the tips of the branches of *B. linearis*.

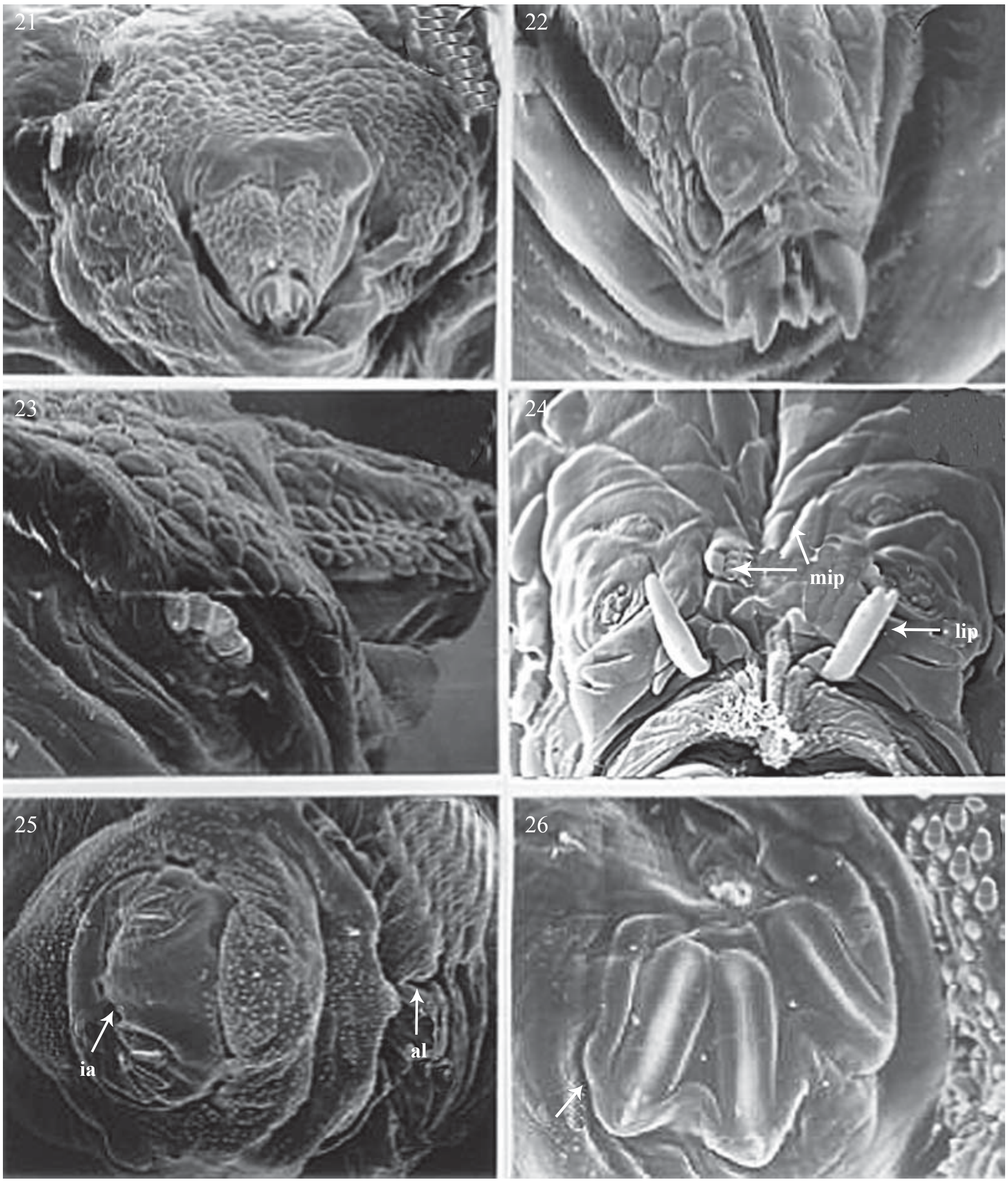
Fig. 19. Chamber of *R. limbata*.

Fig. 20. Third-instar larva of *R. limbata* inside chamber.

Second- and third-instar larvae have the cephalic segment covered with numerous folds and rugose cuticular pads, dorsolaterad and laterad of the mouth lumen; prothorax anteriorly smooth, with minute acanthae, and posteriorly with abundant, elongated, and rounded cuticular pads.

Studies on Nearctic gallicolous Tephritinae species in different tribes and genera such as *Tephritis baccharis* (Coquillett) (Goeden & Headrick 1991)(tribe Tephritini), *Aciurina idahoensis* Steyskal (Goeden & Teerink 1996a), *Aciurina semilucida* (Bates) (Goeden & Teerink 1996b), *A. ferruginea* (Doane), *A. michaeli* Goeden & Teerink (Goeden & Teerink 1996c) (tribe Eurostini Foote, Blanc & Norrbom), *Procecidochares kristineae* Goeden & Teerink, and *P. lisae* Goeden & Teerink (Goeden & Teerink 1997a) (tribe Ditrycini Foote, Blanc & Norrbom) show that first-instar larval cephalic segments are smooth and without rugose pads. The cephalic segment of second- and third-instar larvae of these Nearctic species are smooth, lacking rugose pads or with few elongated rugose pads compared to *R. limbata* larvae. The prothorax of second- and third-instar larvae of these Nearctic species are smooth and without rugose pads, with the only exception being *Procecidochares anthracina* (Doane) that has rugose pads on the dorsal half of the prothorax (Goeden & Teerink 1997b). Third-instar larvae of *Procecidochares blanci* Goeden & Norrbom have rugose pads that circumscribe the anterior halves of the prothorax, and second-instar larvae are circumscribed by many round, oval or elliptical, flattened and rugose pads of different sizes (Goeden & Norrbom 2001).

The numerous cuticular folds and pads on the cephalic segment and prothorax of *R. limbata* could be an apomorphic or adaptative trait for the unique behavior of building the larval chamber with their salival secretions. *R. limbata* larva feed on host plant apical meristem branch tissues and sap, therefore, a highly convoluted cuticle might increase the contact surface area with the salivary secretions liberated by the larvae. This liquid surrounds the larva and invades the folds of the rugose cuticular pads and may act as a means to create the frothy bubbles in the liquid secretions. When the secretions come into contact with the atmosphere the secretion harden to form the larva chamber which constitutes



Figs. 21- 26. Third-instar larva of *R. limbata*. Cephalic segment and prothorax, anterior view (21). Cephalic segment, dorsal view (22). Cephalic segment and prothorax, lateral view (23). Antennomaxillary sense organ, medial and lateral integumental petals (24), medial integumental petals: mip, lateral integumental petals: lip. Caudal segment (25), intermediate area: ia, anal lobe: al. Posterior spiracles (26), the arrow show the cuticle fold that surrounded the spiracular slit.

an important microhabitat to protect the larva and puparium from environmental extremes. During winter, pupal diapause takes place inside this chamber as has been described by Aljaro *et al*. (1984).

First-, second-, and third-instar larvae posterior spiracles of *R. limbata* have three spiracular openings. In other tephritidae species, the number of posterior spiracular openings increase from two to three, from first to second and third instars (Snodgrass 1924, White & Elson-Harris 1992), with the only exception being the first instar of the gallicolous Nearctic Tephritinae species *Aciurina semilucida* which have three spiracular openings similar to *R. limbata* (Goeden & Tererink 1996b).

In first- and second-instar larvae of *R. limbata,* each group

Fig. 27. Puparium of *R. limbata* inside chamber.

of ventral, dorsal, and lateral bundles of posterior spiracles has one single unbranched hair. Third-instar larvae of *R. limbata* do not have hairs on posterior spiracles, and the peritreme is surrounded by a wide cuticular fold. These traits have not yet been described in other tephritid larvae.

In fruit flies, first instars of the Trypetinae species *Bactrocera tryoni* Froggatt have posterior spiracles with two round spiracular openings and four spiracular hair bundles, each with a few relatively long hairs (White & Elson-Harris 1992), *R. pomnonella* (Snodgrass 1924) and *A. ludens* first instars have posterior spiracles with two spiracular openings surrounded by oval rimae and with 2-4 short simple or bifid hairs per group (Carroll & Wharton 1989). Similarly, the number of spiracular bundles in Tephritine first-instar larvae is always four, each with an unbranched hair, such as in: *Aciurina ferruginea* and *A. idahoensis* (Goeden & Teerink 1996a, Goeden & Teerink 1996c), *Procecidochares kristineae*, *P. lisae* (Goeden & Teerink 1997a), *P. anthracina* (Goeden & Teerink 1997b), *P. blanci* (Goeden & Norrbom 2001), *Pararacantha gentilis* Hering (Headrick & Goeden 1990), *Neaspilota signifera* (Coquillett) (Goeden 2000a), *Neaspilota aenigma* Freidberg and Mathis (Goeden 2000b), and *Trupanea jonesi* Curran (Goeden, Teerink & Headrick 1998). For *Trupanea arizonenesis* Malloch, two ovoid rimae and four spatulate bundles were described (Goeden & Teerink, 1999). *Trupanea imperfecta* (Coquillett) has two ovoid rimae and four bundles, each with 1-3 branches (Teerink & Goeden 1999). The absence of posterior spiracular hairs in third-instar larvae of *R. limbata* appear as a recessive and apomorphic trait. More research regarding immature stages morphology among Tephritidae species are needed, specially taking into consideration the current views on phylogenetic relationships.

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