

Description of Eggs of *Anastrepha curitis* and *Anastrepha leptozona* (Diptera: Tephritidae) Using SEM

VIVIAN S. DUTRA,¹ BEATRIZ RONCHI-TELES,² GARY J. STECK,³ AND JANISETE G. SILVA⁴

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ABSTRACT Egg morphologies of *Anastrepha curitis* Stone and *Anastrepha leptozona* Hendel are described using scanning electron microscopy. Eggs were dissected from females captured in McPhail traps in Manaus and Presidente Figueiredo in the state of Amazonas, Brazil. Eggs of *A. curitis* are very long and have no aeropyles but show a seemingly porous surface on the anterior pole. Eggs of *A. leptozona* are robust. Eggs of both species are smooth and have no chorion ornamentation, reticulation, or protuberances. The micropyle is located at the apex of the anterior pole of the egg for both species studied. No distinct respiratory horn was detected either in *A. curitis* or *A. leptozona*. Diagnostic characters include egg length and presence versus absence of a porous surface on the anterior pole.

KEY WORDS fruit fly, chorion ornamentation, micropyle, aeropyle

Anastrepha Schiner (Diptera: Tephritidae) is endemic to the Neotropics with over 250 described species distributed in South and Central America, the West Indies, and the southern United States (White and Elson-Harris 1992; Norrbom et al. 1999; Norrbom and Korytkowski 2009, 2011). In Brazil, a total of 112 *Anastrepha* species have been reported to date in 12 infrageneric species groups based on both morphological characters and host plant use (Norrbom et al. 1999; Zucchi 2007, 2008; Uramoto and Zucchi 2010).

Anastrepha curitis Stone and *Anastrepha leptozona* Hendel studied herein belong to the *pseudoparallela* and *leptozona* species groups, respectively (Norrbom et al. 1999). The *pseudoparallela* group has 20 species and ranges from Texas to Argentina. However, only two species occur north of Panama and one occurs in the Antilles. Most species for which host data are known infest Passifloraceae (Norrbom et al. 1999). Ten species in this group have been reported in Brazil (Zucchi 2008). *Anastrepha curitis* Stone is restricted to the states of Amazonas and Pará in the Amazon region in Brazil (Zucchi 2007, 2008).

The *leptozona* group comprises five species that range from Mexico to Brazil. Only two species in this group have known hosts: *Anastrepha barnesi* Aldrich and *Anastrepha leptozona* Hendel. *Anastrepha barnesi* infests Sapotaceae and *A. leptozona* is known to infest Sapotaceae and also to attack hosts in other plant

families such as Anacardiaceae, Icacinaceae, Myrtales, Quinaceae, Rosaceae, and Rutaceae. In Brazil, *A. leptozona* is widely distributed and has been reported in 15 states in all areas of the country except for the southern region (Norrbom et al. 1999, Norrbom 2004, Zucchi 2008, Frías et al. 2009).

Morphological characters present in immature stages can be useful as diagnostic characters and contribute to the understanding of evolutionary relationships among *Anastrepha* species (Norrbom et al. 1999). However, there is still a lack of information on egg morphology within the genus *Anastrepha*, as so far eggs have been described for only 36 species (Emmart 1933; Seín 1933; Lawrence 1979; Steck and Malavasi 1988; Steck and Wharton 1988; Carroll and Wharton 1989; Murillo and Jirón 1994; Selivon and Perondini 1998, 1999; Norrbom et al. 1999; Selivon et al. 2004; Norrbom and Korytkowski 2009; Dutra et al. 2011a,b; Figueiredo et al. 2011). In the *pseudoparallela* group, the eggs of *Anastrepha pseudoparallela* (Loew) were described only in terms of gross measurements and general shape (Norrbom et al. 1999), whereas those of *Anastrepha consobrina* (Loew) and *A. pseudoparallela* were described in considerable detail by using scanning electron microscopy (SEM) (Figueiredo et al. 2011). In the *leptozona* group, only eggs of *A. leptozona* have had their general shape described (Norrbom et al. 1999).

We describe detailed SEM observations of *A. curitis* and *A. leptozona* eggs. These two species are of economic importance for the Amazon region as they infest hosts that are commercialized and consumed in natura (Cavalcante 1991, Rabelo 2012). Our results can be useful for the identification of *Anastrepha* species at the egg stage and for the understanding of the taxonomy of the genus.

¹ Graduate Program in Entomology, Instituto Nacional de Pesquisas da Amazônia – INPA, CP 478, 69011-970, Manaus, AM, Brazil.

² Coordenação em Biodiversidade, Instituto Nacional de Pesquisas da Amazônia – INPA, CP 478, 69011-970, Manaus, AM, Brazil.

³ Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL 32614–7100.

⁴ Corresponding author: Janisete G. Silva, Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Rodovia Ilhéus/Itabuna km 16, Ilhéus, BA, 45650-000, Brazil. (e-mail: jgs10@uol.com.br).

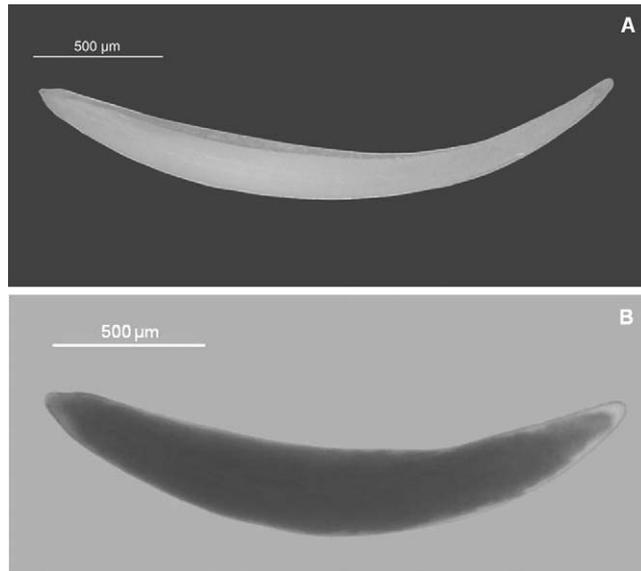


Fig. 1. Eggs of *Anastrepha* species (anterior to left). (A) *A. curitis*. (B) *A. leptozona*.

Materials and Methods

Eggs of *A. curitis* and *A. leptozona* were dissected from ovaries of mature females captured in McPhail traps in Manaus (03° 06' 07" S, 60° 01' 30" W) and Presidente Figueiredo (02° 02' 04" S, 60° 01' 30" W) in the state of Amazonas, Brazil. Eggs were preserved in 1.5-ml plastic microtubes containing 80% ethanol. Eggs taken from ovaries of preserved females have been shown to be identical to those having been oviposited naturally (Selivon et al. 2003). *Anastrepha curitis*: in total, 52 eggs from four females were examined. Three females of *A. curitis* were collected in Manaus and one female was collected in Presidente Figueiredo, state of Amazonas, Brazil. *Anastrepha leptozona*: in total, 51 eggs from four females were examined. The four females were collected in Manaus, state of Amazonas, Brazil.

The length and width measurements of the egg body were taken with an ocular micrometer and a Wild M3C stereoscopic microscope (Leica Geosystems, Heerbrugg, Switzerland) at the Laboratório de Entomologia Agrícola of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil. The eggs were photographed under a Leica M165C stereomicroscope (Leica Microsystems, Inc., Deerfield, IL) at the Laboratório de Prospecção de Bioativos de Insetos of the INPA, Manaus, Amazonas, Brazil. Ten to 20 eggs per female from each of four females of each species were prepared for light microscopy. The anterior pole bears the respiratory horn or a slight projection with the micropyle and aeropyles. The posterior pole is smooth and bluntly rounded and bears no external openings or structures (Headrick and Goeden 1998). The head of the embryo develops oriented toward the anterior pole of the egg. According to the position of the embryo inside the egg, the convex side of the egg is ventral and the

concave side is dorsal (Nascimento and Oliveira 1996, Selivon and Perondini 2000).

In preparation for SEM, eggs of *A. curitis* and *A. leptozona* were transferred to silicone capsules and dehydrated in an ethanol series, then critical point dried in CO₂ for 3 hr, and sputter-coated with a gold layer. The eggs of *A. curitis* were examined in a LEO 1450VP scanning electron microscope (LEO Electron Microscopy Ltd., Cambridge, England) at the Laboratório Institucional de Microscopia Eletrônica de Varredura of the Museu Paraense Emílio Goeldi, Belém, Pará, Brazil. The eggs of *A. leptozona* were examined in a LEO 435 scanning electron microscope (LEO Electron Microscopy Ltd.) at the Laboratório Temático de Microscopia Óptica e Eletrônica of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil. SEM was used to examine at least 10 eggs for each species.

Voucher specimens of eggs and females are deposited at the Coleção de Invertebrados of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil.

Results

The eggs of *A. curitis* and *A. leptozona* are similar in their gross morphology. They are elongate, tapered toward the posterior pole, and with the anterior pole bearing a papilla and the micropyle. The eggs differ in length and presence versus absence of a porous surface on the anterior pole.

General Features. Eggs of both species are creamy-yellow, slightly curved, broader from the middle toward the anterior pole, and tapering gradually toward both ends (Fig. 1A and B).

A. curitis. Eggs are 2.13–2.58 mm long and 0.18–0.26 mm wide, and very elongate (Fig. 1A).

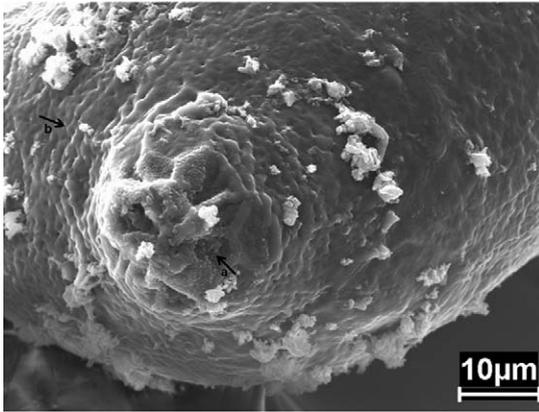


Fig. 2. Scanning electron microscope view of egg of *A. curitis*. Anterior pole showing the micropyle (arrowhead a) and porous surface (arrowhead b).

A. leptozona. Eggs are 1.37–1.87 mm long and 0.25–0.31 mm wide, and elongate (Fig. 1B).

Chorionic Sculpturing. Eggs of both species bear a papilla on which the micropyle is located on the anterior pole. The surface of the entire egg is smooth without any distinct ornamentation, reticulation, or other protuberances (Figs. 2 and 3). No distinct respiratory horn was observed. No aeropyles were detected. The micropyle is located on the apex of the anterior pole on the dorsal side and surrounded by a continuous, smooth rim of the chorion (Figs. 2 and 3). In *A. curitis*, a seemingly porous surface was observed all around the anterior pole (Fig. 2). In *A. leptozona* neither aeropyles nor a porous surface were observed on the anterior pole (Fig. 3) as opposed to what was seen for *A. curitis*.

Discussion

General features of eggs of both species herein studied are similar to those described previously for

other *Anastrepha* species (Emmart 1933; Seín 1933; Lawrence 1979; Steck and Malavasi 1988; Steck and Wharton 1988; Carroll and Wharton 1989; White and Elson-Harris 1992; Murillo and Jirón 1994; Selivon and Perondini 1998, 1999; Norrbom et al. 1999; Selivon et al. 2004; Norrbom and Korytkowski 2009; Dutra et al. 2011a,b; Figueiredo et al. 2011). The only previously published study on egg morphology of *A. leptozona* described the dimensions (length 1.30–1.41 mm; width 0.22–0.27 mm), general shape (“stout”), and sculpture (“absent”) (Norrbom et al. 1999). Our results clearly corroborate the earlier description.

Eggs of both species have an entirely smooth surface and show no chorion sculpturing, reticulation, or protuberances. Many *Anastrepha* species, whose egg morphologies have been described so far, have chorion ornamentation. However, the lack of chorion ornamentation also has been observed several times as reported in three species of the *spatulata* group: *Anastrepha manihoti* Lima, *Anastrepha montei* Lima, and *Anastrepha pickeli* Lima (Dutra et al. 2011b); in *Anastrepha* species in several infrageneric species groups such as *Anastrepha coronilli* Carrejo & González in the *fraterculus* group, *Anastrepha grandis* Macquart in the *grandis* group, *Anastrepha serpentina* (Wiedemann) in the *serpentina* group; and also in two additional species in the *pseudoparallela* group: *Anastrepha consobrina* (Loew) and *Anastrepha pseudoparallela* (Loew) (Emmart 1933, Steck and Wharton 1988, Selivon and Perondini 1999, Dutra et al. 2011a, Figueiredo et al. 2011).

A patch of a seemingly porous surface around the anterior pole was observed on the eggs of *A. curitis* similar to a narrow patch that has been described for *A. montei* (Dutra et al. 2011b), and that is probably related to egg respiration. Thus far, this structure has been observed only for these two species. Interestingly, both species lack distinct aeropyles. In *A. leptozona*, we did not observe any structure or chorion ornamentation that could be related to respiration.

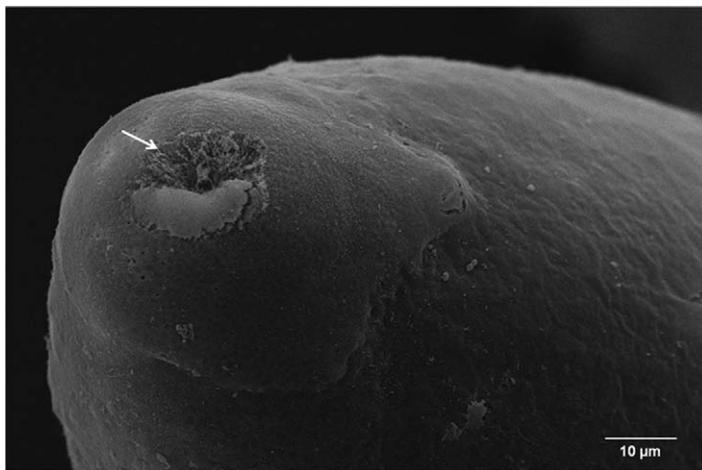


Fig. 3. Scanning electron microscope view of egg of *A. leptozona*. Anterior pole showing the micropyle (arrowhead).

The eggs of both species studied here lack a distinct respiratory horn such as observed for *Anastrepha barbiellinii* Lima, *A. manihoti*, *Anastrepha nigrifascia* Stone, *Anastrepha obliqua* (Macquart), *A. pickeli*, and *Anastrepha pittieri* Caraballo (Murillo and Jirón 1994, Norrbom et al. 1999, Norrbom and Korytkowski 2009, Dutra et al. 2011b).

The micropyle of both *A. curits* and *A. leptozona* is located at the anterior apex of the egg as observed previously for most *Anastrepha* species whose egg morphology already has been described (Carroll and Wharton 1989; Selivon and Perondini 1998, 1999, 2000; Selivon et al. 2004; Norrbom and Korytkowski 2009; Dutra et al. 2011a,b; Figueiredo et al. 2011), except for *Anastrepha* sp. 1 aff. *fraterculus* (Wiedemann) and *Anastrepha turpiniae* (Stone), whose micropyle is slightly dislocated from the apex (Murillo and Jirón 1994; Selivon and Perondini 1998, 2000; Dutra et al. 2011a) and for *A. barbiellinii*, *A. manihoti*, *A. nigrifascia*, *A. obliqua*, *A. pickeli*, and *A. pittieri* that have a respiratory horn on the apex of the anterior pole (Murillo and Jirón 1994, Norrbom et al. 1999, Norrbom and Korytkowski 2009, Dutra et al. 2011b).

It has long been argued that morphological characters of insect eggs such as surface sculptures, respiratory filaments, and horns may be adaptations of the species to their different habitats, such as the diversity of ovipositional substrates. These adaptations reflect the important role of the chorion in protecting the embryo during development, allowing gas exchange and controlling water loss (Kambysellis 1993, Cónsoli et al. 1999, Biron et al. 2003). In *Drosophila*, particularly in the Hawaiian species, it is believed that chorionic structures involved in respiratory exchange have undergone divergence in response to the ecological diversity of ovipositional substrates (Kambysellis 1993). There have been some reports on these structures in eggs of different *Anastrepha* species but their evolutionary significance largely has remained unexplored. In a recent study analyzing eggs of 17 *Anastrepha* species in five different species groups, the authors found no congruence between egg chorionic structures and infrageneric species groups (Figueiredo et al. 2011). According to the authors, the morphological similarity "besides reflecting phylogenetic relationship may be due to ecological or life strategy adaptive convergence." As the information currently available on egg morphology is still restricted to a small fraction of *Anastrepha* species, when further detailed observations on egg morphology of additional *Anastrepha* species, both in ancestral and derived species groups, become available, it will be possible to analyze the evolution of chorionic structures, to understand its significance in phylogenetic relationships of the infrageneric species groups, and to investigate the role of adaptive convergence to understand its significance.

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