Structural and Genetic Investigation of the Egg and First-Instar Larva of an Egg-Laying Population of *Blaesoxipha plinthopyga* (Diptera: Sarcophagidae), a Species of Forensic Importance

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Structural and Genetic Investigation of the Egg and First-Instar Larva of an Egg-Laying Population of *Blaesoxipha plinthopyga* (Diptera: Sarcophagidae), a Species of Forensic Importance

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ABSTRACT Flies in the family Sarcophagidae incubate their eggs and are known to be ovoviviparous (i.e., ovolarviparous), but a laboratory-maintained colony of *Blaesoxipha plinthopyga* (Wiedemann) deposited clutches of viable eggs over 10 generations. A description of the egg and first-instar larva of this species is provided along with genetic data (genome size and cytochrome oxidase I sequences). The egg is similar to previously described eggs of other Sarcophagidae but differs in the configuration of the micropyle. In the first-instar larva, the oral ridges are much more developed than has been described for other species. *B. plinthopyga* has forensic importance, and the present descriptive information is critical for proper case management.

KEY WORDS flesh fly, morphology, genome size, ovoviviparity

While most flies lay eggs, their strategies for optimizing fitness vary widely. Some culicine mosquitoes (Culicidae) deposit their eggs in rafts (Strickman and Fonseca 2012), whereas other insects such as the torsalo (also known as the human bot fly), *Dermatobia hominis* (L. Jr.) (Oestridae), attaches its eggs to the underside of a porter (typically an insect such as a mosquito), which brings the eggs to the vertebrate host (Catts 1982). Flesh flies (Sarcophagidae) are well-known for using the reproductive strategy of ovoviviparity (or ovolarviparity; Ferrar 1987, Meier et al. 1999, Byrd and Castner 2010), which is a reproductive strategy in which females produce eggs and incubate them after fertilization in a specialized structure called a uterus (Abasa 1970). The eggs hatch at deposition or are deposited in advanced embryonic development and hatch soon during or immediately following deposition (Meier et al. 1999). Viviparity is a derived trait in which the eggs hatch within the uterus and the larvae are nourished in utero before being deposited, and is best exemplified in Diptera by the tsetse flies (Glossinidae) (Tobe and Langley 1978, Cantrell 1981, Meier et al. 1999).

It is considered a ground-plan trait of Sarcophagidae that the female larviposits or ovilarviposits (Pape 1996, 1998). The common oviduct has a bilobed pouch (the uterus) where the fertilized eggs are incubated, and eggs usually hatch immediately before or during deposition. Deposition of eggs is believed to be uncommon. Oviposition has been documented in the kleptoparasitic species *Oebalia minuta* (Fallén) in the subfamily Miltogramminae. In this species, females glue incubated eggs onto their host wasp as a means of bringing eggs into the nest (Day and Smith 1980, Sanborne 1982). Deposition of eggs has also been reported for several species in the subfamily Sarcophaginae, including *Sarcophaga cruentata* (Meigen), *Sarcophaga exuberans* (Pandellé), *Sarcophaga nodosa* (Engel), *Sarcophaga tibialis* (Macquart), and *Liosarcophaga aegyptica* (Salem) (Knipling 1936, Arthur and Coppel 1953, Aspoas 1991, Sukontason et al. 2005, Saloña Bordas et al. 2007, Majumder et al. 2012). Such eggs were not typically viable, and in these studies eggs lacking well-developed embryos were prone to desiccation and rarely or never hatched. Only laboratory-bred individuals with constant access to a suitable breeding medium were observed to oviposit, and in all these studies oviposition was mentioned but not investigated further; the main goals of such studies being the measurement or prediction of some other life-history trait or descriptions of larval instars.

We describe oviposition by *Blaesoxipha plinthopyga* (Wiedemann) maintained in a colony. Flies in this colony deposited clutches of viable but not fully incubated eggs on beef liver over multiple generations. *Blaesoxipha* (Loew) (Sarcophagidae), in the broad sense of Pape (1994), is comprised of a large clade of koinobiont endoparasitoids of mainly Orthoptera and Coleoptera (Pape 1994) plus more basal necrophagous lineages. *B. plinthopyga* is native to the Americas, though it was recently intercepted in China (Jia et al. 2010).

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Contrary to all other species of the genus, *B. plinthopyga* can cause myiasis and has been recorded from multiple vertebrate species. In the southwestern United States, it has been reported from cases of traumatic injuries in spotted whiptail lizards (*Cnemidophorus gularis*) (Whitworth and Wangberg 1985), jackrabbits (*Lepus californicus texianus*) (Roberts 1931), and man (Patton and Evans 1929, Knipling and Rainwater 1937), and it has been reported as an agent of myiasis in an unidentified South American gecko (Marmels 1994, as *Cistudinomyia* sp., corrected in Pape and Dahlem 2010). This species can occur in nuisance numbers, and can be so common in some areas that it has been recorded that it "constantly flies around and land on people who are walking in or along the edge of the desert" (Pratt 2000). *B. plinthopyga* breeds in vertebrate carrion (Wells and Greenberg 1994, Vasconcelos and Araujo 2012) and has been bred from human remains in forensic investigations (Wells and Smith 2013).

A population of *B. plinthopyga* expressing a reproductive trait (oviposition) was discovered on the campus of Texas A&M University, providing a unique opportunity to study newly deposited eggs of a sarcophagid fly. The objective of this study was to describe the egg and first-instar larva of *B. plinthopyga* and provide genetic data for this species. Included in the genetic description is the genome size of this and two related species. Previous work has demonstrated that genome size may be useful for species-level identification (Picard et al. 2012) of blow flies. Very little is currently known about the genome sizes of sarcophagids.

Materials and Methods

Colony Maintenance. Two female flesh flies were collected in February 2012 on the campus of Texas A&M University in College Station, TX, using rotting beef liver as bait. Each female was isolated and allowed to deposit her offspring on the beef liver. Upon eclosion of the offspring, both cohorts were combined in a single BugDorm-1 299-cm^3 plastic cage (MegaView Science, Taichung, Taiwan) at 28°C, ≈50% relative humidity, and a photoperiod of 16:8 (L:D) h and allowed to interbreed. They were given deionized water, honey, and beef blood ad libitum, and this was refreshed daily for 10 d posteclosion. The colony was then denied blood or other potential breeding substrate for 24 h. For sample collection for this work, the first and second generation colonies were offered a beef liver oviposition substrate over a 4-h period to ensure that the eggs and larvae were of a known age. Liver was covered with a Kim-wipe (Kimberly-Clark Corp., Irving, TX) to reduce the rate of desiccation and provide protected oviposition sites. The substrate was then removed from the colony and eggs were either processed immediately (see "Results"), or allowed to hatch to collect first-instar larvae 12 h later. Five males were collected and pinned, their terminalia spread, and identification as *B. plinthopyga* confirmed (Fig. 1A–D, compare with, e.g., Fig. 128 in Aldrich (1916) as *Sarcophaga robusta* (Aldrich), and Fig. 78 in Carvalho and Mello-Patiu (2008)). This approach was repeated for 10 successive generations to ensure that the colony consisted of a single species. Representative samples of males, females, were collected for DNA and the immature stages collected from the colony are vouchered in the Texas A&M University Insect Collection (TAMUIC) under voucher no. 704.

Scanning Electron Microscopy (SEM) Sample Preparation. Samples were processed similarly to other studies on eggs and first-instar larvae (e.g., Sukontason et al. 2005). Freshly collected eggs were placed into a 2.5% acrolein solution to preserve and fix them. Larval samples were pretreated for 15–20 s in subboiling deionized water to extend the pseudocephalon before fixation and preservation in the 2.5% acrolein solution. Next the samples were suspended in a 1% osmium tetroxide solution. In this study, samples were then dehydrated in increasing concentrations of methanol (rather than ethanol) from 10 to 50% in 10% increments, and then from 50 to 100% in 5% increments, followed by three final washes in 100% methanol for 30 min. The samples were critical-point-dried with liquid carbon dioxide in a DCP-1 Vacuum Critical Point Dryer (Denton Vacuum, Moorestown, NJ), mounted on 10-mm aluminum stubs with double sticky tape, and coated by ruthenium chloride fuming. Ruthenium chloride fuming has been shown to be a valuable replacement for gold sputtering to increase conductivity without occluding fine detail (Dal Molin et al. 2011).

Cephaloskeleton Sample Preparation. Samples of first-instar larvae were preserved as described above. Whole first-instar larvae were macerated in a 10% KOH solution for 24 h at 28°C. After being washed in deionized water, the samples were washed in 85% ethanol. Whole larvae were mounted in polyvinyl alcohol on well slides. An additional cephaloskeleton was dissected from a macerated individual and slide mounted with the same mounting medium.

Imaging. Digital scanning electron micrographs were captured with a JEOL 6400 (JEOL Ltd., Akishima, Tokyo). Light microscopy images of the male terminalia spread for identification purposes, embryos within the chorionic sheath, and slide mounted cephaloskeleton samples were captured with a Leica IC D camera mounted on a Leica M205 microscope with a Planapo 0.63× lens and the Leica Application Suite v. 3.5.0 software (Leica Microsystems Ltd., Heerbrugg, Switzerland). The images of the egg clutches were captured with an Infinity1–3C camera (Lumenera Corp., Ottawa, Canada) mounted on a Meiji Techno EM2–8TR microscope (Meiji Techno, Tokyo, Japan) with Infinity Capture Application v. 4.4.0 software (Lumenera Corp., Canada). Structures of the egg and first-instar larva documented by SEM were confirmed via observations on at least two other specimens. Structures were described using terminology from Courtney et al. (2000).

Genome Sizes. Genome sizes of five females and five males from the colony of *B. plinthopyga* were estimated using flow cytometry following methods described by Hare and Johnston (2011). Nuclei were isolated from the pseudocephalon of single maggots by

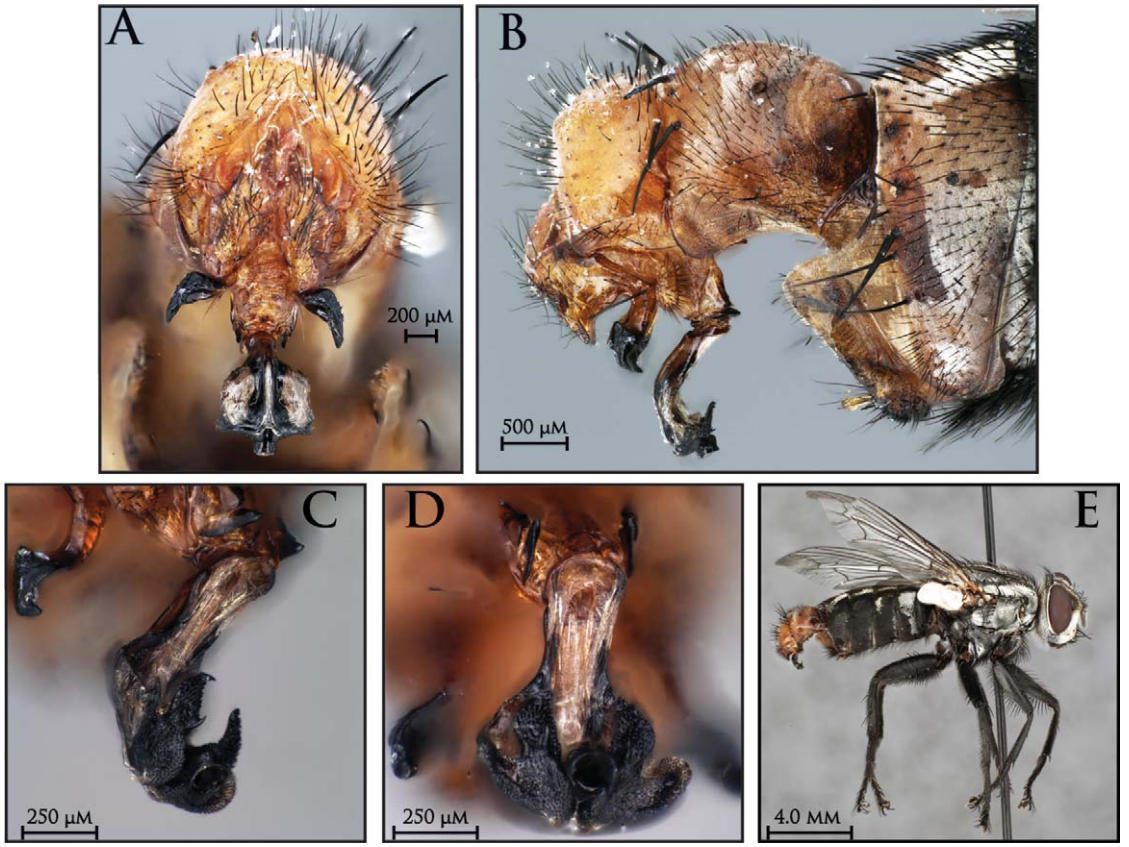

Fig. 1. *B. plinthopyga*, adult male habitus, and terminalia. (A–D) Male terminalia. (A) Posterior view. (B) Lateral view. (C) Distiphallus, antero-lateral oblique view. (D) Distiphallus, distal view. (E) Habitus with terminalia extended, lateral view.

grinding with the "A" pestle in a Dounce tissue grinder. These were stained along with co-prepared nuclei from a *Drosophila virilis* Sturtevant (Drosophilidae) standard (1C = 328 Mbp; 1C is a statistic for the average amount of nuclear DNA, nDNA, in a gamete) with propidium iodide at a final concentration of 25 μg/ml. Relative fluorescence of nDNA in sample and standard nuclei were compared using a Cy-flow (Partec, Münster, Germany) with 432 nm excitation and the nDNA amount in the sample calculated as the ratio of fluorescence in the sample and standard times the amount of nDNA in the standard. For comparison, genome sizes of a sample of eight females and eight males from a colony of *Sarcophaga bullata* Parker and four females and four males of a colony of *Sarcophaga crassipalpis* Macquart (both species of forensic importance, see Greenberg 1991, Velásquez et al. 2010) were estimated. Comparisons between genome size estimates were done using a *t*-test for multiple comparisons and pooled standard deviations.

Cytochrome Oxidase I (COI) Sequencing. Whole DNA was extracted from individual heads of four males and four females using a lithium chloride DNA extraction method. Heads were individually homogenized in 200 μl of Buffer A (100 mM Tris-HCl, 100 mM EDTA, and 100 mM NaCl) using a pestle and incubated at 65°C for 30 min. Following incubation, 400 μl of lithium chloride solution (1M KAc and 3M LiCl) was added and samples cooled on ice for 15 min. Samples were spun at 12,000 rpm for 15 min and the supernatant transferred to 300 μl of isopropanol. Samples were mixed and spun for 15 min at 12,000 rpm. Supernatant was removed and pellets washed with 80% ethanol. Pellets were air-dried and resuspended in 150 μl of Tris-EDTA buffer.

A ≈550-bp sequence of the barcoding region of the COI gene of the mitochondrial DNA was amplified. The polymerase chain reaction (PCR) mix consisted of: PCR Master Mix (2×; Thermo Scientific, Fischer Scientific, Pittsburgh, PA), 25 pM of each primer, C1-J-1751 (5′-GGATCACCTGATATAGCATTCCC-3′) and C1-N-2293 (5′-CGAGGTATTCCAGCAAGTCC-3′; Wells and Sperling 1999), and 100 ng of template DNA. PCR reactions were run on a Bio-Rad MyCycler thermocycler (Bio-Rad Laboratories Inc., Hercules, CA). Cycling conditions consisted of 35 cycles at 95°C for 1 min, followed by 45°C for 1 min, followed by 72°C for 2 min. A final extension period at 72°C for 5 min was used. Products were visualized on a 1% agarose gel using UV *trans*-illumination.

Forward and reverse sequencing reactions were performed by the Laboratory of Genome Technology

at Texas A&M University on an ABI 3130xl Genetic Analyzer (Life Technologies, Gaithersburg, MD) following manufacturer's instructions. Sequences were visualized in 4Peaks (http://nucleobytes.com/index.php/4peaks) for assessment of quality and transferred into MEGA5 (Tamura et al. 2011) for sequence trimming. A dataset of *Blaesoxipha* spp. and some *Sarcophaga* spp. known to be carrion breeders were assembled from NCBI nucleotide database. *Cochliomyia macellaria* (F.) was used as an outgroup for rooting the cladogram. A neighbor-joining bootstrap tree was constructed in MEGA5 under the Tamura–Nei model (Tamura et al. 2011) with 1,000 replicates.

The consensus sequences have been submitted to GenBank (Accession numbers KF859847–KF859854).

Data Collection for Forensic Relevance. Data on the prevalence of *B. plinthopyga* in casework collections were analyzed from cases processed by the Harris County Institute of Forensic Sciences from 14 January 2013 through 31 December 2013. Males were obtained through rearing of specimens during routine casework to estimate minimum postmortem interval following standard operating procedures (Howard County Institute of Forensic Science Standard Operating Procedures: ENTO.001, ENTO.002, and ENTO.003).

Results

Egg. All observed lab generations have at least partially reproduced via oviposition.

Egg Shape. Egg was elongate oval, ≈1.8 mm on the longest axis and 500 μm at the widest point (Fig. 2A), slightly convex dorsally and concave ventrally, with one end more acute than the other. The acute end had a small, flat, ovoid, ridged, and serrated micropyle at its apex (Fig. 2F and G), ≈45 μm on its long axis and 30 μm on the short axis. The opposite, broader end had a round indentation in the center of an irregularly shaped, spongy aeropyle (Fig. 2H). No plastron or median area was observed on any surface.

Chorion. The chorion was multilayered (Fig. 2B) with an underlying polygonal structure (Fig. 2C and D). The exochorion was comprised of spongy tissue with a system of transverse trabeculae and air pockets between them (Fig. 2E),

Development. Eggs from individual clutches exhibited a range of appearances. Some eggs within a clutch were a uniform, creamy yellow, whereas in others well-developed embryos could be clearly distinguished through the chorion in that they were dark colored and striped dorsally (Fig. 3A and B). These more developed embryos, while still enveloped within the chorionic sheath (Fig. 3C), were clearly distinguishable by their cephaloskeleton and transverse rows of darkened cuticular spines at intersegmental boundaries.

First-Instar Larva. Samples of first-instar larvae were either obtained after eggs hatched or immediately after larviposition.

Body Shape. About 4.4 mm in length, with a typical maggot form; tapered anteriorly and blunt posteriorly (Fig. 4A). Under SEM, the anterior spiracles were not visible externally (Fig. 4B), confirming that the specimens were in the first instar. However, the developing second-instar anterior spiracles could be observed through the cuticle in late first instars (Fig. 3D).

Pseudocephalon. Collar well-developed (Fig. 4A, B, C, and D), narrower dorsally and forming a protruding lip ventrally posterior to the pseudocephalon (Fig. 4B). The oral ridges extend along the entire lateral aspect (Figs. 4D, and 5). Each oral ridge consisted of three sections: anteriorly, a narrow, somewhat smooth band of cuticle; in the middle, a band of long and narrow ridged cuticle; and posteriorly, a row of broad and irregularly patterned cuticle.

There were two pairs of major sensory organs anteriorly on the pseudocephalon (Fig. 4C); the antennae on the dorsum (Fig. 4E), and the maxillary palpi ventrally (Fig. 4F). The antenna consisted of a large styloconic sensillum. The conical peg of the sensillum was smooth, longer than its widest diameter, and longer than the pocket into which it was socketed. The cuticle of the socket was finely textured with three pores: a circular swirl laterally, a small pore ventrally, and a hemispherical depression medially adjacent to the peg (Fig. 4E). The maxillary palp (Fig. 4F) consisted of a large plate of interleaved cuticular ridges and eight small papillae (three sensilla basiconica plus three sensilla coeloconica in a central cluster and two additional sensilla coeloconica).

Posterior Spiracles. The spiracular atrium was located posterodorsally on the anal division and surrounded by a ring of hair-like spines and six papillae (Fig. 4H). Posterior spiracles on spiracular discs were set within this depression (Fig. 4G), each disc with two almost vertical, subparallel spiracular slits, and ≈25 μm apart dorsally and 15 μm apart ventrally. The medial slit on each disc was slightly shorter than the lateral slit. There were four perispiracular tufts (or "palmate hairs"), the two lateral and the median tuft of similar size and with few and mostly short branches, the dorsal tuft reduced to a slender, unbranched structure. The broad bases of the ventrolateral, dorsolateral, and medial tufts originate were ≈5, 30, and 30 μm, respectively, from the ventral base of their closest spiracular slit. The ventrolateral and medial tufts exhibited deeper invaginations than the dorsolateral one.

Genetic Data. DNA was successfully extracted, the COI gene of the mitochondrial DNA was sequenced from four male and four female samples, and all consensus sequences were uploaded to GenBank. Using a neighbor-joining bootstrap tree under the Tamura–Nei model (Tamura et al. 2011) with 1,000 replicates, all sequences were assigned species identity as *B. plinthopyga* (Fig. 6). This was supported by a high bootstrap value (100%) for inclusion with the clade *B. plinthopyga.*

Genome Size. The genome sizes of *B. plinthopyga* were estimated for five individuals of each sex (Table 1). The size of the female genome was 1C = 1,244 ± 5 Mbp, and was significantly larger than the size of the male genome, which was 1C = 1,179 ± 6 Mbp ($P < 0.0001$). Also determined were the genome sizes of both sexes of two other species of Sarcophagidae (Ta-

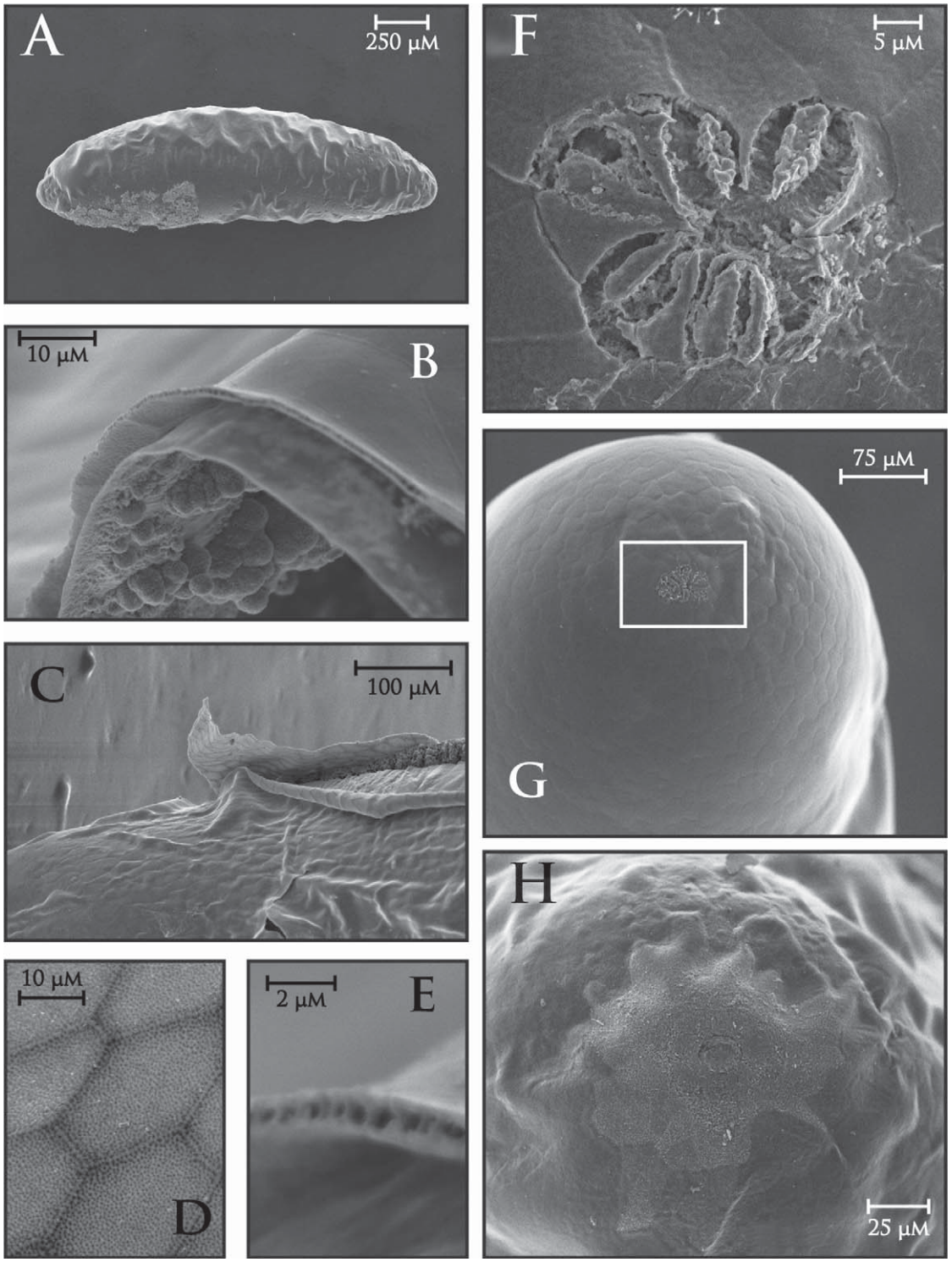

Fig. 2. *B. plinthopyga*, SEM of egg. (A) Egg, lateral view (anterior pole to the right). (B) Chorion, showing both outer and inner layer. (C) Exochorion, underside showing polygonal structure. (D) Exochorion, external surface showing polygonal structure. (E) Exochorion, cross-section showing presence of transverse pillars. (F) Micropyle showing irregular, serrated structures surrounding a depressed area of chorion. (G) Anterior pole, showing polygonal structure of chorion and the micropyle (white rectangle). (H) Aeropyle at blunt posterior pole showing an irregular distribution of spongy tissue around a central circular depression.

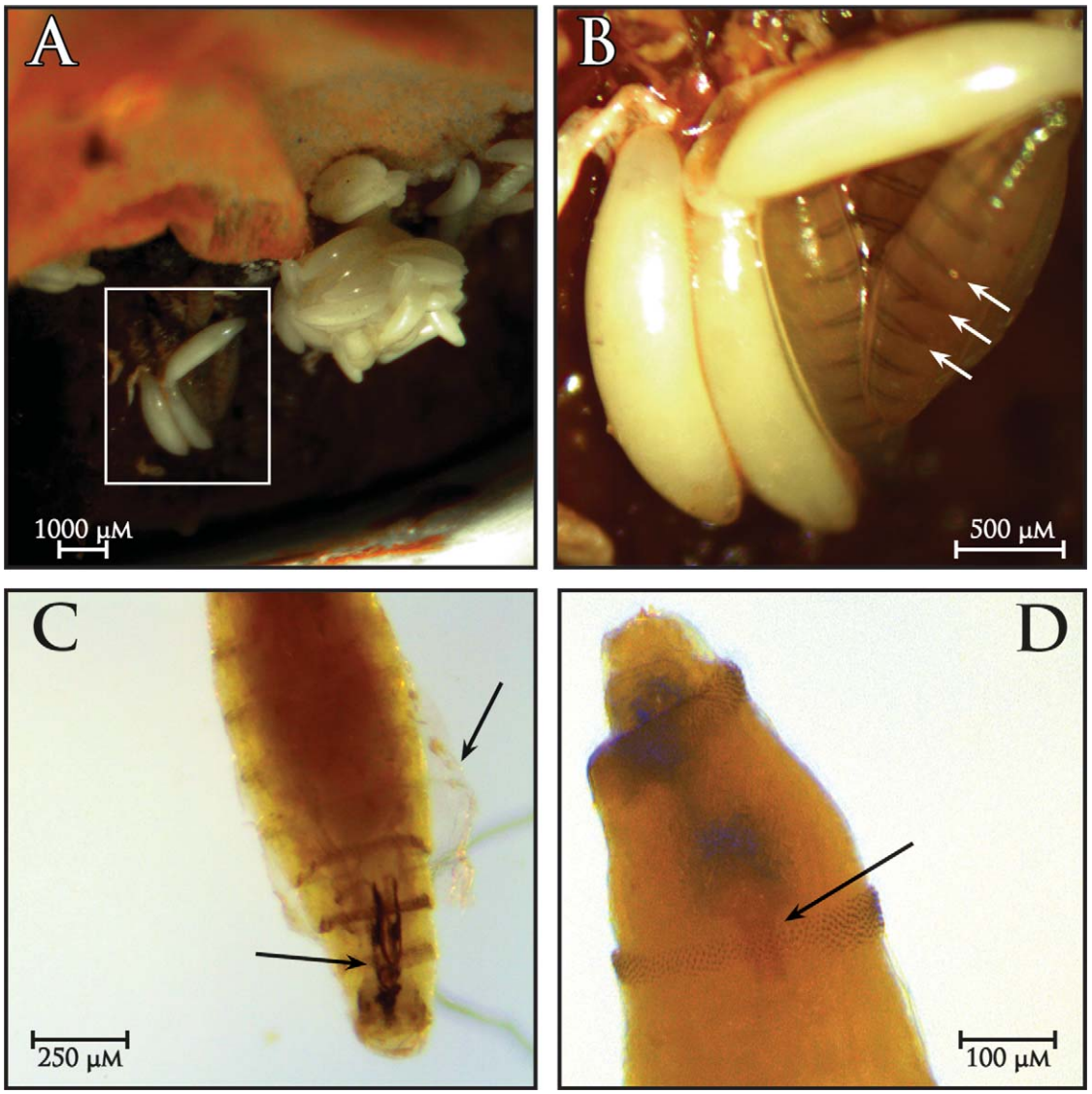

Fig. 3. *B. plinthopyga* egg clutches, late embryos within their chorion, and first-instar larva. (A) Large clutch of eggs (center) laid on beef liver (dark red) covered with a moistened wipe (light red); clutch in the white rectangle contains both undeveloped (creamy and opaque) eggs and mature embryos. (B) Six eggs, three creamy white and undeveloped, three with mature embryos within their chorion; white arrows indicate paired spine bands at intersegmental boundaries on a single embryo. (C) An embryo within its partly shed chorion (upper black arrow); cephaloskeleton clearly visible (lower black arrow). (D) First instar using backlighting to demonstrate lack of externally visible anterior spiracles; second instar spiracles can be seen developing through the first instar cuticle (arrow).

ble 1). *B. plinthopyga* females had significantly larger genomes than either *S. crassipalpis* or *S. bullata* females ($P < 0.0002$; $P < 0.0001$).

Evaluation of the Forensic Relevance of *B. plinthopyga*. Sarcophagidae were collected at 77.3% of indoor death investigation scenes (34 of 44) from January through December of 2013. Larval Sarcophagidae specimens collected and reared to adults ($n = 24$) from four different bodies recovered from indoor scenes between January and December 2013 were all identified as *B. plinthopyga.* In Harris County, TX, Sarcophagidae are collected more often than Calliphoridae during indoor death investigation scenes with only 50.0% (22 of 44) of bodies recovered from indoor scenes which exhibit insect colonization with blow flies.

Discussion

The laboratory-maintained colony of *B. plinthopyga* has been depositing a mixture of first-instar larvae and eggs with a significant postdeposition development over multiple generations. Aspoas (1991) studied four

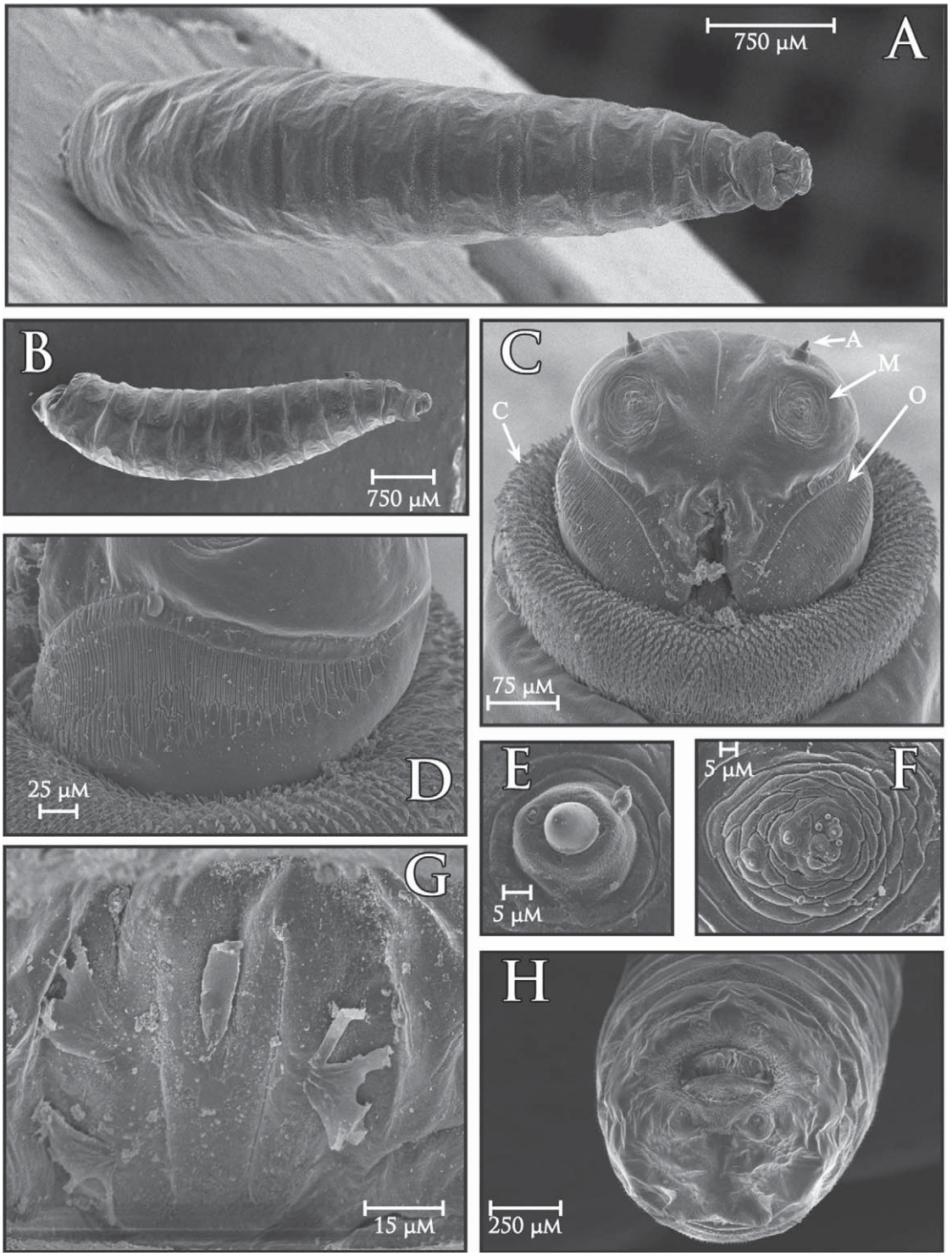

Fig. 4. *B. plinthopyga*, SEM of first-instar larva. (A) Ventral view, showing distribution of transverse rows of spines at the intersegmental boundaries. (B) Lateral view, showing the spined collar, which is narrow dorsally and protrudes ventrally into a lip-like structure. (C) Ventral view of anterior end, showing the collar C), oral ridge O), antenna A), and maxillary palp M). (D) Oral ridge, anterolateral view, showing tapering shape, broader ventrally and narrowing out dorsally. (E) Antenna. (F) Maxillary palp. (G) Left spiracular plate, showing paired vertical slits and perispiracular tufts with slender, unbranched dorsal tuft. (H) Posterior view, showing distribution of papillae around the spiracular atrium and paired spiracular plates within.

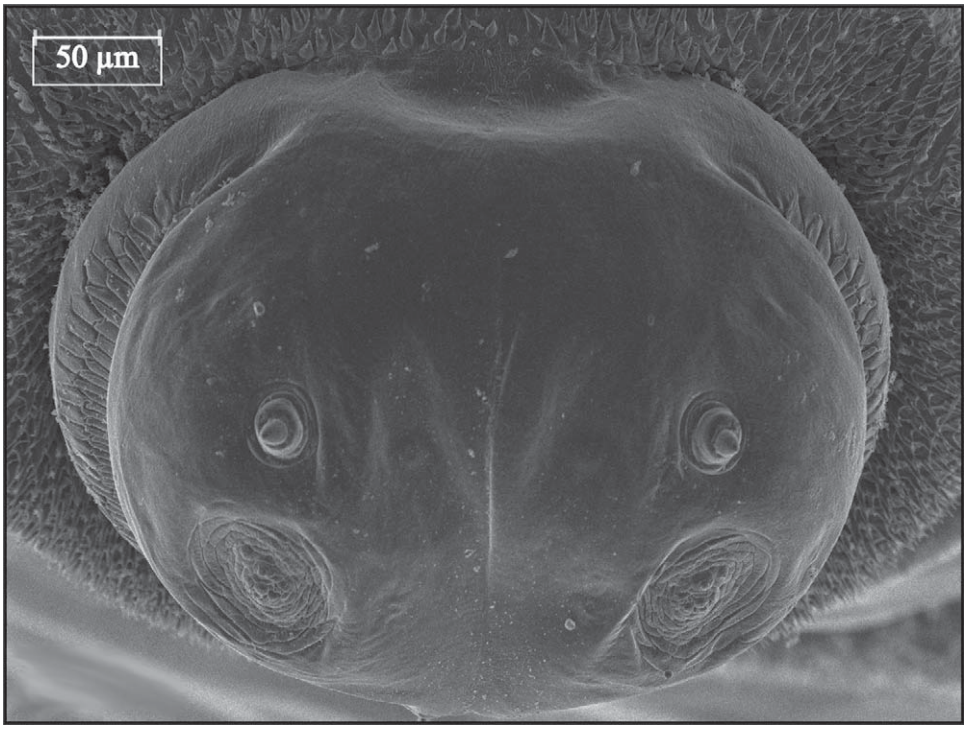

Fig. 5. *B. plinthopyga*, SEM of anterior of first-instar larva. The length of the oral ridges is clearly visible, demonstrating that the paired oral ridges extend dorsally until they almost meet.

species of *Sarcophaga* (*S. cruentata*, *S. exuberans S. nodosa*, and *S. tibialis*) and found that wild-caught gravid females did not deposit eggs, only larvae, whereas laboratory-bred females always deposited eggs, and these eggs would hatch within 2–20 min of deposition. Saloña Bordas et al. (2007) reported how females from the first generation of a laboratory culture of *S. aegyptica* "began to lay eggs instead of larvae," and although "most of the eggs were not viable and did not complete their development, deteriorating in a few days," some eggs with larvae in an advanced state of development would hatch within 30–60 min. In the current study, eggs have been observed to hatch 4 h postoviposition. Our study is the first to integrate egg and larval morphology, genome size, and gene sequence information for a forensically important sarcophagid.

The egg of *B. plinthopyga* is similar in general appearance to those described from other sarcophagids (Ferrar 1987, Lopes and Leite 1989, Sukontason et al. 2005); unlike the previous studies, however, these eggs were obtained without dissection. The micropyle of the *B. plinthopyga* egg appears to be quite different from that observed on the eggs of flies in other oestroid families. In *Pollenia* spp. (Calliphoridae), the micropyle is situated in a deep depression or funnel (Grzywacz et al. 2012), whereas in *Calliphora vicina* (Robineau-Desvoidy) and *Lucilia cuprina* (Wiedemann) (Calliphoridae) the depression is shallow (Greenberg and Singh 1995). Evidence concerning the shape and position of the micropyle from the dissected eggs of other sarcophagids suggests that the structure may vary considerably in the family. The micropyle of some species of Sarcophagidae appears to be a simple hole in a depression created by a lip of chorion [*Peckia lambens* (Wiedemann), see Lopes and Leite (1989); *Sarcophaga aratrix* Pandellé, see Baudet (1985)]. Saloña Bordas et al. (2007) noted that the micropyle of the egg of *S. aegyptica* is centrally located at the anterior pole, but the documentation provided is insufficient and their figure showing the micropyle appears to point to shallow, anterolateral flanges reminiscent of the hatching pleats found widespread within Diptera (Grzywacz et al. 2012). In one species of the subfamily Miltogramminae, the micropyle is stellate in appearance, lacking toothed ridges and situated in a shallow depression (Szpila and Pape 2008). In comparison, the micropyle in *B. plinthopyga* appears to be complex in shape and flush with the egg surface.

The first instar of *B. plinthopyga* was described for the first time by Knipling (1936, as *S. plinthopyga*). His description focused on the length and distribution of spines on the cuticle, and the only illustration was a drawing of the cephaloskeleton. Lopes (1983, as *Hystricocnema plinthopyga*) provided additional drawings of the first-instar cephaloskeleton at various angles but without further data. Our images of the cephaloskeleton (Fig. 7) are comparable in shape. The oral ridges observed in this species are broader and more well-developed compared with first-instar larvae of other sarcophagids (Sukontason et al. 2003; Szpila and Pape

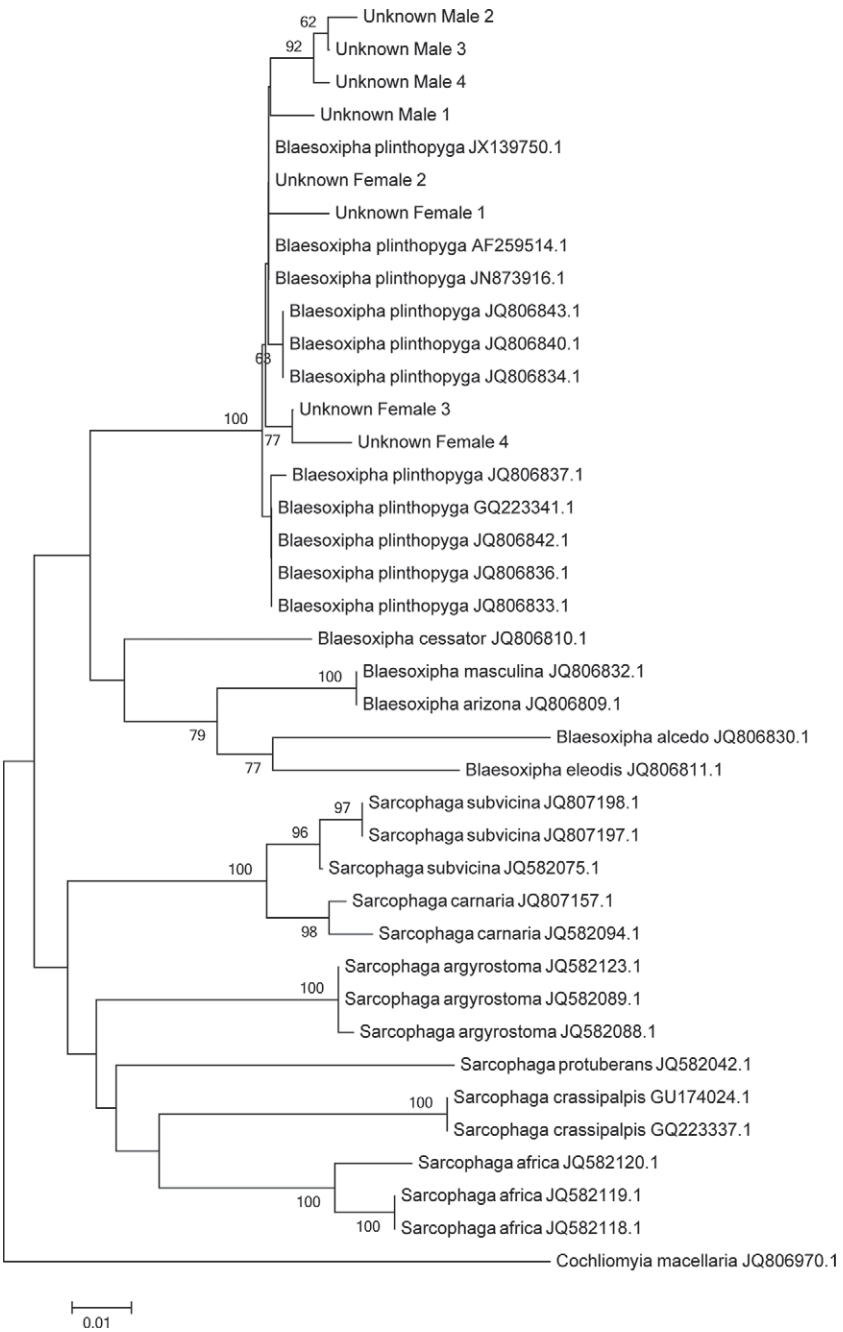

Fig. 6. Genetic confirmation using COI. Neighbor-joining bootstrap phylogeny of Sarcophagid flies of *Blaesoxipha* spp. and carrion-breeding *Sarcophaga* spp. The analysis is presented in the form of a phylogram in which branch length indicates the number of base substitutions. The phylogram was based on 519 bp of the COI gene. Numbers indicate the bootstrap percentage support for each branch (1,000 replicates). Position of *B. plinthopyga* in a distinct clade indicates correct identity of specimens.

2005a,b, 2007, 2008; Brink 2009; Draber-Mońko et al. 2009). Species with narrow, noncoprophagous diets have simpler, less developed oral ridges (Schmidt 1993; K. Szpila and T. P., unpublished). Therefore, the size and complexity of the oral ridges in first-instar *B. plinthopyga* may be related to aspects of its broadly necrophagous lifestyle.

B. plinthopyga has been frequently observed as a carrion-breeding fly in the southern United States since the 1920s (Patton and Evans 1929), a behavior

Table 1. Genome sizes of males and females of three species of Sarcophagidae flies

Species	Sex	Size (Mbp)	SE (Mbp)	*n*	Levels	Sexual dimorphism
B. plinthopyga	F	1244	±5	5	A	Yes
	M	1179	±6	5	B	$P < 0.0001$
S. crassipalpis	F	1203	±3.7	4	C	Yes
	M	1173.25	±7.26	4	B	$P = 0.0343$
S. bullata	F	597.3	±6.9	8	D	No
	M	590.5	±5.3	8	D	$P = 0.7547$

Reported here are the genome sizes of males and females three species of sarcophagid flies from colony specimens as estimated with flow cytometry. Sizes of 1C (average amount of nDNA in a gamete) are reported in mega basepairs (Mbp) along with SE, also in Mbp, number of specimens (*n*) used to estimate the genome size, the statistically significant groups based on differences in genome sizes between the eight samples (species and sex) using a *P* value cutoff of 0.05, a *t*-test for multiple comparisons and pooled standard deviations (Levels), and whether the species exhibits sexual dimorphism in genome size based on a *t*-test corrected for multiple comparisons (sexual dimorphism).

which is in strict contrast to the endoparasitoid strategy used by the large majority of species within this genus (Pape 1994, Allen and Pape 1996, Danyk et al. 2005). One morphological realization of these different strategies may be observed in the shape of the cephaloskeleton, which in *B. plinthopyga* presents a pair of robust mouthhooks and a complete dorsal bridge (clypeal arch; Lopes 1983) like other generalist necrophages. In all species of the subgenera *Acridiophaga* Townsend, *Acanthodotheca* Townsend, *Blaesoxipha* (s.str.), *Servaisia* Robineau-Desvoidy, and *Tephromyia* Townsend within *Blaesoxipha*, the mouthhooks are reduced to a varying degree, and the dorsal bridge is incomplete anteriorly or even absent. This is possibly because these parasitoids have less need for forcefully operating their mouthhooks and thus for the associated strong muscle attachments, as the primary function of the mouthhooks would seem to be to penetrate a nonsclerotized part of the host cuticle to enter the hemocoel (Léonide and Léonide 1986). Similarly, for the first-instar posterior spiracles, which in all species of the abovementioned subgenera except for *Acanthodotheca* have their rim extended into a sclerotized bifid spine (Léonide and Léonide 1986, Pape 1994), the hypothesized function of this specialized morphology is to pierce a trachea of the grasshopper host to gain access to oxygenated air (Ferrar 1987). *B. plinthopyga*, classified within the subgenus *Gigantotheca* Townsend by Pape (1994, 1996), does not share this specialized morphology. However, the subgeneric classification of *Blaesoxipha* is based on adult male characteristics "for most of the peculiar species for which no information on females exist(s)" (Pape 1994), and there is an associated paucity of available information on the larval characteristics. At least for *B. plinthopyga*, this deficiency in immature description has now been amended.

The posterior spiracles are remarkable by showing perispiracular tufts with a slender, unbranched dorsal tuft, while the others are of a more typical palmate shape. This configuration is reminiscent of (and convergent to) that found in blow flies of genus *Lucilia* Robineau-Desviody (Diptera: Calliphoridae) (Szpila et al. 2013), but the biological significance of this is unknown.

The importance of this species for forensic casework in the United States is only now becoming recognized. The first record of *B. plinthopyga* on decomposing human remains within the United States was recently published (Wells and Smith 2013) on specimens from Idaho. In Harris County, TX, the Sarcophagidae appear to have an important role in indoor decomposition of human remains. Indeed, *B. plinthopyga* appears to be among the primary colonizers of indoor human remains in Harris County. Therefore, there is a need for the development of

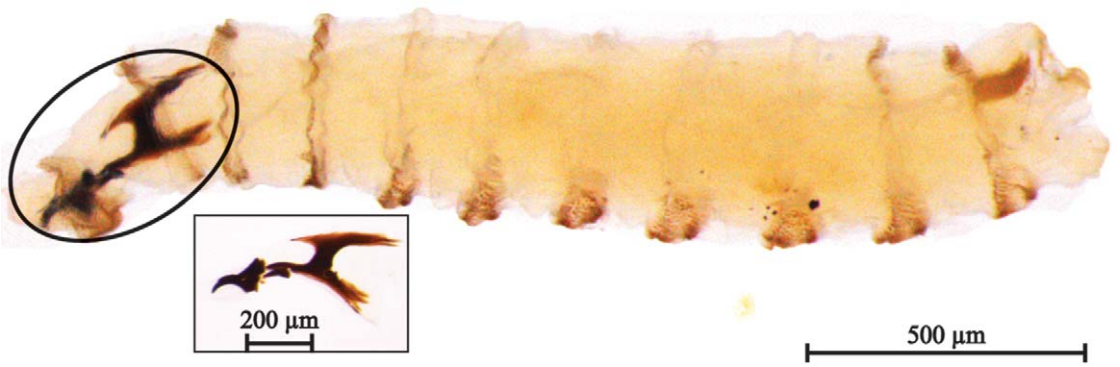

Fig. 7. *B. plinthopyga*, first-instar larva, habitus and cephaloskeleton. The cephaloskeleton (in circle) is clearly visible through the cuticle of this slide-mounted first-instar larva. Inset shows excised cephaloskeleton.

temperature-based developmental datasets for this species that are life-stage-specific (Denlinger et al. 1988 includes an egg-to-adult development time). Furthermore, the difficulty in species-level identification has led researchers to turn to molecular methods for identification (Wells et al. 2001, Picard et al. 2012). The results reported here suggest that genome size estimates may be a useful tool for differentiating adult female specimens where morphological tools are currently lacking.

B. plinthopyga was only recently reported as collected during a forensic investigation for the first time in the United States (Wells and Smith 2013), though it had been found associated with human remains in Costa Rica 30 yr earlier (Jirón et al. 1983). The genetic information and the descriptions of the egg and first-instar larva will facilitate the correct identification of this and other flesh flies of forensic importance to species and thereby assist investigators in the production of more accurate estimates of time of colonization (Byrd and Tomberlin 2010). It also highlights an important assumption that is often made regarding eggs found on a body during casework, such that one should not assume that creamy white oblong eggs are from Calliphoridae. In the absence of high-powered microscopy, a portion of the egg samples should be reared to confirm identification whenever possible.

It is unknown how often *B. plinthopyga* exhibits egg laying rather than ovovivipary, as reported here. Nor is it known if the ovoviviparity is a simple response to resource availability. It has been suggested that an overabundance of protein sources available to the adults will induce egg-laying in some laboratory colonies of other Sarcophagidae (Aspoas 1991, Saloña Bordas et al. 2007; C. C. Heo, personal communication). The information garnered from this work generates the opportunity to investigate the evolutionary and ecological implications of choice in a plastic reproductive system (the behavior appears to be environmentally dependent). Further studies, in this and other species, to investigate the factors that affect the timing, induction and regulation of deposition of incubated offspring in the laboratory may help researchers to better understand the evolution and ecology of inter- and intraspecific plasticity in reproductive strategies and the implications for forensic entomology and casework.

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