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A DESCRIPTION AND MORPHOMETRIC ANALYSIS OF THE EGGS OF FOUR SOUTH AMERICAN POPULATIONS OF *ANOPHELES (NYSSORHYNCHUS) AQUASALIS* (DIPTERA: CULICIDAE)

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ABSTRACT. The egg of *Anopheles aquasalis* is described from electron micrographs of material collected at Moron (MO), near Puerto Cabello, west central Venezuela. For comparisons, eggs from three other populations were examined, one from Cano Rico (CR), close to the Moron population in Venezuela, one from near Paramaribo (PA), Suriname, and one from about 100 km inland from Rio de Janeiro (RJ), Brasil. Electron micrographs were used as the source of an extensive series of morphometric measurements of the four populations, which were comparatively analyzed by multivariate statistics. Eggs from the four localities were divisible into two visually distinct groups, MO/CR and PA/RJ. The Venezuelan (first) group was distinguished by possession of proportionately shorter floats, larger decks that were not tapered posteriorly, and greater egg width in relation to egg length. Surinamese and Brasilian eggs, conversely, were comparatively narrower and had longer floats, which almost invariably wrapped around the posterior end of the posteriorly narrowed deck. Discriminant function analysis indicated that PA eggs much more closely resembled those from RJ than the MO and CR forms, despite much greater geographical proximity to the latter.

INTRODUCTION

Anopheles (Nyssorhynchus) aquasalis Curry is one of nine species forming the Oswaldoi Complex in the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles* (Faran 1980). It is primarily a coastal species distributed along the eastern coastline of Central and South America from Nicaragua to southern Brasil, on the western coastline south to Ecuador, as well as in the Lesser Antilles north to St. Kitts and Antigua, and Trinidad and Tobago (Senior-White 1951, Faran 1980, Faran and Linthicum 1981).

Whether *An. aquasalis* should more correctly be considered a single, polymorphic species or a complex of closely related ones has been a matter of interest to researchers for some time. A range of evidence, reviewed in some detail by Conn et al. (1993), has provided support for both viewpoints. Morphological (Causey et al. 1945, Faran 1980), cytological (Kitzmiller and Chow 1971, Moncada Perez and Conn 1992), and electrophoretic (Steiner et al. 1981) studies have suggested a single entity, but reports of variation in vector competence and behavior between populations, as well as differences in the egg (Cova-Garcia 1964) and additional electrophoretic evidence (Steiner et al. 1982), have suggested the possibility of a complex of species.

To elucidate the status of *An. aquasalis* more thoroughly, Conn et al. (1993) have recently completed an analysis, using mitchondrial DNA (mtDNA), of the relationships of five *An. aquasalis* populations from Venezuela, Trinidad, and Brasil (Fig. 1). Eggs from females of two of the populations studied by mtDNA, as well as samples from two other areas within the geographic range of the mtDNA analysis (Fig. 1), were examined with an electron microscope and analyzed morphometrically.

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MATERIALS AND METHODS

Source of material and fixation. Eggs were obtained from females taken at human or animal bait in four locations (Fig. 1). Two, in Venezuela, were Moron (MO), 10° 29′ N 68° 1′ W, near Puerto Cabello, Carabobo State, and Cano Rico (CR), 10° 11′ N 67° 36′ W, Aragua State. One of the other two sites, Paramaribo (PA), 5° 50′ N 55° 11′ W, was in Suriname; the other, Mage (RJ), 23° 47′ S 43° 49′ W, was in Rio de Janeiro State. Brasil. Figure 1 indicates, also, that eggs were available from two populations (MO, RJ) of the five recently studied by mtDNA (Conn et al. 1993). The eggs from seven (MO) or six (CR, PA, RJ) females were allowed to embryonate for 24 hr and then were fixed in alcoholic Bouin's solution in small vials.

Electron microscopy, micrographs, data acquisition, and analysis. For each female from each site, three eggs were examined; thus, 21 eggs were examined from MO and 18 from each of the other three localities. The preparative procedures for scanning electron microscopy were as described by Linley et al. (1993), and the same sets of micrographs were taken except for those of the lobed tubercles and ventral plastron cells, which are both absent in *An. aquasalis*. The micrograph (4,000×) of the anterior deck tubercles in *An. aquasalis* had to be taken with special care with respect to position. In this species, there is a patch of unusually large tubercles, more apparent in some eggs than others, on the extreme anterior part of the deck (see description below). These were not recorded; instead, a micrograph was taken just posterior to the area of size transition, that is, about 0.25 of the length from the anterior end of the egg. Measurements were digitized from the micrographs as previously described (Linley et al. 1993), and statistical analysis was performed with the same software. Means cited in the text are given as ± SE.

Definitions of attributes and terminology. From the low-power (200×) ventral views of whole eggs, 15 attributes were measured, counted, or calculated (percentages, ratios).

Fig. 1. Collection sites (solid symbols) of the four populations of *An. aquasalis* populations for which eggs were studied. Three of the five sites used by Conn et al. (1993) are shown by open symbols; the other two are MO and RJ.

Nine more, descriptive of the dorsal plastron cells and micropyle, were obtained from other micrographs at higher magnification. As in previous work (Linley et al. 1993), the names of the attributes are tabulated as acronyms, defined alphabetically in the appendix. One of them, antposdkrat, was intended to reflect differences in the anterior and posterior deck widths. It is defined as the ratio of anterior deck width at the widest point to the posterior deck width at 1/6 of the egg length from the posterior end.

For the terminology applied to the eggs, we have followed Harbach and Knight (1980), with the addition of "outer chorionic cell field" (Linley 1989). Cell length is the dimension in the longitudinal axis of the egg, and width is the circumferential dimension.

Table 1. Attributes[1] of the eggs of four populations of *An. aquasalis* measured from three eggs from each of seven females (MO) or six females (CR, PA, RJ).

Attribute	Mean (±SE)[2] for population			
	MO (n = 21)	CR (n = 18)	PA (n = 18)	RJ (n = 18)
Linear dimensions[3]				
Egglen	383.8 ± 3.9a	448.7 ± 2.7c	415.8 ± 5.5b	427.7 ± 7.8b
Eggwid	161.1 ± 2.2a	182.7 ± 1.4b	159.1 ± 2.8a	161.8 ± 2.3a
Lenwidrat	2.38 ± 0.03a	2.46 ± 0.02a	2.62 ± 0.03b	2.64 ± 0.04b
Dklenpcn	92.0 ± 0.4b	92.0 ± 0.4b	88.9 ± 0.6a	88.9 ± 0.4a
Antposdkrat	1.24 ± 0.04a	1.39 ± 0.06a	3.09 ± 0.29b	3.44 ± 0.40b
Float attributes				
Mnfltlen	278.7 ± 3.2a	336.0 ± 3.6b	329.6 ± 5.4b	338.5 ± 7.6b
Fltpcn	72.4 ± 0.3a	74.8 ± 0.6b	79.4 ± 0.6c	78.8 ± 0.4c
Mnribs	26.9 ± 0.3a	29.4 ± 0.3b	29.7 ± 0.4b	31.8 ± 0.3c
Fltlenprib	10.6 ± 0.2a	11.4 ± 0.1b	11.1 ± 0.2ab	10.7 ± 0.3a
Deck area dimensions[4]				
Arwhlegg	444.5 ± 8.8a	595.9 ± 6.1c	493.6 ± 13.6b	521.7 ± 16.9b
Ardk	168.6 ± 5.4b	219.8 ± 6.3c	144.0 ± 8.5ab	125.3 ± 9.6a
Dkpcn	37.8 ± 0.7c	36.0 ± 0.9c	29.6 ± 1.9b	23.8 ± 1.5a
Anterior deck tubercles				
Anttbden[5]	62.2 ± 1.9a	57.4 ± 2.5a	64.8 ± 2.6a	91.2 ± 2.7b
Mnanttbar[6]	2.22 ± 0.06c	2.26 ± 0.10c	1.76 ± 0.09b	1.34 ± 0.04a
Mnanttbfm[7]	0.58 ± 0.02b	0.41 ± 0.02a	0.47 ± 0.02a	0.64 ± 0.02b

[1] For definitions, see appendix.
[2] Means followed by same letter do not differ significantly.
[3] All linear measurements in μm.
[4] All area measurements in μm².
[5] Number in an area of 400 μm².
[6] Five tubercles measured from one egg of each female.
[7] Form factor = 4 × pi × area/perimeter².

RESULTS

Egg of *An. aquasalis* (MO Population)

Size: As in Table 1. *Color*: Black. *Shape, overall appearance*: Broadly boat-shaped in ventral view (Fig. 2A), floats large and fairly wide, anterior and posterior ends rather blunt. Dorsal surface strongly and uniformly curved in lateral view (Fig. 2B), ventral surface flat or only slightly concave, float deep.

Dorsal (lower) and lateral surfaces: Plastron (Hinton 1968) made up of hexagonal or pentagonal outer chorionic cells, boundaries indistinct, mean length 25.4 ± 0.5 μm (n = 21), mean width 17.3 ± 0.4 μm, mean area in Table 2. Field of each cell slightly raised, covered with small nodules interspersed with several pores, larger ones tending to be more central (Fig. 3G,H). Openings may consist of single pores, a few to several pores coalesced (Fig. 3G), or many joined to form long gaps extending across more than one cell (Fig. 3F). More lateral cells with fewer pores, especially at anterior and posterior ends of egg (Fig. 4B,E) and adjacent to float (Fig. 3G). Pore size and area characteristics in Table 2. Floats long, extending about 72% of length of egg (Table 1), deep (Fig. 2B); 23–30 ribs (mean 26.4 ± 0.3) clearly defined to boundary with dorsal plastron (Fig. 3E), rib width 10.6 ± 0.2 μm.

Ventral (upper) surface: Anteriorly, floats terminating at about 80% of deck length from anterior end of deck (Fig. 2A), 85–90% of deck length from posterior end. Area of deck quite large, about 38% of whole area of egg, usually slightly narrowed in middle, with anterior portion somewhat wider and larger than

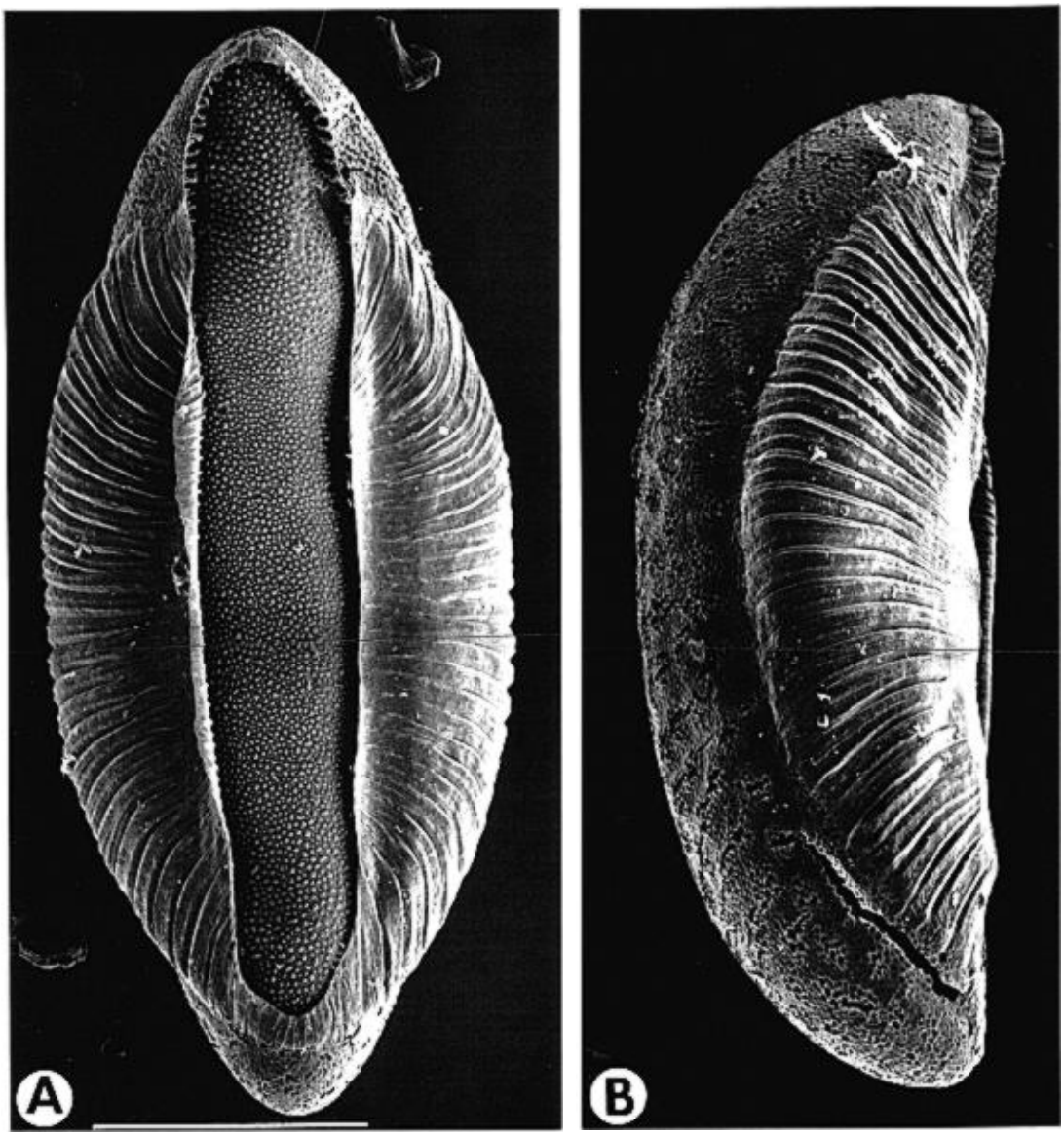

Fig. 2. *Anopheles aquasalis* (MO, Venezuela). A, Entire egg, ventral (upper) view, anterior end at top; B, entire egg, lateral view, ventral surface at right, anterior end at top. Scale = 100 μm.

posterior (Fig. 5). Boundaries of outer chorionic cells not visible. A patch of distinctly larger tubercles usually present on most anterior part of deck, anterior tubercles immediately posterior to patch also larger than those in middle or posterior deck regions, middle deck tubercles smallest (Fig. 3A,B,C; Table 3). Larger tubercles structurally prominent, walls convoluted and recessed, tops domed and smooth, often with shallow indentations (Fig. 3D), distinctly smaller tubercles in middle deck less prominent (Fig. 3B).

Anterior end, micropyle: Anterior end rounded (Figs. 4A, 5), frill well developed (Fig. 4B), with deep interior ridges (Fig. 4C). Micropylar collar quite widely separated from anterior extremity of frill, surrounding plastron cells with few small pores, except openings often more numerous immediately

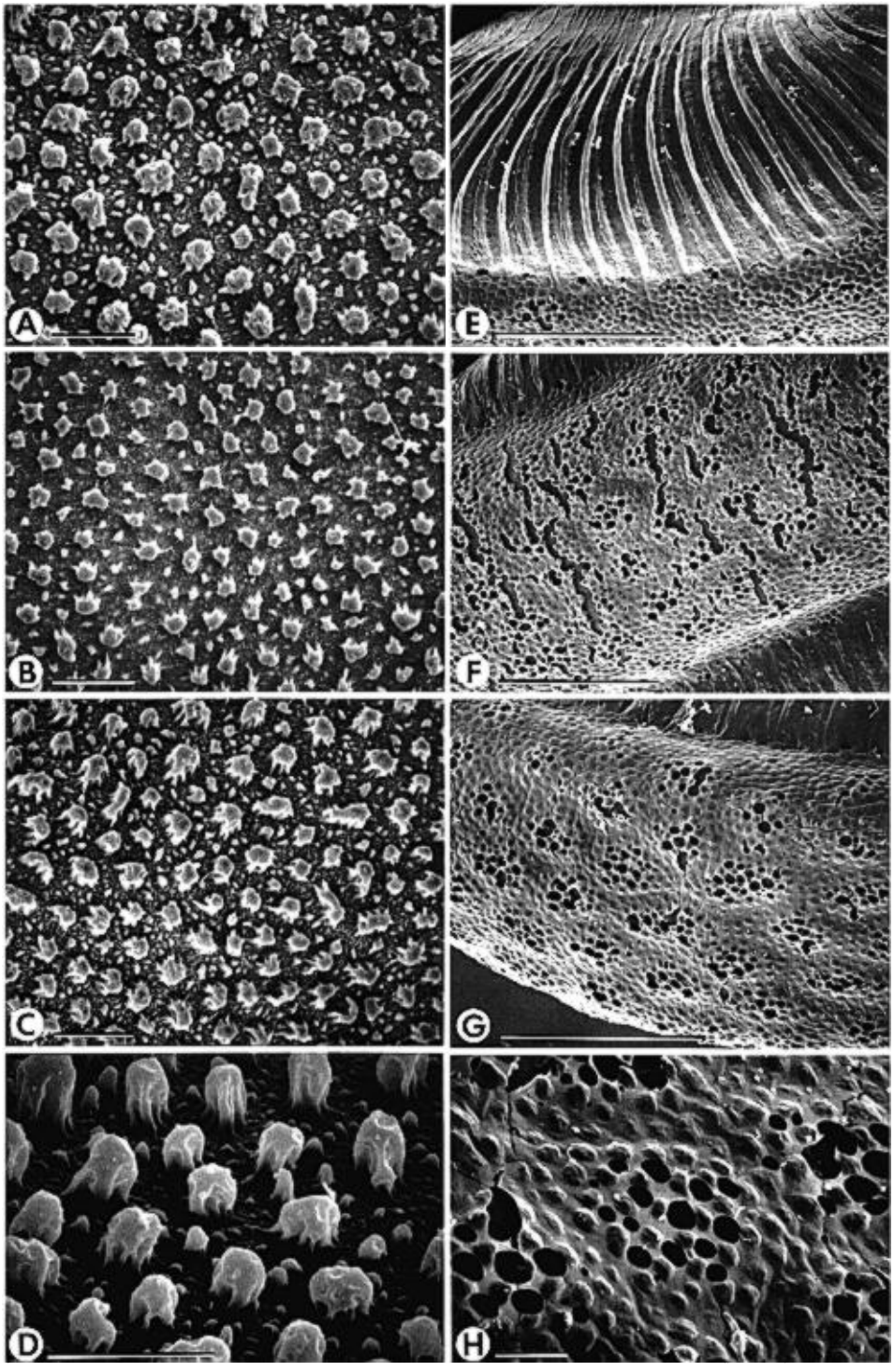

Fig. 3. *Anopheles aquasalis* (MO, Venezuela). A, Outer chorionic tubercles, middle of anterior deck; B, tubercles, middle deck; C, tubercles, posterior deck; D, detail, tubercles on anterior deck; E, dorsal margin of float, lateral surface; F, chorionic cells (plastron), middle of dorsal surface; G, chorionic cells of dorsal surface, alternative form; H, cell detail, middle of dorsal surface. Scale = 50 μm (E–G), = 5 μm (A–D, H).

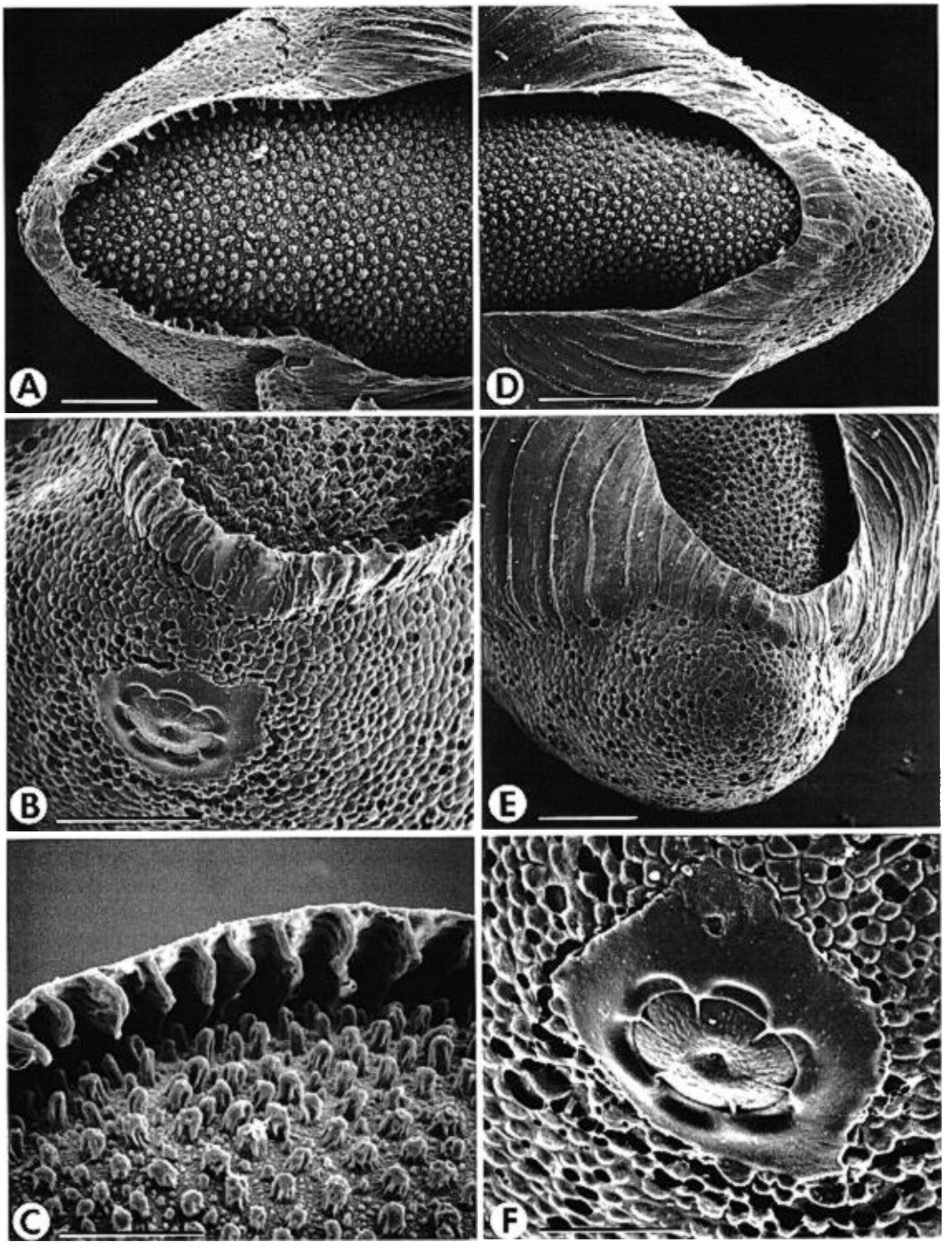

Fig. 4. *Anopheles aquasalis* (MO, Venezuela). A, Anterior end, ventral (upper) surface; B, anterior end, end-on view; C, tubercles and interior wall of frill, anterior deck; D, posterior end, ventral surface; E, posterior end, end-on view; F, micropylar apparatus. Scale = 20 μm (A,B,D,E), = 10 μm (C,F).

Table 2. Attributes[1] of the dorsal plastron and micropylar apparatus in eggs of four populations of *An. aquasalis*.

Attribute	Mean (±SE)[2] for population			
	MO	CR	PA	RJ
Dorsal plastron cells[3]	(n = 21)	(n = 18)	(n = 18)	(n = 18)
Celar[4]	377.2 ± 16.5ab	387.9 ± 11.7b	330.8 ± 17.3a	353.7 ± 13.8ab
Mnnopor	12.6 ± 1.1a	11.3 ± 1.3a	9.4 ± 1.1a	9.1 ± 0.5a
Mnporar	4.5 ± 0.5a	9.5 ± 1.4b	5.2 ± 0.9a	3.8 ± 0.4a
Porarpcn	13.6 ± 1.1b	20.9 ± 1.3c	11.8 ± 0.9ab	9.5 ± 0.9a
Micropyle[5]	(n = 14)	(n = 12)	(n = 12)	(n = 12)
Totarmic	465.3 ± 20.7a	544.6 ± 21.4b	474.7 ± 18.3ab	530.3 ± 13.2ab
Colarmic	339.4 ± 16.4a	420.6 ± 20.6b	342.7 ± 19.4a	384.1 ± 10.5ab
Dskarmic	125.8 ± 7.3a	123.9 ± 4.6a	131.9 ± 5.9a	146.3 ± 9.8a
Dskarpcn	27.1 ± 1.0ab	23.1 ± 1.1a	28.2 ± 1.5b	27.5 ± 1.4ab
Nosect	6.5 ± 0.1a	7.1 ± 0.2b	6.8 ± 0.1ab	6.9 ± 0.1ab

[1] For definitions, see appendix.
[2] Means followed by same letter do not differ significantly.
[3] Three cells measured from one egg of each female.
[4] Area measurements in μm^2.
[5] One micropyle measured from each of two eggs of each female.

around collar (Fig. 4B). Area and sector data relating to micropylar apparatus in Table 2. Disk perimeter variable, with conspicuous excavations, sometimes partially overlain by collar (Fig. 4F), or excavations absent. Surface of disk rather rough, area around micropylar orifice slightly raised (Fig. 4F), diameter of orifice 0.4–1.4 μm (mean 0.87 ± 0.12 μm, n = 9).

Posterior end: Shape rounded (Figs. 2A, 5), float extending almost to extremity of deck, frill well developed, plastron cells with few pores, especially at extreme end of egg (Fig. 4E).

Table 3. Mean diameters (widest point) and areas of tubercles on anterior, middle, and posterior deck regions of *An. aquasalis* eggs (MO population).

Deck region	Mean (±SE)[1]	
	Diameter (μm)	Area (μm^2)
Anterior	1.92 ± 0.09c	2.29 ± 0.08c
Middle	1.32 ± 0.04a	1.20 ± 0.05a
Posterior	1.59 ± 0.06b	1.50 ± 0.05b

[1] Means followed by same letter do not differ significantly.

Simple Comparison of Populations

The low-power ventral views of six whole eggs of each population (Figs. 5, 6) reasonably simulate the maximum resolution that would be attainable with a stereomicroscope. Only the ventral surfaces are of interest in this respect as no dorsal surface attributes can be seen at this magnification. In the ventral aspects, there are three characters that indicate separation of the populations into two broad groups, one comprising MO and CR and the other including PA and RJ. Among these differences, the most obvious is in the area and shape of the deck. It is similar and significantly larger in relative area (dkpcn) in the two geographically close Venezuelan populations (MO, CR, Table 1), in which there also is very little or no tapering toward the posterior end, whereas eggs from PA and RJ are tapered (Figs. 5, 6). Measurements also indicate that the length of the deck proportionate to egg length (dklenpcn) is significantly greater in the two Venezuelan populations (Table 1), a difference that is visible though not intrusively obvious (Figs. 5, 6). Along with and related to deck area and shape, the two groups differ secondly in the posterior

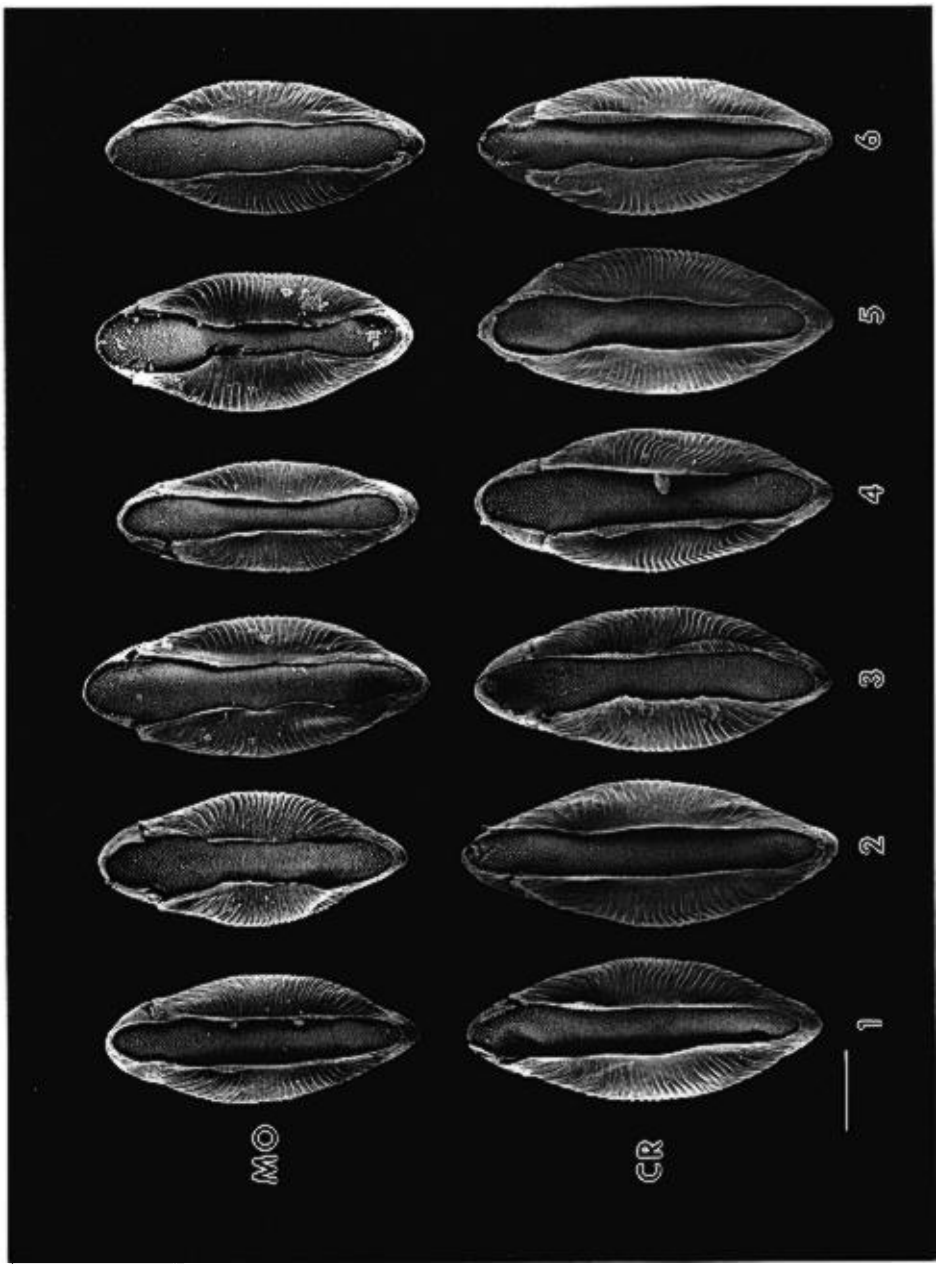

Fig. 5. Eggs of *An. aquasalis* populations (labelled at left); one egg each from six of the seven MO females studied (numbered at bottom) and from the six CR females. Scale = 100 μm.

extent of the floats relative to the deck. MO and CR eggs have floats that terminate a short distance before (sometimes almost at) the posterior extremity of the deck, whereas PA and RJ floats always extend at least to the end of the deck and almost always beyond, often wrapping around the tapered end (Figs. 5, 6). Float length as a percentage of egg length (fltpcn) is significantly greater in the PA and RJ eggs (Table 1), and this is visible, but it is the effect of this difference on the relative posterior extensions of the floats and deck that is most clearly defined. A third though less apparent distinction is in the length to width ratio of the egg (lenwidrat). Venezuelan eggs have significantly lower values for this ratio (Table 1), indicating greater width relative to length. PA and RJ eggs therefore are expected to be more slender in appearance, as borne out by inspection (Figs. 5, 6), but individual eggs are exceptions. PA egg 3, for example, is unusually broad (Fig. 6), and MO egg 4 is rather slender (Fig. 5). All three attributes taken together, however, make it rather easy to recognize two apparently similar groups, MO/CR and PA/RJ. No distinctions within these groups are discernible at this level of resolution.

Synoptic Appraisal of Attributes

As reflected in the two primary indicators of egg size, egg length (egglen) and area of whole egg (arwhlegg), the populations were dissimilar. CR eggs were larger in both measurements than those from any other locality, particularly with respect to the relatively nearby MO population (Table 1), as seen in the whole eggs (Fig. 5). Egg widths were proportionately smaller in the PA and RJ populations, leading to significantly higher values for length to width ratio (lenwidrat, Table 1). As already mentioned, deck length as a percentage of egg length (dklenpcn) was significantly greater at the two Venezuelan sites, but the ratio anterior/posterior deck width (antposdkrat) was much higher (Table 1) in eggs from Suriname and Brasil because of the deck's posteriorly tapered configuration. The reduced posterior deck areas in these populations also caused these sites to have smaller values (Table 1) for total deck area (ardk) and deck area as a percentage of whole egg area (dkpcn).

The floats in PA and RJ eggs were, as previously mentioned, proportionately longer with respect to the egg (fltpcn) than those in the Venezuelan specimens (Table 1). They also had more ribs (mnribs), but the length of float occupied by each rib (fltlenprib) was significantly greater only in the CR population. Tubercles on the anterior deck region were significantly larger (mnanttbar) in MO and CR eggs (Table 1) than in the other two populations, as is apparent in the micrographs (Fig. 7), from which it can be seen, also, that the tubercles on RJ eggs were significantly smaller. Anterior tubercle density (anttbden) was negatively correlated (Fig. 8) with tubercle size (mnanttbar), so RJ eggs had the highest values and Venezuelan eggs the lowest (Table 1). Form factor (mnanttbfm), as an index of roundness, indicated that tubercles in RJ eggs conformed most closely to round (Fig. 7), along with eggs from MO, whereas PA and particularly CR tubercles were more irregularly shaped (Table 1).

Cells of the dorsal plastron were smallest in area (celar) in the PA and RJ populations and larger in the Venezuelan eggs, particularly in the large eggs from CR (Table 2). Numbers of pores (mnnopor) were lowest in the two populations with smaller cells (PA, RJ). However, the numbers in CR eggs were unexpectedly low owing to the significantly larger pore size in this population (Table 2), which also, consequently, had the largest total pore area as a percentage of cell area (porarpcn).

The total area of the micropylar apparatus (totarmic) was greatest in the large CR eggs, and, correlated with total area, the collar areas (colarmic) showed much the same differences (Table 2). Surprisingly, however, disk area (dskarmic) was least in the CR population, and, consequently, disk area as a percentage of the whole micropylar area (dskarpcn) was smaller than in any other group (Table 2). Although the disks of CR eggs were divided into more sectors than those

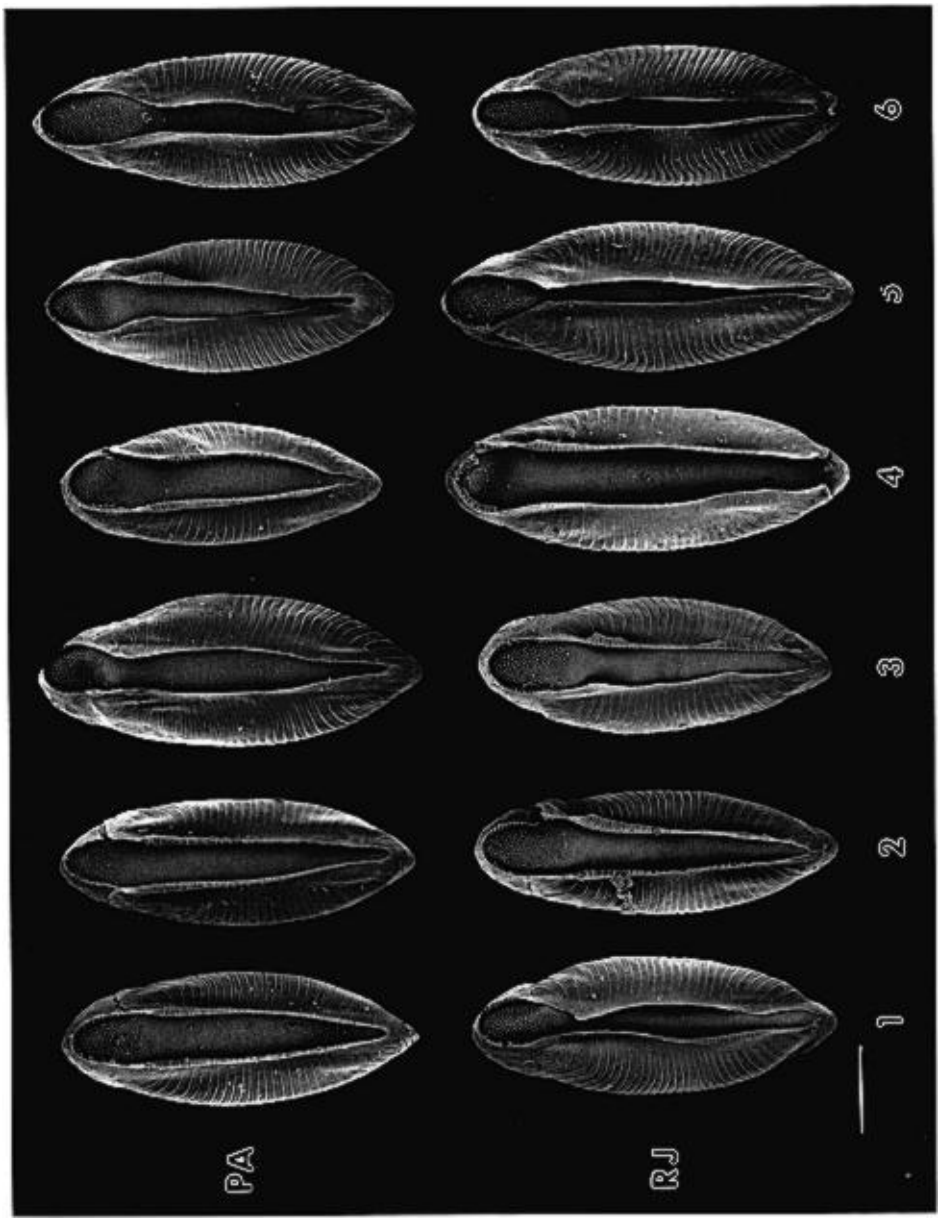

Fig. 6. Eggs of *An. aquasalis* (populations labelled at left); one egg each from the six PA females and the six RJ females studied (numbered at bottom). Scale = 100 μm.

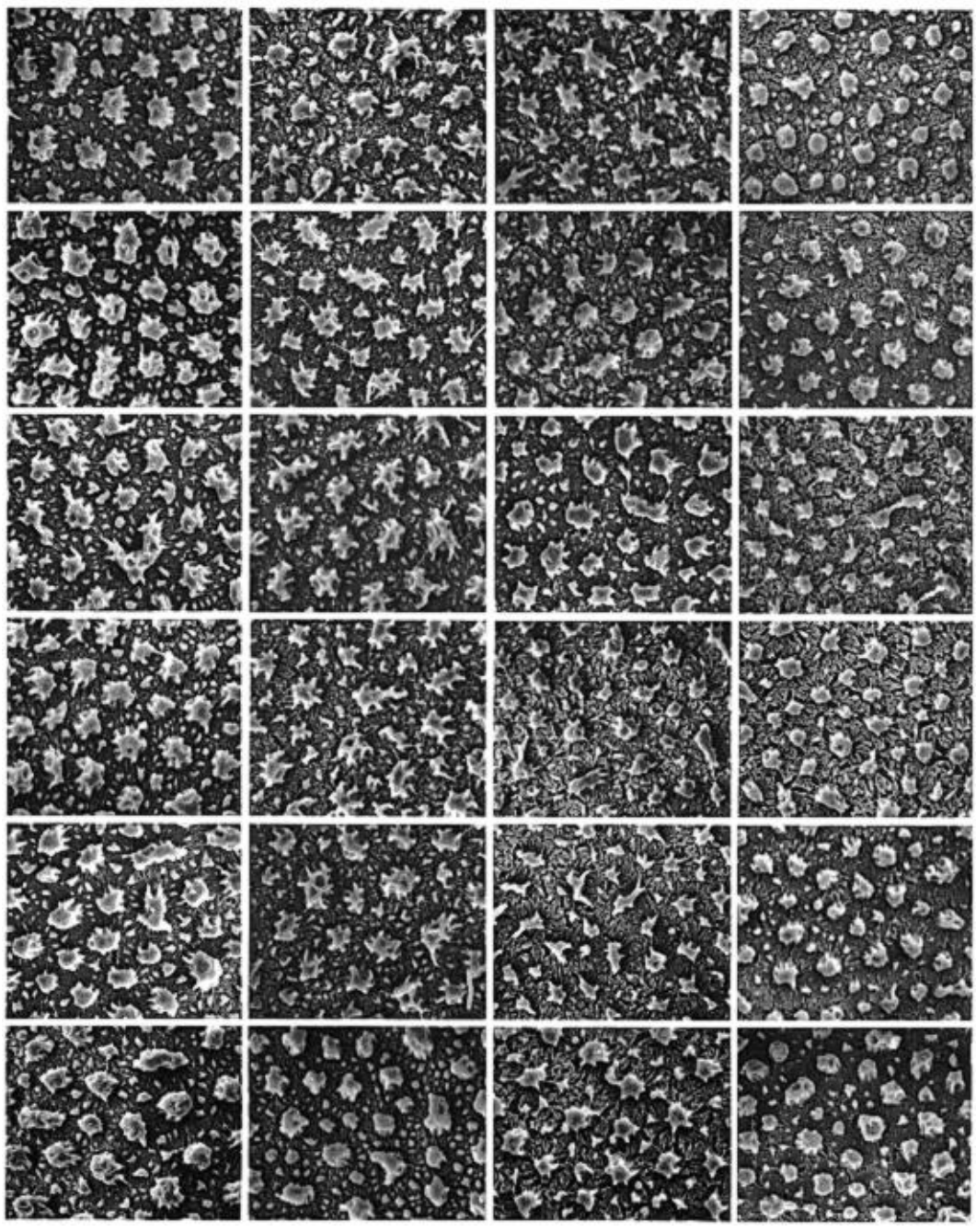

Fig. 7. Tubercles of the mid-anterior deck of four populations (labelled at left) of *An. aquasalis*; one egg each from six of the seven MO females studied and from the six CR, PA, and RJ females (numbered at bottom). Scale = 10 μm.

in the other three populations (Table 2), the differences were not significant.

Multivariate Analysis

We have adopted the same rationale as applied in a previous study (Linley et al. 1993) with regard to attributes used for multivariate analysis. Absolute measures related to overall egg size (e.g., egg length, area of deck) have been avoided in favor of derived characters, such as ratios and percentages, which were considered more likely to reveal true population differences. Of the nine attributes used (antposdkrat, anttbden, dklenpcn, dkpcn, fltlenprib, fltpcn, lenwidrat, mnanttbar, mnanttbfm), only the three concerned with the anterior deck tubercles (anttbden, mnanttbar, mnanttbfm) were direct measures, and regression calculations showed that none was related to egg size as measured by either egg length or whole egg area.

Principal components analysis with these nine attributes first required standardization of the unequally scaled variables to zero mean and unit variance. A partial tabulation of the results (Table 4) showed that the first component accounted for 45.93% of the variation and, with the first six components explaining 92.75%, that it was reasonable to discard components 7–9. Interpretation of the first two components serves to indicate the attributes in which the main interpopulational differences resided and is facilitated by a plot of the appropriate eigenvectors (Fig. 9A) and individual components for the 75 eggs (Fig. 9B). Component 1 carried heavy positive weightings (Fig. 9A) for anterior tubercle density (anttbden, A), float length as a percentage of egg length (fltpcn, F), and anterior/posterior deck width ratio (antposdkrat, J), along with somewhat smaller loadings for length/width ratio (lenwidrat, R) and anterior tubercle form factor (mnanttbfm, M). These positive values contrasted with large negative ones for deck area as a percentage of total egg area (dkpcn, D), mean anterior tubercle area (mnanttbar, T), and deck length as a percentage of egg length (dklenpcn, E), whereas float length per rib (fltlenprib, P) had little

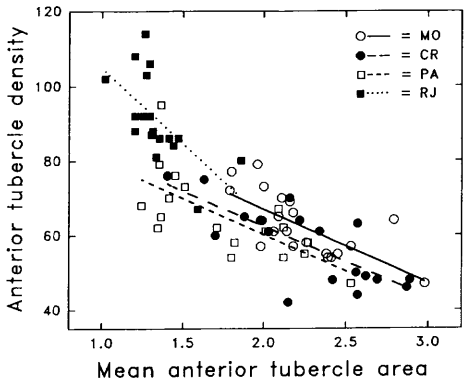

Fig. 8. Regression of anterior tubercle density (no./400 μm^2) on mean anterior tubercle area (μm^2) for four populations of *An. aquasalis*. Regressions for all four populations were highly significant ($P < 0.001$, < 0.01, < 0.005, < 0.001 for MO, CR, PA, and RJ, respectively).

effect on this component (Fig. 9A). As expected from these weightings, the majority of which reflected visible differences in the whole eggs (Figs. 5, 6), individual eggs from PA and RJ populations separated at the positive end of the component 1 axis from the two Venezuelan groups on the negative side (Fig. 9B). One Suriname female was an exception (Fig. 9B). In component 2, a heavy positive loading for float length per rib (fltlenprib, P) and more modest ones for float length as a proportion of egg length (fltpcn, F), mean anterior tubercle area (mnanttbar, T), egg length/width ratio (lenwidrat, R), and anterior/posterior deck width ratio (antposdkrat, J) were contrasted with negative values for mean anterior tubercle form factor (mnanttbfm, M) and anterior tubercle density (anttbden, A). PA eggs were differentiated somewhat more clearly from RJ eggs along this component than were the MO and CR populations (Fig. 9B), although two eggs of one of the RJ females fell within the positive range occupied by the PA group. Explicit description of components 3–6 is omitted, as their interpretation with respect to weighting can be reviewed from the eigenvectors (Table 4).

Derivation of discriminant functions showed that all three were significantly different between populations (Table 5), with chi-square probabilities particularly low for functions 1 and 2, which captured 70.37 and

Table 4. Partial tabulation of principal components analysis of nine attributes of eggs of four populations of *An. aquasalis*.

Principal component	Eigen-value	% of variance	Lenwidrat	Fltpcn	Fltlenprib	Dkpcn	Dklenpcn	Antposdkrat	Anttbden	Mnanttbar	Mnanttbfm
1	4.133	45.93	0.288	0.390	0.013	−0.408	−0.349	0.382	0.376	−0.402	0.164
2	1.758	19.53	0.187	0.304	0.583	−0.054	−0.077	0.185	−0.321	0.207	−0.586
3	1.029	11.43	0.548	−0.049	0.416	0.372	0.357	−0.285	0.268	−0.290	0.142
4	0.542	6.02	−0.623	0.232	0.546	−0.129	0.275	0.043	0.107	−0.011	0.391
5	0.484	5.38	0.073	−0.034	−0.244	−0.221	0.783	0.467	−0.049	−0.123	−0.189
6	0.402	4.46	0.415	−0.004	0.049	−0.154	0.031	0.169	−0.405	0.462	0.628

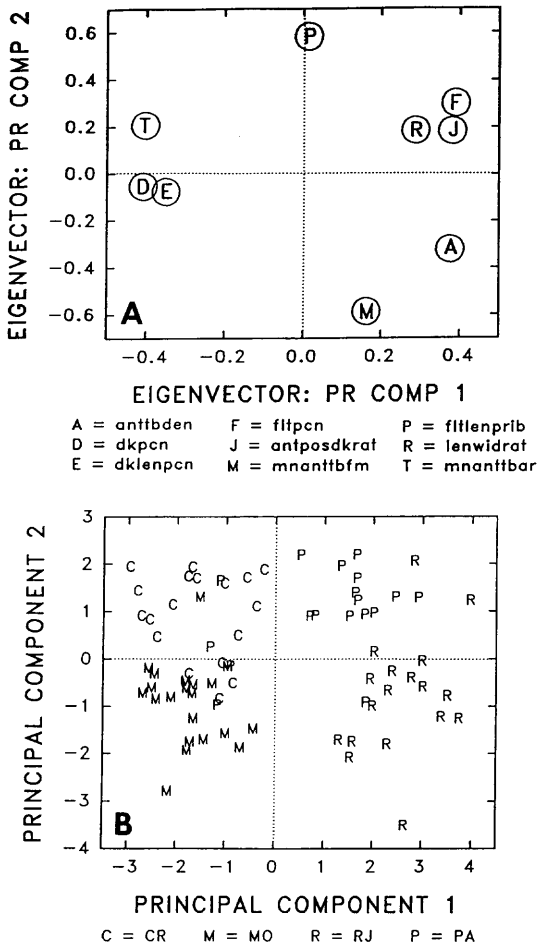

Fig. 9. A, Plot of the eigenvectors of the first two principal components, based on nine attributes, of the eggs of four populations of *An. aquasalis*; B, plot of the individual principal components of 75 eggs.

26.02% (total 96.39%) of the population differences, respectively. The first two functions plotted for the 75 individual eggs (Fig. 10) indicated good discrimination of the four groups. RJ (R) and PA (P) eggs separated clearly on the positive side of function 1 from MO (M) and CR (C) from Venezuela on the negative, with no overlap, even in one PA egg with a slightly negative value in function 1 (Fig. 10). In function 2, it was perhaps not surprising that the geographically widely separated (Fig. 1) RJ (R) and PA (P) populations would be well differentiated, albeit with two RJ eggs having negative values in the PS range (Fig. 10). However, the two Venezuelan groups, only about 130 km apart, also were

separated to a perhaps surprising degree (Fig. 10).

DISCUSSION

From the standpoint of egg morphology, the four populations divide into two major, visually distinct groups, the first comprising the two geographically close Venezuelan populations (MO, CR) and the second containing the PA and RJ populations. At low magnification under the electron microscope, eggs from the latter two (Fig. 6) are clearly distinct, the main differences involving characters of the deck, floats, and shape of the egg, which would be distinguishable relatively easily under a stereomicroscope. The three important characters are float length as a percentage of egg length (floats are long and wrap around the posterior end of the deck), anterior/posterior deck width ratio (deck tapers posteriorly), and egg length/width ratio (eggs are narrow relative to length), all of which are greater in the Suriname (PA) and Brasil (RJ) eggs (Figs. 5, 6).

Both the two Venezuelan and Surinamese and Brasilian populations separate along principal component 2 (Fig. 9B), but because this accounted for only 19.53% of the variance, the differences were considerably less obvious, especially as the heavily weighted characters could not be appreciated at low magnification (Fig. 9A). Between MO and CR, there are no perceptible differences, and these populations clearly could not be distinguished stereomicroscopically. The same applies to the PA and RJ groups, where the clearest differences are in the density, size, and shape of the anterior deck tubercles (Table 1), with no differences apparent in the easily visible attributes associated with the deck or floats (Fig. 6).

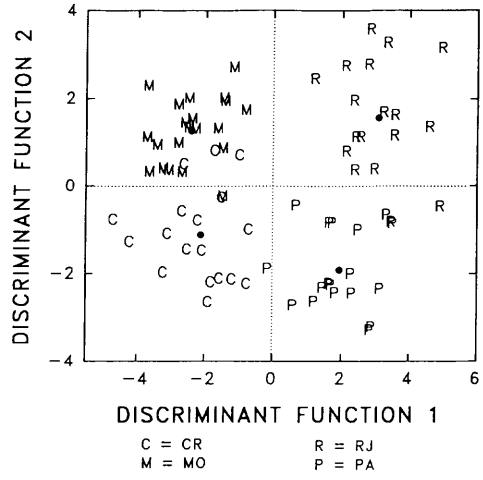

Fig. 10. Plot of the first two discriminant functions, based on nine attributes, of 75 individual eggs of four populations of *An. aquasalis*. Group centroids are indicated by small filled circles.

In their recent study of mitochondrial DNA in five populations of *An. aquasalis*, Conn et al. (1993) concluded that the two Venezuelan (MO, SF, Fig. 1) and one Trinidadian (PS) populations were probably a single species, which might suggest that their eggs would be likely to resemble the MO type (Fig. 5)—wide relative to length and with short floats and no posterior tapering of the deck. Eggs from the SF and PS localities were not available for the present study; however, Cova-Garcia (1964) claimed that the eastern Venezuelan form, recognized by him as *An. emilianus* Komp (see discussion in Conn et al. 1993), was distinguishable from the more western forms in the morphology of the egg. However, careful examination of Cova-Garcia's paper provided no clues for reconciling these observations, or those of Rozeboom (1942), with present findings. Not only are there possible questions as to the identity of the females from which eggs were obtained, but

Table 5. Summary of discriminant function analysis of nine attributes of *An. aquasalis* eggs.

Discriminant function	Eigenvalue	Percentage	Chi-square	df	*P*
1	6.327	70.37	234.801	27	<0.00001
2	2.340	26.02	100.368	16	<0.00001
3	0.324	3.61	18.963	7	<0.01

the appearance of the deck in these eggs can be very much affected by desiccation. Any degree of drying or shrinkage, such as might easily occur under the heat of illumination for stereomicroscopy, causes the gap separating the floats to close, markedly altering the egg's appearance. The possible existence of interpopulational differences in Venezuelan eggs can be resolved conclusively only through further collection and systematic examination of material from relevant localities in eastern Venezuela and also, if possible, in Trinidad. For the present, it is at least certain from comparison of MO/CR eggs (Fig. 5) with those from PA (Fig. 6) that substantial morphological change occurs between these two general localities. In addition, discriminant function analysis (Fig. 10) indicates that eggs of the PA population differ much more from MO/CR eggs than from RJ specimens, despite the enormously greater geographical distance that separates them from the latter (Fig. 1). Conn et al. (1993) examined a population from Marajo Island (MJ, Fig. 1) that, in terms of genetic distance, appeared to be distinct from all other populations examined, although small sample size precluded thorough assessment. Similarly, specimens from the RJ site in southern Brasil again proved (Conn et al. 1993) to be different from the remainder, as indicated also by an electrophoretic analysis (Steiner et al. 1982).

At the current level of genetic understanding of these populations, therefore, at least three (Venezuela/Trinidad, Marajo Island, southern Brasil) may be considered sufficiently different (by estimates of sequence divergence based on mtDNA) to be considered separate species. In terms of egg morphology, the Brasilian forms are expected to be visually very similar to each other and to populations at least as far north and west as Suriname. There are differences between these eggs (Fig. 10), for example, in the size and shape of the anterior deck tubercles (Fig. 7), but the overall appearance at stereomicroscopic magnifications is quite uniform (Fig. 6). Eggs of the Marajo Island population, although not available, would probably conform generally with the RJ and PA groups. The situation in Venezuela may be more difficult to understand. Conn et al. (1993) considered two Venezuelan (MO, SF, Fig. 1) and a Trinidadian population (PS) to be a single entity, which might suggest that Cova-Garcia's (1964) interpretation of the eggs was erroneous. Otherwise, confirmation of Cova-Garcia's interpretation would indicate egg polymorphism in the Venezuelan populations.

In a species possessing the enormous and essentially linear, unidimensional range of *An. aquasalis,* it was apparent at the outset that examination of only four populations, of which two were spatially very close, would represent only a preliminary understanding of egg morphology in this probable species complex. More collections are needed from possible transitional areas in eastern Venezuela, from Trinidad and other Caribbean islands, from Central America, and from the west coast of South America before a more complete understanding, in conjunction with genetic studies, will become possible.

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APPENDIX

Definitions of abbreviations (acronyms) of attributes of eggs of *An. aquasalis*.

Ant-posdkrat	—anterior deck/posterior deck width ratio
Anttbden	—anterior deck tubercle density
Arwhlegg	—area of whole egg (ventral view)
Ardk	—area of deck
Celar	—cell area in dorsal plastron
Colarmic	—collar area of micropylar apparatus
Dklenpcn	—deck length as % egg length
Dkpcn	—area of deck as % area whole egg
Dskarmic	—disk area of micropylar apparatus

Dskarpcn —disk area as % total apparatus area
Egglen —egg length
Eggwid —egg width (widest point, across floats)
Fltlenprib —mean float length/mean number of ribs
Fltpcn —mean float length as % egg length
Lenwidrat —length/width ratio
Mnanttbar —mean anterior deck tubercle area
Mnanttbfm —mean anterior deck tubercle form factor
Mnfltlen —mean float length (of the two floats)
Mnnopor —mean number of pores/cell in dorsal plastron
Mnporar —mean pore area in dorsal plastron
Mnribs —mean number of ribs (of the two floats)
Nosect —number of sectors in micropylar disk
Porarpcn —pore area in dorsal plastron as % cell area
Totarmic —total area of micropylar apparatus