Description of the Egg of *Ochlerotatus japonicus japonicus* (Diptera: Culicidae) Using Variable Pressure Scanning Electron Microscopy

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ABSTRACT The egg of *Ochlerotatus japonicus japonicus* (Theobald) is described with the aid of variable pressure scanning electron micrographs. The egg is black, cigar shaped, and tapers ventrally. The length is ≈591 μm and the width is ≈172 μm. The outer chorionic cells are irregular in shape, either hexagonal or pentagonal, and decrease in size toward the anterior and posterior poles. Ventral tubercles typically range from three to six and contact the chorionic reticulum. Dorsal tubercles contain two large tubercles with small oval-shaped tubercles grouped around them. A large thread-shaped tubercle extends from this grouping either as a single tubercle or as a series of connected tubercles. The micropylar collar is low and discontinuous and is seldom complete. This description will aid researchers in the identification of this invasive vector species.

KEY WORDS *Aedes albopictus*, *Ochlerotatus triseriatus*, *Ochlerotatus japonicus japonicus*, egg, La Crosse virus

The previous range of *Ochlerotatus* (*Finlaya*) *japonicus japonicus* (Theobold) (=*Aedes japonicus japonicus*) (Reinert 2000) included Korea and Paleartic Japan (Tanaka et al. 1979); it had also been collected in the former USSR (Gutsevich and Dubinsky 1979). Since its introduction into the United States in 1999 (Peyton et al. 1999, Morris et al. 2007), it has spread rapidly and poses a significant threat to the maintenance and transmission of arboviruses. *Oc. japonicus* has been shown to be a competent laboratory vector of La Crosse virus (Sardelis et al. 2002b), eastern equine encephalitis virus (Sardelis et al. 2002a), St. Louis encephalitis (Sardelis et al. 2003), West Nile virus (Sardelis and Turell 2001), and Japanese encephalitis virus (Takashima and Rosen 1989). Recently, this vector has been found at several case sites of La Crosse encephalitis in eastern Tennessee (A.D.H., unpublished data).

Herein we describe the egg of *Oc. japonicus* with the aid of a variable pressure scanning electron microscope (VP-SEM). This is the first use of a VP-SEM to describe a culicid egg. There are two partial descriptions of the egg of *Oc. japonicus*, both using traditional low-power SEMs. The first shows the ventral (upper) chorionic surface and cells of a deformed egg (Matsuo et al. 1972), and the second shows the ventral (upper) chorionic cells (Moriya et al. 1973). We present these new data to assist field biologists in the identification of predominantly collected tree-hole/container species and to provide more detailed information on characters of Aedini eggs. This brings the number of more complete descriptions and micrographs of the micropyle and associated structures of the subgenus *Finlaya* to three.

Materials and Methods

Embryonated eggs were obtained from a laboratory colony at Rutgers University, NJ. The colony was derived from field-collected individuals from Ocean and Somerset Counties, NJ, collected from 2000 to 2001. Blood-fed mosquitoes were provided moist seed germination paper on which to oviposit. Eggs originated from five different females. Three eggs were randomly selected for analysis from each female.

Microscopy was conducted using a Hitachi S-4300 SE/N VP-SEM (Hitachi High Technologies America, Rexdale, Ontario, Canada). VP-SEM is more versatile than traditional SEM for a number of factors (Goldstein et al. 2003), most notably the ability to view specimens in their natural state, leading to a reduction in specimen preparation cost and time. Measurements were made using Image J (National Institutes of Health). Terminology follows that of Harbach and Knight (Harbach and Knight 1980), and the definition of Linley (Linley 1989a) of "outer chorionic cell" and "cell field." Previous work used the mean length and width of the outer chorionic cells, but this procedure

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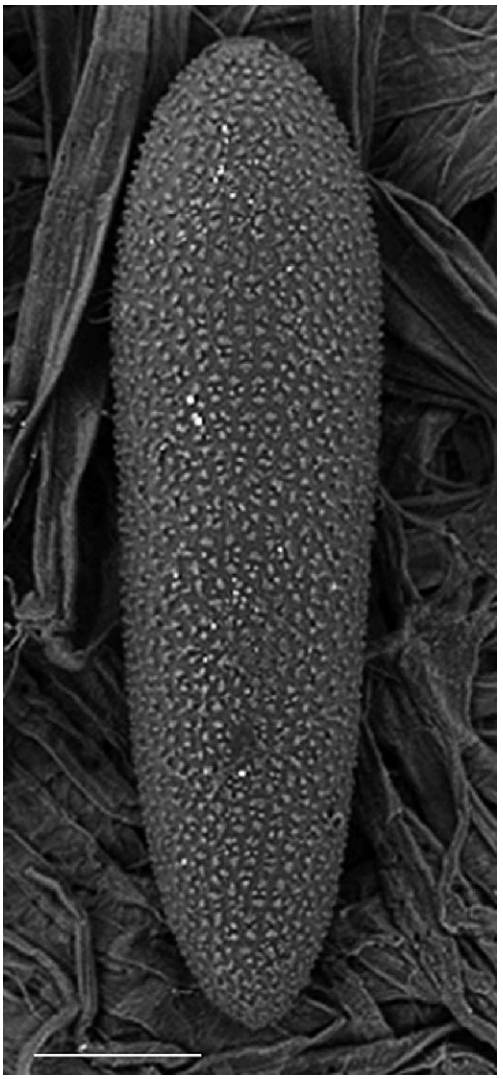

Fig. 1. Entire egg. Scale = 100 μm.

does not lend itself well to replication between researchers. To enhance repeatability, the area of the cells was determined. Descriptive statistical analyses were performed using STATA 10.0 (STATA, College Station, TX).

Results

General Features. The egg is black and cigar-shaped, tapers ventrally, and has a conspicuous micropylar collar (Fig. 1). The mean length was 591 ± 7.6 (SE) μm; mean width was 172.3 ± 2.6 μm; and the mean length/width ratio was 3.44 ± 0.06. The outer chorionic cells are mainly hexagonal, although some are pentagonal. Individual cell areas range from 177 to 355 μm^2. Dehiscence occurs transversely approximately one fourth from the anterior pole (Fig. 2a and b).

Ventral (Upper) Surface. With the exception of the anterior and posterior poles, the chorionic cells are primarily hexagonal and are variable in size (Fig. 3a). Within the cell fields, tubercles range from three to six in number. These tubercles are typically irregular in size and appear trapezoidal in shape, are flat topped or gently rounded, and are present throughout the cell field (Fig. 3b). Spoke-like bridges attach them on at least one side to cell floor and/or to the inner rim of the outer chorionic reticulum. The surface of the cell field is smooth in texture.

Anterior Pole and Micropyle. The outer chorionic cells diminish in size approaching the anterior pole (Fig. 4a). Tubercles decrease in number to two or three within the cell, eventually fusing to each other and the reticulum walls (Fig. 4b and c). The micropylar collar is low and discontinuous, with three to four narrow notches present, and is seldom complete (Fig. 4d). The micropylar collar width is highly variable: mean width at the narrowest point was 3.77 ± 0.15 μm, mean width at the widest point was 7.27 ± 0.27 μm, micropylar collar mean diameter was 31.2 ± 0.98 μm, the internal diameter was 17.8 ± 0.47 μm, and micropylar collar mean depth was 3.18 ± 0.19

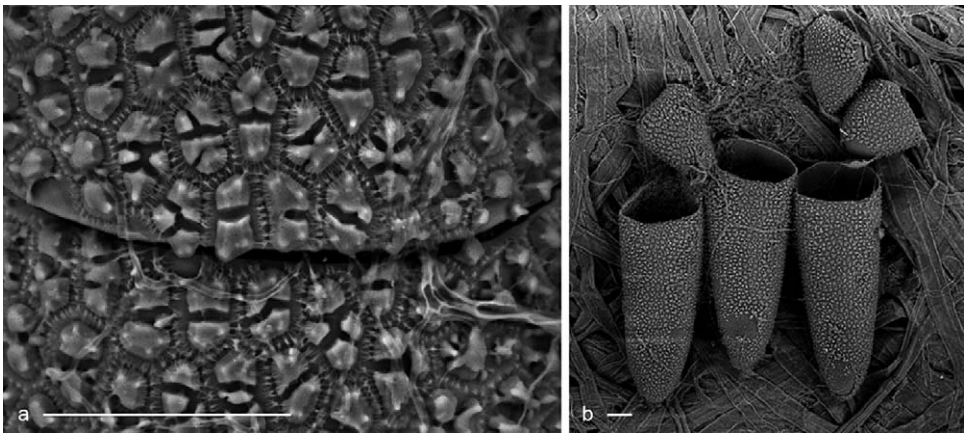

Fig. 2. (a) Transverse dehiscence, ventral (upper) surface, approximately one fourth the distance from the anterior pole. (b) Hatched eggs with associated egg caps. Scale = 50 μm.

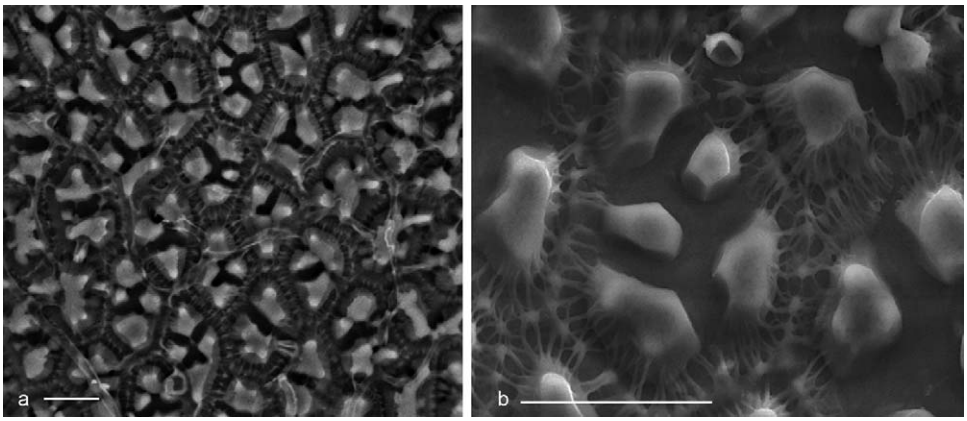

Fig. 3. (a) Ventral (upper) surface, typical outer chorionic cells, midway along the length of the egg. (b) Detail of the ventral chorionic cell structure showing five raised tubercles connected to inner rim of the outer chorionic reticulum by spoke-like bridges. Scale = 10 μm.

μm. The micropylar disk is not clearly defined, is slightly domed, and has a mean diameter of 11.5 ± 0.38 μm. The micropyle itself has a mean diameter of 1.69 ± 0.04 μm.

Posterior Pole. The outer chorionic cells diminish in size approaching the posterior pole (Fig. 5a). Tubercles may fuse to each other and the reticulum walls.

Ventral-dorsal Transition Areas and Dorsal (Lower) Surface. The transitional zone in *Oc. japonicus* is narrow and occurs rapidly. The dorsal (lower) surface outer chorionic cells become extremely irregular in shape. Each cell contains two large tubercles with small oval shaped tubercles grouped around them; a large thread-shaped tubercle extends from this group-

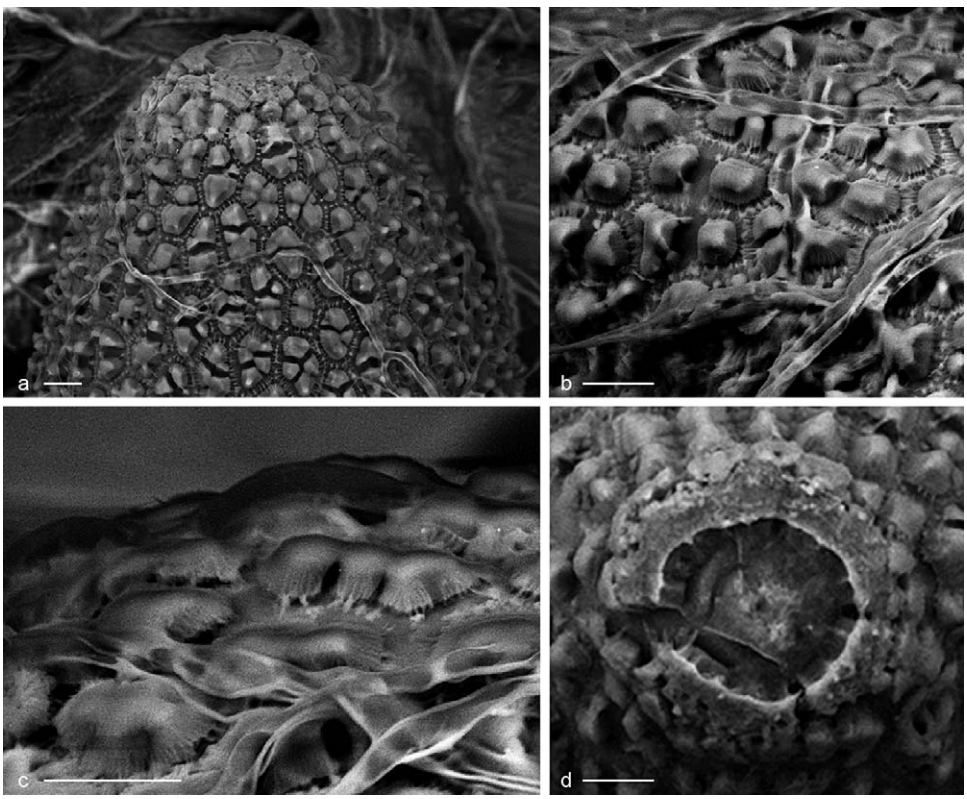

Fig. 4. (a) Anterior pole, ventral surface. (b) Outer chorionic cells, ventral surface, approaching the anterior pole. (c) Anterior pole, ventral surface, outer chorionic cell detail. (d) Top view, anterior pole and detail of the micropylar apparatus. Scale = 10 μm.

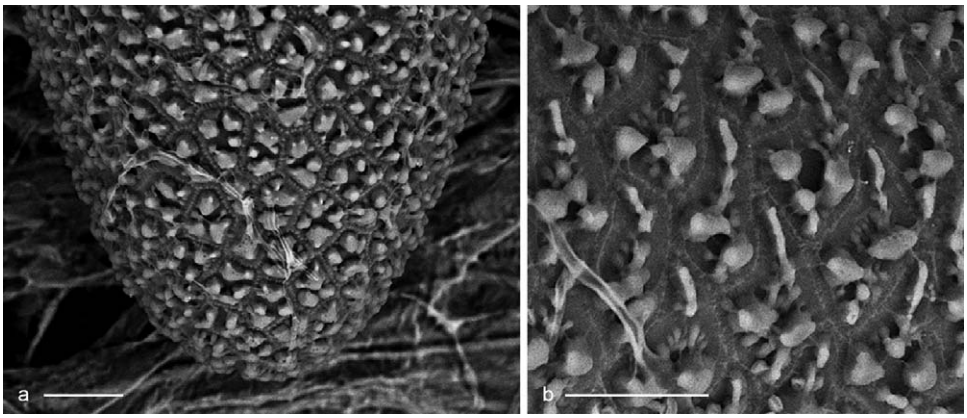

Fig. 5. (a) Posterior pole, ventral (upper) surface. (b) Detail of the outer chorionic cells, dorsal (lower) surface. Scale = 20 μm.

ing either as a single tubercle or as a series of connected tubercles (Fig. 5b).

Discussion

Partial descriptions of *Finlaya* species eggs exist for *Oc. togoi* (Theobald), *Oc. melanopterus* (Giles), *Oc. albolateralis* (Theobald), *Oc. formosensis* (Yamada), and *Oc. japonicus* (Matsuo et al. 1972, 1974). Two more complete descriptions exist for the eggs of *Oc. alboannulatus* (Macquart) and *Oc. rubrithorax* (Macquart) (Linley et al. 1991). The characteristics listed in Table 1 form a basis of dimensions that could be useful for the separation of these species. When compared with the descriptions of *Oc. alboannulatus* and *Oc. rubrithorax* (Linley et al. 1991), the *Oc. japonicus* specimens examined did not exhibit the mid-ventral strip of cells along the dorsal-ventral transition zone. Of the seven *Finlaya* species for which partial and/or complete descriptions exist, there are noticeable differences in the fine structure of the eggs (Matsuo et al. 1972, 1974, Linley et al. 1991). Further work within this subgenus is needed to determine further differentiating characteristics.

Ochlerotatus japonicus has been shown to be a competent laboratory vector of La Crosse virus (Sardelis et al. 2002b), although its role in the natural maintenance and transmission of the virus has yet to be determined. La Crosse virus was first isolated from the brain of a deceased pediatric patient who died of the virus in 1960 (Thompson et al. 1965). Since that time, confirmed cases of La Crosse encephalitis have been reported to the Centers for Disease Control in 29 of the lower 48 states (Nasci et al. 2000). The primary vector in the upper Midwestern United States is *Ochlerotatus triseriatus* (Say) (=*Aedes triseriatus*) (Watts et al. 1972, Calisher 1994, Reinert 2000). La Crosse virus has been isolated from *Ae. albopictus* (Skuse) in eastern Tennessee, and evidence suggests that this species may be involved in the maintenance and transmission of the virus in the region (Gerhardt et al. 2001, Erwin et al. 2002). *Oc. japonicus* was collected in eastern Tennessee in 2003 and has since become established (Caldwell et al. 2005). All three species' eggs have been collected together in tree-holes, ovitrap cups, and EPS float traps within their range, and therefore differentiating characteristics would prove useful in identifying these species (A.D.H., unpublished data; Table 2).

The eggs of *Oc. japonicus* and *Oc. triseriatus* are similar in superficial appearance, whereas the egg of *Ae. albopictus* is noticeably different. With regard to size, *Oc. triseriatus* is the largest of the three species and likely could be differentiated from *Oc. japonicus* by length. In terms of fine structure, the eggs of *Ae. albopictus*, *Oc. triseriatus*, and *Oc. japonicus* differ con-

Table 1. Dimensions of the described eggs of *Ocherlotatus* (*Finlaya*) species[a]

Species	Length (μm)		Width (μm)		Length/width (μm)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Oc. alboannulatus[b]	700.5 ± 5.9	669.6–762.0	187.8 ± 1.9	177.2–203.8	3.74 ± 0.05	3.46–4.05
Oc. albolateralis[c]	495 ± 2	470–510	151 ± 3	120–170	NA	
Oc. formosensis[c]	611 ± 4	550–650	167 ± 3	120–180	NA	
Oc. melanopterus[c]	572 ± 3	550–590	169 ± 5	130–200	NA	
Oc. rubrithorax[b]	682.3 ± 6.1	632.9–727.8	179.6 ± 1.9	165.8–192.4	3.81 ± 0.06	3.49–4.37
Oc. togoi[c]	516 ± 6	450–560	159 ± 4	140–180	NA	

[a] *Ochlerotatus* = *Aedes*, see Reinert 2000.
[b] Linley et al. 1991.
[c] Matsuo et al. 1974.
NA, not available.

Table 2. Differentiating characteristics useful for identifying the tree-hole/container inhabiting mosquitoes involved in the transmission and/or maintenance of La Crosse virus in the United States

	Ochlerotatus japonicus	*Ochlerotatus triseriatus*[a]	*Aedes albopictus*[b]
Total length range (μm)	545.3–636.7	NA	558.8–629.4
Mean length ± SE (μm)	591.0 ± 7.6	680.8 ± 9.6	609.8 ± 5.9
Total width range (μm)	161.7–186.7	NA	170.6–211.8
Mean width ± SE (μm)	172.3 ± 2.6	201.6 ± 2.0	192.9 ± 2.4
Total L/W ratio range (μm)	3.13–3.85	NA	1.97–3.42
Mean L/W ratio ± SE (μm)	3.44 ± 0.06	3.15 ± 0.05	3.28 ± 0.04
Micropylar collar range (μm)	25–38	36.0 (range not given)	45–50
Width of micropylar disk range (μm)	Not clearly defined, slightly domed, 9.04–15.3	Raised, 18.0	Not clearly defined, domed, 18–21
Micropylar orifice (μm)	1.7 ± 0.04 (1.36–1.93)	2.0	3.3
Outer chorionic cell shape	Primarily hexagonal, some pentagonal	Irregularly hexagonal, some pentagonal	Hexagonal, occasionally pentagonal
Egg color	Matte black	Dull black	Shiny jet black

[a] Linely (1989a).
[b] Linely (1989b).
NA, not available.

siderably and can be distinguished when examined under a stereomicroscope. The egg of *Ae. albopictus* is shiny black in color, cigar shaped, tapering anteriorly and posteriorly, chorionic cells are regular in shape and contain one large rounded tubercle with small tubercles present on the inner walls of the outer chorionic reticulum (Linley 1989a). The egg of *Oc. triseriatus* is dull black in color, cigar shaped, the chorionic cells are irregular in shape, and one large tubercle is usually present touching the outer chorionic reticulum (Linley 1989b). The egg of *Oc. japonicus* is matte black, cigar shaped, tapering ventrally, the chorionic cells are irregular in shape; tubercles usually range from three to six and contact the chorionic reticulum.

Acknowledgments

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