The Chorionic Morphology of Eggs of the *Psorophora confinnis* Complex in the United States. I. Taxonomic Considerations[1, 2, 3, 4]

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ABSTRACT. The patterns of the endochorionic sculpturing and the thickness of the endochorion of eggs from the females of the *Psorophora confinnis* complex collected from 10 geographic locations in the United States were studied with scanning electron microscopy. Eggs deposited by members of the *Ps. confinnis* complex in California have 3 distinct patterns not found in eggs of this complex collected from Arkansas, Florida, Louisiana, Mississippi, New Jersey and Texas; thus indicating that *Ps. columbiae* in California may be genetically different from the *Ps. columbiae* found elsewhere in the United States. No seasonal or geographic variations in the inner chorionic patterns or endochorionic thickness were detected for the eggs from eastern populations of the *Ps. confinnis* complex.

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[3]The research described herein was conducted in part as a cooperative effort between the Texas Agricultural Experiment Station (TAES) and the USDA, ARS and in partial fulfillment of objectives included in USDA, CSRS Southern Regional Project S-122 (Biology, Ecology and Management of Riceland Mosquitoes in the Southern Region of the U. S.) and is approved for publication by the Director of TAES as TA 18631.

[4]Opinions contained herein are those of the authors and should not be construed as official or reflecting the views of the Department of the Army, USDA or TAES. Also, any mention of a commercial or proprietary product in this paper does not constitute a recommendation or endorsement of this product by the Department of the Army, USDA or TAES.

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INTRODUCTION

The distinctive spinose pattern of the outer chorion of the eggs of *Psorophora* spp. characterizes this genus of mosquitoes (Mitchell 1907, Howard et al. 1912, 1917), while, the reticulations of the inner chorion (endochorion) have been used for identifying eggs of different species of *Psorophora* (Howard et al. 1912, 1917; Horsfall et al. 1952; Ross and Horsfall 1965). More recently, Horsfall et al. (1970) presented scanning electron micrographs (SEM) which suggest the endochorionic reticulations of eggs are more or less consistent within each subgenus of *Psorophora* (i.e., *Psorophora, Janthinosoma* and *Grabhamia)*. On this basis, it might be assumed that eggs of each species within a given subgenus of *Psorophora* would have the same endochorionic pattern. However, intraspecific and intraracial differences have been recently reported for the endochorionic patterns of certain *Aedes* species (Horsfall et al. 1970; Olson and Meola 1976). Since previous SEM studies of *Psorophora* eggs involved a small number of eggs from a limited geographic area, a more detailed study of the chorionic structure of *Psorophora* eggs from a variety of geographic areas was undertaken to determine the extent (if any) of intraspecific variation of endochorionic patterns that might occur for mosquitoes belonging to this genus.

Psorophora columbiae (Dyar and Knab) of the *Ps. confinnis* complex was investigated due to its wide distribution in the United States (Darsie and Ward 1981), its importance to human and animal health (Steelman et al. 1972; Olson and Grimes 1974; Sudia and Newhouse 1971; Sudia et al. 1971), and the recent taxonomic uncertainty that has arisen concerning the species. The taxonomic status of *Ps. columbiae* is not clear; specifically whether it is synonymous with *Ps. confinnis* (Lynch Arribalzaga) (Aitken 1940) or whether the members of the *Ps. confinnis* complex found in the eastern and southern United States are *Ps. columbiae* and those in the western states (California, Arizona, New Mexico), are *Ps. confinnis* (Belkin et al. 1970). Bohart and Washino (1978) stated that the California population appears to be *Ps. columbiae.*

We investigated the reliability of using the endochorionic sculpturing of the eggs of *Ps. columbiae* as a means of species identification and to identify populations of this species from different geographic areas. Variations in the inner chorionic structure and thickness were also studied with regard to identifying populations of *Ps. columbiae* collected during different seasons of the year.

MATERIALS AND METHODS

SOURCES AND PROCESSING OF MOSQUITO EGGS.

The sources and location of the sources of the eggs used in this study are summarized in Table 1. Most of the eggs were obtained from wild-caught females which were allowed to deposit their eggs on moistened cotton gauze pads in the laboratory. The eggs were rinsed from the pads into a small casserole and then pipetted onto small filter paper squares. The paper squares of eggs were placed on moistened cellucotton pads in petri dishes and stored at 26° C for at least 3 days to allow completion of embryonic development.

In 2 instances mosquitoes were collected as larvae from field sites in Texas, reared to the adult stage in the laboratory, force-mated and then allowed to oviposit on gauze pads. In 2 other instances, mosquito eggs were obtained from soil samples taken during the fall and winter months at field sites in Texas using the soil sampling and egg separation techniques of Horsfall (1956). The eggs obtained from force-mated females and from soil samples were stored in the same manner as those deposited by wild-caught females.

In studies requiring the collection of eggs from individual females, each mosquito was aspirated into a 10 ml beaker cage containing a moistened filter paper disc for oviposition. If additional batches of eggs were required from the same female, a new moistened disc was placed in the beaker at the start of each new gonotrophic cycle. Females that did not readily deposit eggs, were induced to oviposit by a decapitation technique similar to that described by Clements (1963). All adult mosquitoes were maintained at 26° C and 85% RH throughout the period of egg deposition in the laboratory.

SCANNING ELECTRON MICROSCOPY.

SAMPLE PREPARATION. The exochorion was removed from the eggs by rolling each egg on a strip of double stick tape (Scotch®) affixed to an index card (Horsfall et al. 1970). When free of the exochorion, the eggs have a glossy black appearance. The eggs were mounted on specimen stubs with either double stick tape, silver electrical paint or Television Tubecoat®. They were then coated with 25-30 nm gold palladium in a sputterer and viewed with either a Joel JSM-U3 or a Cambridge S4 Stereoscan at 10-15 Kv. Voltages higher than 15 Kv damaged the specimens.

PATTERN DETERMINATION AND MEASUREMENT OF THE ENDOCHORION. The categories of endochorionic patterns were determined on the basis of geographic location, season, and differences between batches of eggs from the same female. Twenty-five mosquitoes were used to test the frequency of the occurrence of specific patterns between different batches of eggs deposited by a female. Investigation of seasonal variations of the endochorionic reticulation involved eggs collected from 4 Texas counties during different seasons of 1978-80.

Only the anteriolateral surface of embryonated eggs was scanned for endochorionic patterns. The micropyle and dorsal and ventral regions of some of the eggs were also viewed in order to compare these regions with those reported by Horsfall et al. (1970) in their study of *Psorophora* eggs.

Measurements of the thickness of the endochorion were also made at the anteriolateral region. Assessment of inner chorionic thickness of *Psorophora* eggs, based on geographic location, used eggs from California, Texas and New Jersey. Pieces of endochorion were positioned on an SEM stub to measure the width of this structure. The average of several measurements was taken at various points along the edge of the shell (areas where reticula occurred were excluded). The thickness recorded for all the eggs in a given population of eggs was then averaged and the average thickness for the 4 populations of eggs was then analyzed for significant difference using a randomized design analysis of variance (AOV). Since there was an unequal number of observations between egg populations, Fisher's protected least significant difference (lsd) method was used to compare the means (Steel and Torrie 1980). The level of significance was 5%.

EGG MORPHOLOGY TERMINOLOGY.

We used the terminology of Harbach and Knight (1980). The structure we call endochorion is analogous to the chorion of Harwood and Horsfall (1959), whereas the endochorion of these authors appears to be the structure denoted as the vitelline membrane by Christophers (1960). The latter author also used the terms exo- and endochorion to designate the outer two layers of the egg shell and thus is in accord with Harbach and Knight. When terms are used in this study to describe unnamed structures, care has been taken to use names and abbreviations that are compatible with the accepted terminology.

RESULTS AND DISCUSSION

ENDOCHORIONIC PATTERNS.

The SEM study of 491 eggs of the *Ps. confinnis* complex, collected from 12 counties in 7 states, revealed at least 13 distinct variations in endochorionic pattern (Fig. 1). The general appearance of the endochorionic pattern of the various eggs studied was usually consistent with previously published descriptions of this complex. The imprint of the follicle cells on the endochorion of the eggs was most commonly a polygonal cells pattern outlined by a network of raised ridges or reticula; the network of ridges and the area enclosed by these ridges is termed the inner chorionic reticula (ICR) and the inner chorionic cell (ICC) or cell, respectively (Fig. 2). Variations observed were based on the degree of clarity of the polygonal pattern on a given egg which in turn, was determined by deviations in the fine structure of the ridges (R) and the surface of the cell (ICC). The different patterns noted were labeled A through M (Fig. 1) and are further described below.

Patterns A through I possess a distinct polygonal cell pattern. The reticular network of patterns A, B, C, E, G, H, and I is relatively continuous, with infrequent breaks along the length of the ridges. At these breaks, short segments of reticula or subreticula occur and extend into the cell or ICC. The subreticula are longer in pattern B than pattern A, and the inner cell surface of the latter is smooth while that of pattern B contains occasional ridges (Fig. 1, A & B). Pattern C differs from A or B in that it contains an oval disc (CeD) centrally located or slightly posterior to the center of the cell (Fig. 1C). Pattern D (Fig. 1D) also contains a disc in the posterior half of the cell, but this disc is characterized by a network of ridges that extend outward from the periphery of the disc. The reticula of pattern D are less elevated than that of other patterns that have a distinct reticular network with the exception of pattern I (Fig. 1I). Pattern E is characterized by a network of interlocking ridges in the anterior portion of each cell (Fig. 1E). The reticula of pattern F are frequently interrupted by subreticula extending into the cell, which contains a round or oval disc (Fig. 1F). Pattern G has a reticular and subreticular network similar to pattern B; but, it contains a number of ridges and papules that extend from the region of the cell where a disc would occur. Some of these ridges impinge upon the subreticula (Fig. 1G). Pattern H is characterized by ridges within the cell that extend from the subreticula, 1/3-1/2 (or 0.3-0.5) the length of the cell (Fig. 1H). This pattern, like pattern G, contains no disc but 10 or more ridges and/or papules radiate from the area of the cell where a disc is usually found. The reticula of pattern I are not as elevated as that of pattern D but the polygonal shape of the cell is more distinct with some lengths of the reticula higher than others. The reticula and cells of this pattern become more pronounced toward the micropylar region of the egg (Fig. 1I).

The polygonal cell imprint of patterns J through M is indistinct or absent (Fig. 1J-M). Pattern J has very unusual cells. Each cell contains a large, centrally located, depressed ovoid disc (ca. 12-15 X 6.0 μm) and enclosed by a ring of 15-20 smaller ovoid pits (CeP, Fig. 1J). Reticula on eggs having this pattern are discernible because the pits, encircling each cell, highlight the margins of these structures. Numerous short, thin ridges extend the width of the cells, through the pits, reticula and sometimes through the cellular discs. Pattern K is unique in that it contains no cells or reticulations. However, matrices of ridges are directed toward smoother areas (SmA), which are more regularly spaced (Fig. 1K). Reticula are not present in pattern L, instead, the outline of the cells is delineated by variable sized, small, smooth pits (CeP). The central posterior region of each cell has a disc (CeD) anterior to which are numerous filamentous ridges similar to those in pattern C, which extend across the width of the cells to the anterior pits (Fig. 1L). Endochorionic pattern M is characterized by poorly defined reticula on the anteriolateral surfaces. These reticula surround cells that have fairly smooth surfaces (Fig. 1M). Generally the reticula are relatively low, widely marginated and contain subreticula with 1 or 2 short, straight, narrow grooves, that extend from the posterior cell margin. As in pattern D, the polygonal cell shape is more clearly discernible toward the micropylar region of eggs containing pattern M.

The importance of restricting the description of inner chorionic patterns to embryonated eggs is demonstrated in Figures 2 and 3 which compare the chorionic patterns of embryonated and unembryonated eggs. The shape of the inner chorionic cells (ICC) of the embryonated *Ps. columbiae* egg is typically polygonal with the inner chorionic reticulations (ICR) more or less continuous and with infrequent ridges extending into the cell (Fig. 2). In contrast, unembryonated eggs from the same egg batch had frequent notches extending from the ICR into the cell (Fig. 3). Since the unembryonated egg is more wrinkled and shrunken, these features prevent an accurate determination of the chorionic pattern of an egg. Thus, all eggs from a given sample with a wrinkled appearance were simply designated "W".

The frequency with which the various inner chorionic patterns occurred among eggs deposited by females of the *Ps. confinnis* complex collected in 7 different regions of the United States is shown in Table 2. From these frequencies, it appears that eggs from the California mosquitoes (which would be identified as *Ps. columbiae*, using the keys of Bohart and Washino 1978) are distinct from the ones deposited by *Psorophora* from other states. The California mosquito eggs fell into one of 3 inner chorionic pattern categories: K, L, or M (predominantly M) and were the only eggs of the 491 examined whose chorionic patterns were of these types (Table 2; Fig. 1 K-M). As for the eggs of *Psorophora* populations collected from other states, pattern B (Fig. 1B) occurred on at least some eggs from each of these regions (Table 2). In fact, the present study indicates that except for eggs from the Mississippi populations, pattern B is the most common pattern found on *Ps. confinnis* complex eggs collected outside California. Eggs from Mississippi populations usually contained patterns C and D (Fig. 1C, D and Table 2).

The widest range of endochorionic patterns occurred among eggs from the Texas populations (Table 2). Whether this is a unique feature of the *Ps. confinnis* complex in Texas or the result of a much larger sample is unknown. It is probable that the endochorionic pattern can be used to differentiate Californian populations of the *Ps. confinnis* complex from those occurring in other states. Apparently the endochorionic patterns of the *Ps. confinnis* complex cannot be used to differentiate populations of this species occurring in different regions of Texas (Table 3). Endochorionic patterns A, B, D, and E occurred on some eggs in each of the samples obtained from the Texas counties surveyed. Patterns A, B, and D were the most common. The widest range of endochorionic patterns were found in eggs deposited by females from Chambers County, while the narrowest range occurred among eggs from Cameron County. The latter may be due to a smaller sample size. Some differences were noted among egg populations collected from different counties based on the presence or absence and/or frequency of occurrence of a specific endochorionic pattern; but these differences are not adequate for distinguishing populations from different geographic areas of Texas.

The results of the seasonal study of endochorionic pattern formation are shown in Table 4. As in the regional survey, patterns A, B, and D were most frequently found on eggs deposited at different times of the year in Texas. Patterns A and B were always present on some eggs in each of the collections, regardless of the season of the year or the county from which they were collected.

Eggs of the *Ps. confinnis* complex deposited by mosquitoes during the spring and summer in Texas contained a greater diversity of endochorionic patterns than those deposited in the fall. For example, the 146 eggs collected from Brazos, Chambers, and Jefferson counties during the spring of 1979 included specimens containing 9 of the 10 endochorionic categories established for the populations of the *Ps. confinnis* complex. Whereas, the total number of patterns occurring among eggs deposited by late fall populations was 7, with a notable absence of patterns C and E and the addition of pattern I for the first time (Table 4). However, the low frequency of occurrence, or even the presence or absence of a given pattern may not be a reflection of an actual difference among eggs deposited at different times of the year. This view is supported by a comparison of endochorionic pattern data of eggs from other regions of the United States during the summer of 1979 (Tables 1 and 2) with data of eggs collected in Texas during the late fall (Table 4). In this comparison, pattern I occurred on 2 eggs from Mississippi, and patterns C and E were absent among the eggs collected from Florida during the summer of 1979. This is similar to observations made of eggs from the late fall populations of Texas mosquitoes. Thus, on the basis of the present study, it does not appear that variations of endochorionic patterns can be used to effectively discern eggs of the *Ps. confinnis* complex deposited at different times of the year.

Identification of different geographic populations of the *Ps confinnis* complex may also be difficult since an individual mosquito may deposit eggs with different endochorionic patterns. Seventy percent (16 out of 23) of the mosquitoes from which the consecutive batches of eggs were studied for variations of endochorionic patterns, deposited an initial batch of eggs that contained 2 or more of the 10 endochorionic pattern categories established for non-Californian populations of *Ps. confinnis* (Tables 2 and 5). An individual mosquito may deposit eggs with as many as 5 endochorionic patterns (Table 5, mosquitoes No. 12 and 22). Endochorionic patterns A, B, and D were the usual patterns found in batches of eggs deposited by the same mosquito. These patterns were also most frequently found on the eggs deposited by groups of mosquitoes from states other than California.

The variation in patterns among eggs deposited by individual mosquitoes could not be correlated with source, date of deposition, or age of mosquito. Thus, inferences made from the frequencies of specific endochorionic patterns in different geographical areas (except California) or season of the year are a consequence of variation of pattern formation between individual mosquitoes (Table 5). The patterns on the California eggs were very different from all other patterns. In this instance, the occurrence of patterns K, L, and M may be due to the presence of a different *Psorophora* species of the *confinnis* complex in California.

INNER CHORIONIC THICKNESS.

Comparison of the thickness of the inner chorion of eggs of *Ps. confinnis* complex females collected in California, New Jersey and 2 counties in Texas (Cameron and Jefferson) is shown in Table 6. Application of Fisher's (protected) lsd test at 5% level of significance shows the average thickness of the inner chorion of eggs from California differed significantly from that of eggs from New Jersey and Jefferson County, Texas, but is similar to that of eggs from Cameron County, Texas. The average thickness of the inner chorion of eggs from New Jersey is similar to that of both Texas mosquito populations, but dissimilar to that of eggs from California mosquitoes. Thus, the average thickness of the inner chorion of eggs from these 4 geographical areas suggests a cline from relatively thin shells in the western United States to relatively thick shells in the northeast. If the California mosquito is indeed a different species then differences in shell thickness may prove to be a useful diagnostic character.

CONCLUSIONS

The inner chorionic sculpturing of eggs of the *Ps. confinnis* complex is more variable in appearance than inferred by previous workers. There are at least 13 different inner chorionic patterns that may occur on eggs of this species complex in the United States (Fig. 1A-M). Three of these patterns (Fig. 1K, L and M) are unique to eggs deposited by mosquitoes from California which are considered to be *Ps. columbiae* by Bohart and Washino (1978). The most common inner chorionic patterns (i.e., patterns A and B, Fig. 1A, B, respectively) found on eggs from the other states resemble the pattern shown in the SEM micrograph of the mid-lateral region of a *Ps. confinnis* (now *Ps. columbiae*) egg from northern Alabama (Horsfall et al. 1970). A description did not accompany the micrograph published by these authors; but, in an earlier publication by Horsfall et al. (1952), the inner chorion pattern was described as having "walls of the reticulation carinate and sharply defined over the whole egg," and "discs of the reticulation depressed and only faintly crinkled." Similarly, Ross and Horsfall (1965) described the inner chorion of *Ps. columbiae* eggs as "margins of cells of reticulation forming sharp ridges with branches radiating onto discs of cells." Both of these descriptions generally fit the inner chorionic pattern here designated as A and B.

As suggested by Harbach and Knight (1980), the term "discs" used in previous papers has the same meaning as our term "cell." Our results indicate that *true* cellular discs may occur in the cells of inner chorionic patterns on certain of the eggs of *Ps. columbiae* (see CeD, Fig. 1C, D, F and J). Cellular discs are also common to the inner chorion found in the micropylar areas of eggs of members of the subgenera *Psorophora* and *Janthinosoma* (Horsfall et al. 1979). Pattern "J" (Fig. 1J) appears to be remarkably similar to eggs of *Ps. (Jan.) cyanescens* (Coquillett). Since this pattern was observed in a group of eggs deposited by a female *Ps. columbiae* (mosquito No. 24, Table 5), the possibility of this pattern occurring from contamination by another species has been ruled out. In the subgenus *Psorophora*, cellular discs occur on the inner chorions of the eggs of *Ps. howardii* (Coquillett) and *Ps. ciliata* (Fabricius), and are clearly shown in scanning electron micrographs of the eggs of these 2 species (Horsfall et al. 1970); these latter authors described the discs as "spiral pitting" around the egg. Ross and Horsfall's (1965) definition of a true cellular disc is a "distinct circular spot covering the posterior third" of the cells or a "spot on the posterior third not circular" in the case of *Ps. ciliata* and *Ps. howardii*, respectively. Thus, this and previous studies demonstrate that cellular discs are a common occurrence on the eggs of the genus *Psorophora* and the morphology of these discs (i.e., shape, size and location) within the inner chorionic cells may be useful for identifying eggs of *Psorophora*. We propose that the term "cellular disc (CeD)" be used to describe that portion of the inner chorion of mosquito eggs.

We conclude that the inner chorionic patterns, frequency of occurrence of these patterns and the thickness of the inner chorion of eggs deposited (excluding the California populations) are indicative of neither the season when the eggs were deposited nor the geographic area where oviposition occurred (see Tables 2-6). This probably is because one mosquito can deposit eggs with as many as 5 different inner chorionic patterns of their surfaces. However, the inner chorionic patterns of eggs from California mosquitoes are convincingly different from those of *Ps. columbiae* eggs collected in Texas and the 5 other states. The patterns L and M (Fig. 1L, M) resemble the inner chorionic patterns of species of the subgenus *Psorophora* illustrated by Horsfall et al. (1970). The absence of reticulations on the anteriolateral side make it difficult to discern the cellular patterns of *Ps. columbiae* eggs from California populations of this species, since the typical polygonal cell pattern of this species are detectable only on the area nearest the micropyle of the egg in this population. The value of this observation may only be to suggest that the inner chorionic sculpturing on the eggs from the California mosquitoes is morphologically similar to the eggs of *Ps. (Gra.) columbiae* (= *Ps. confinnis*) rather than to the eggs of *Ps. (Pso.) ciliata* as shown by Horsfall et al. (1970). The level of variation in the inner chorionic morphology of other species of mosquitoes in the subgenus *Grabhamia* is unknown. The only other species of *Grabhamia* whose egg chorion morphology has been examined is *Ps. discolor* (Coquillett). Horsfall et al. (1952) reported that neither the reticulations nor surface patterns of eggs of this species resemble those on eggs of *Ps. confinnis*. In their discussion of inner chorionic patterns of *Psorophora* eggs, Horsfall et al. (1970) stated "the subgenus *Grabhamia* is represented by *Ps. confinnis*," and did not mention the unusual pattern on the eggs of *Ps. discolor*. Before any further interpretation can be made concerning the classification of eggs of the subgenus *Grabhamia*, additional morphological studies of the eggs of other members of this subgenus are needed.

The observation of the unique inner chorionic patterns on the surfaces of the eggs deposited by California mosquitoes purported to represent a population of *Ps. columbiae* provides evidence to support the hypothesis of Belkin[1] that *Ps. columbiae* of the eastern states (including Texas) is not conspecific with populations from the western states. Distinguishing taxonomic characters of other life stages of the eastern and western species have not been found (Aitken 1940). Further studies are needed to substantiate the renaming of the California mosquito as a separate species from *Ps. columbiae*. It is suggested, that the California species should remain as an unnamed member of the *Ps. confinnis* complex until such time as the actual distribution of *Ps. confinnis*, described by Lynch Arribalzaga (1891) in Argentina, is studied in South America. If the surface morphology of eggs of this species in South America can be studied and compared to that of the eggs of mosquitoes in the *Ps. confinnis* complex in North America, then taxonomic and zoogeographic questions which have developed since Dyar and Knab (1906) described *Ps. columbiae* may be resolved.

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[1]See note by J. N. Belkin, 1976, in the "Editorial Notes" Section of Mosquito News 36:376.

ACKNOWLEDGMENTS

Appreciation is expressed to Mark Thompson of the United States Department of Agriculture, Agricultural Research Service, Veterinary Toxicology and Entomology Laboratory, College Station, Texas, for his support during this study. Thanks are also extended to the following individuals and agencies for their contributions of time and effort in acquiring specimens: M. Yates, Chambers County, Texas, Mosquito Control; R. Shelton, Jefferson County, Texas, Mosquito Control; L. Terracina, Calcausieu Parish, Louisiana, Mosquito Control; Dr. M. Meisch, University of Arkansas; Dr. D. Sutherland, Rutgers University, New Jersey; Dr. R. Washino, University of California, Davis; J. Haeger, Florida Medical Entomology Laboratory, Florida; and to J. Welch for making field collections of mosquito larvae and eggs.

REFERENCES CITED

Aitken, T. H. G. 1940. The genus *Psorophora* in California (Diptera, Culicidae). Rev. Entomol. (Rio de Janerio) 11:672-682.

Belkin, J. N., S. J. Heinemann and W. A. Page. 1970. Mosquito studies (Diptera: Culicidae). XXI. The Culicidae of Jamaica. Contr. Am. Entomol. Inst. 6: 1-458.

Bohart, R. M. and R. K. Washino. 1978. Mosquitoes of California. 3rd ed. Univ. Calif., Berkeley. 153 pp.

Christophers, S. R. 1960. *Aedes aegypti* (L.). The Yellow Fever Mosquito. Its Life History, Bionomics and Structure. Cambridge University Press. London. 739 pp.

Clements, A. N. 1963. The Physiology of Mosquitoes. Pergamon Press, New York. 393 pp.

Darsie, R. F., Jr. and R. A. Ward. 1981. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. Mosq. Syst. Supplement 1:1-313.

Dyar, H. G. and F. Knab. 1906. Diagnoses of new species of mosquitoes. Proc. Biol. Soc. Wash. 19:133-142.

Harbach, R. E. and K. L. Knight. 1980. Taxonomists' Glossary of Mosquito Anatomy. Plexus Publishing Inc., Marlton, New Jersey. 415 pp.

Harwood, R. F. and W. R. Horsfall. 1959. Development, structure and function of coverings of eggs of floodwater mosquitoes. III. Functions of coverings. Ann. Entomol. Soc. Am. 52:113-116.

Horsfall, W. R. 1956. A method for making a survey of floodwater mosquitoes. Mosq. News 16:66-71.

Horsfall, W. R., R. C. Miles and J. T. Sokatch. 1952. Eggs of floodwater mosquitoes. I. Species of *Psorophora* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 45:618-624.

Horsfall, W. R., F. R. Voorhees and E. W. Cupp. 1970. Eggs of floodwater mosquitoes. XIII. Chorionic sculpturing. Ann. Entomol. Soc. Am. 63: 1709-1716.

Howard, L. O., H. G. Dyar and F. Knab. 1912. The Mosquitoes of North and Central America and the West Indies. Plates. Vol. 2. Carnegie Inst. Wash. Pub. 159:1-520.

__________. 1917. The Mosquitoes of North and Central America and the West Indies. Systematic Description. Vol. 4. Ibid. 525-1064.

Lynch Aribalzaga, F. 1891. Dipterologia argentina. Revta. Mus. La Plata. 2: 131-174.

Mitchell, E. G. 1907. Mosquito Life. G. P. Putnam Sons, New York. 281 pp.

Olson, J. K. and J. E. Grimes. 1974. Mosquito activity in Texas during the 1971 outbreak of Venezuelan equine encephalitis (VEE): II. Virus incidence in mosquito samples from the South Texas plains. Mosq. News 34:178-182.

Olson, J. K. and S. M. Meola. 1976. Variation in chorionic sculpturing of eggs of *Aedes sollicitans* (Walker). Ann. Entomol. Soc. Am. 69:96-100.

Ross, H. H. and W. R. Horsfall. 1965. A synopsis of the mosquitoes of Illinois (Diptera: Culicidae). Ill. Nat. Hist. Surv. Biol. Notes 52. 50 pp.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics, 2nd ed. McGraw Hill Co., New York. 633 pp.

Steelman, C. D., T. W. White and P. E. Schilling. 1972. Effects of mosquitoes on the average daily gain of feedlot steers in southern Louisiana. J. Econ. Entomol. 65:462-466.

Sudia, W. D. and V. F. Newhouse. 1971. Venezuelan equine encephalitis in Texas, 1971: Informational Report. Mosq. News 31:350-351.

Sudia, W. D., R. D. Lord, V. F. Newhouse, D. L. Miller and R. E. Kissling. 1971. Vector-host studies of an epizootic of Venezuelan equine encephalomyelitis in Guatemala. 1969. Am. J. Epidemiol. 93:137-143.

Table 1. Collection data for specimens of the *Psorophora confinnis* complex used as sources of eggs for chorion variation studies.

State	Location		Date of collection			Stage collected or received
	County or Parish	City or area	Day	Mo.	Yr.	
ARKANSAS	Lonoke	Stuttgart	1	July	79	Adult
CALIFORNIA	Riverside	Coachella Valley	12	Oct.	79	Adult
	San Bernardino	San Bernardino	20	Oct.	79	Egg[1]
FLORIDA	Indian River	Vero Beach	15	Aug.	79	Egg[1]
LOUISIANA	Acadia	Crowley	15	July	78	Egg[1]
	Calcasieu	Lake Charles	9	Aug.	79	Adult
MISSISSIPPI	Bolivar	Benoit	6	July	79	Adult
NEW JERSEY	Ocean	Eversham Township	15	Sept.	79	Egg[1]
TEXAS	Brazos	College Station	19	Sept.	78	Adult
			14	May	79	Adult
			31	May	79	Adult
			5	June	79	Adult
			17	June	79	Larva
			15	July	79	Adult
			19	Sept.	79	Adult

Continued

Table 1. (Continued)

State	Location		Date of collection			Stage collected or received
	County or Parish	City or area	Day	Mo.	Yr.	
TEXAS	Cameron	Cavazos	21	Sept.	79	Adult
	Chambers	Anahuac	12	Aug.	77	Adult
			28	June	78	Adult
			19	May	79	Adult
			27	June	79	Adult
			20	Sept.	79	Adult
			3	Nov.	79	Egg[2]
			10	Nov.	79	Larva
			4	Jan.	80	Egg[2]
	Jefferson	China	30	May	78	Adult
			28	Aug.	79	Adult
			18	Oct.	79	Adult

[1] Eggs collected from wild-caught adults and shipped to Texas A&M by cooperators in California, Louisiana and New Jersey.

[2] Collected from soil samples taken from field sites in Chambers and Jefferson Counties, Texas.

Table 2. Frequency of various inner chorionic patterns occurring on eggs from females of the *Psorophora confinnis* complex collected in 7 states during 1979-1980.

Source of	Total	Frequency of occurrence of given inner chorionic pattern[2]													
Mosquitoes[1]	eggs examined	A	B	C	D	E	F	G	H	I	J	K	L	M	W
Arkansas	25	.24	.24	.08	.16	--	.08	.16	--	--	.04	--	--	--	--
California	21	--	--	--	--	--	--	--	--	--	--	.10	.10	.81	--
Florida	21	.05	.67	--	.14	--	--	.14	--	--	--	--	--	--	--
Louisiana	26	.12	.58	.04	.08	--	.04	.04	--	--	--	--	--	--	.12
Mississippi	19	--	.05	.37	.26	--	--	.10	.05	.10	--	--	--	--	.05
New Jersey	10	.10	.40	.20	--	.30	--	--	--	--	--	--	--	--	--
Texas	369	.23	.40	.05	.09	.03	.02	.05	.06	.01	.01	--	--	--	.06

[1]Exact sources of mosquitoes for each are listed in Table 1.

[2]Capital letters A-M correspond to those of inner chorionic patterns shown in Fig. 1. The letter W indicates wrinkled pattern typical of unembryonated eggs (see Fig. 3).

Table 3. Frequency of various inner chorionic patterns occurring on eggs of females of the *Psorophora confinnis* complex collected in 4 Texas counties during 1979-1980.

County[1]	Total eggs examined	Frequency of occurrence of given inner chorionic pattern[2]										
		A	B	C	D	E	F	G	H	I	J	W
Brazos	143	.17	.41	.06	.08	.04	.01	.06	.06	--	.01	.10
Cameron	29	.34	.38	--	.14	--	--	.10	.03	--	--	--
Chambers	97	.11	.40	.07	.12	.03	.08	.04	.05	.02	.01	.05
Jefferson	100	.40	.37	.03	.07	.01	--	.02	.07	--	.01	.02
TOTAL	369	.23	.40	.05	.09	.03	.02	.05	.06	.01	.01	.06

[1]Exact sources of eggs are listed in Table 1.

[2]Capital letters A-J correspond to those of inner chorionic patterns shown in Fig. 1. The letter W indicated wrinkled pattern typical of unembryonated eggs (see Fig. 3).

Table 4. Frequency of various inner chorionic patterns occurring on eggs of females of the *Psorophora confinnis* complex collected during different seasons of the year in Texas in 1979-1980.

Season[1] County	Total eggs examined	Frequency of occurrence of given inner chorionic pattern[2]										
		A	B	C	D	E	F	G	H	I	J	W
SPRING												
Brazos	103	.17	.40	.05	.10	.06	--	.01	.06	--	.02	.13
Chambers	38	.15	.37	.05	.13	.08	.08	.03	--	--	.03	.08
Jefferson	5	.40	.60	--	--	--	--	--	--	--	--	--
TOTAL	146	.18	.40	.05	.10	.06	.02	.01	.04	--	.02	.11
SUMMER												
Brazos	40	.15	.42	.10	.02	--	.02	.18	.05	--	--	.05
Chambers	43	.07	.42	.12	.16	--	.07	.05	.07	--	--	.05
Jefferson	73	.33	.36	.04	.10	.01	--	.03	.10	--	.01	.03
TOTAL	156	.21	.39	.08	.10	.01	.03	.07	.08	--	.01	.04

Continued

Table 4. (Continued)

Season[1] County	Total eggs examined	Frequency of occurrence of given inner chorionic pattern[2]										
		A	B	C	D	E	F	G	H	I	J	W
LATE FALL												
Cameron	29	.34	.38	--	.14	--	--	.10	.03	--	--	--
Chambers	16	.12	.44	--	--	--	.12	.06	.12	.12	--	--
Jefferson	22	.64	.36	--	--	--	--	--	.04	--	--	--
TOTAL	67	.39	.39	--	.06	--	.03	.06	.04	.03	--	--

[1]Exact sources and dates of egg collections are given in Table 1. Seasonal designations of spring, summer and late fall represent the dates of 21 March - 21 June 1979, 22 June - 20 September 1979, 21 September 1979 - 20 March 1980, respectively.

[2]Capital letters A-J correspond to those of inner chorionic patterns shown in Fig. 1. The letter W indicates wrinkled pattern typical of unembryonated eggs (see Fig. 3).

Table 5. Frequency of various inner chorionic patterns occurring on eggs deposited by individual females of the *Psorophora confinnis* complex.

Source County/State	Collection date	Female no.[1]	No. of eggs Deposited	No. of eggs Scanned	Frequency of occurrence of given egg pattern[2] A	B	C	D	E	F	G	H	I	J	W
BRAZOS/TX	5 June 79	1a	109	9	.22	.44	--	--	--	--	--	.33	--	--	--
		b	100	10	--	--	--	--	--	--	--	--	--	--	1.00
		2a	78	6	.17	.50	.17	.17	--	--	--	--	--	--	--
		b	49	5	--	.60	--	--	.20	--	--	--	--	--	.20
		3a	123	6	--	.50	--	--	--	--	.17	.33	--	--	--
		b	72	6	--	.33	--	--	.33	--	--	.17	--	--	.17
		4	106	2	--	1.00	--	--	--	--	--	--	--	--	--
		5	82	2	--	1.00	--	--	--	--	--	--	--	--	--
		6	96	7	--	--	--	1.00	--	--	--	--	--	--	--
		7	NC3/	8	--	.62	--	.12	--	--	--	--	--	--	.25
	15 July 79	8	81	5	--	--	--	--	--	.20	.60	--	--	--	.20
		9	59	5	.40	.60	--	--	--	--	--	--	--	--	--
		10	27	5	--	1.00	--	--	--	--	--	--	--	--	--
		11	8	5	--	1.00	--	--	--	--	--	--	--	--	--
CAMERON/TX	21 Sept. 79	12	84	21	.48	.28	--	.10	--	--	.10	.05	--	--	--
CHAMBERS/TX	12 Aug. 77	13	64	9	.22	.22	.44	.11	--	--	--	--	--	--	--
		14	28	7	--	.57	--	--	.14	--	--	.28	--	--	--
		15a	65	4	.25	.50	--	--	--	--	--	.25	--	--	--
		b	90	2	--	--	--	--	--	--	--	--	--	--	1.00
		16	NC3/	5	--	1.00	--	--	--	--	--	--	--	--	--
	19 May 79	17	28	4	--	.25	--	.25	--	.25	.25	--	--	--	--
		18	NC3/	2	--	--	--	.50	--	.50	--	--	--	--	--
		19	83	6	--	.33	.17	--	.50	--	--	--	--	--	--
		20	68	8	--	1.00	--	--	--	--	--	--	--	--	--
JEFFERSON/TX	28 Aug. 79	21	NC3/	21	.38	.33	.05	--	--	--	--	.24	--	--	--
		22	65	8	.25	.25	--	.12	--	--	.12	.12	--	--	.12
		23	64	19	.63	.37	--	--	--	--	--	--	--	--	--
SUBTOTAL (Texas)				197	.20	.42	.04	.08	.04	.02	.04	.08	--	--	.11
LONOKE/AR	1 July 79	24	127	8	.25	.25	--	--	--	.25	.12	--	--	.12	--
		25	60	3	.66	--	--	--	--	--	.33	--	--	--	--
SUBTOTAL (Arkansas)				11	.36	.18	--	--	--	.18	.18	--	--	.09	--

1/ Mosquito specimen number followed by letters a and b reflect 1st and 2nd batch of eggs deposited by the same individual, respectively. Specimen numbers not followed by a letter indicate the use of eggs from only the 1st batch deposited by the individual.

2/ Capital letters A-J correspond to those of inner chorionic patterns shown in Table 1. The letter W indicates wrinkled pattern typical of unembryonated eggs (see Fig. 3).

3/ NC = Not counted.

Table 6. Average thicknesses of the inner chorion of eggs of the *Psorophora confinnis* complex from California, New Jersey and two counties in Texas.

Sources of mosquitoes	Date of adult collection	Total eggs examined	Average[1] thickness (μm)	Standard error (μm)
California	12 Oct. 79	19	1.41a	0.17
Texas				
Cameron County	21 Sept. 79	11	1.61ab	0.27
Jefferson County	18 Oct. 79	15	1.66b	0.12
New Jersey	15 Sept. 79	10	1.85b	0.23

[1]Average numbers followed by the same letter are not significantly different according to Fisher's (protected) lsd at the 5% level of significance.

Figure 1. Scanning electron micrographs of the types of patterns of inner chorionic sculpturing observed on the anterio-lateral surfaces of embryonated eggs deposited by *Psorophora columbiae* females collected from different geographic areas of the United States. Inner chorionic patterns shown include: (A) Pattern Type A; (B) Pattern Type B; (C) Pattern Type C with an example of a cellular disc (CeD) indicated; (D) Pattern Type D with an example of a cellular disc (CeD) and ridges (R) radiating out from the disc indicated; (E) Pattern Type E; (F) Pattern Type F with an example of the cellular disc (CeD) indicated.

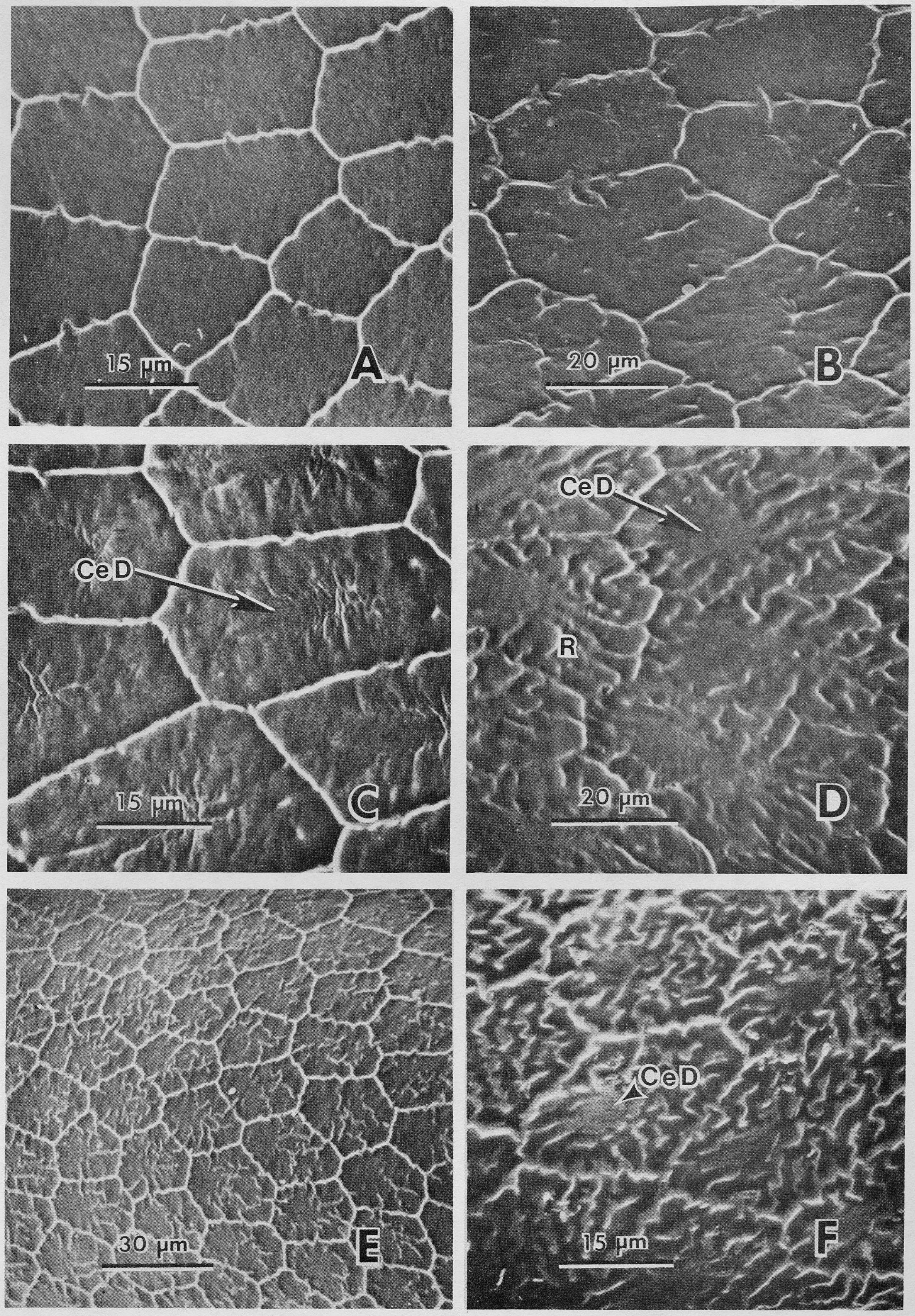

15 µm
A
20 µm
B
CeD
15 µm
C
CeD
R
20 µm
D
30 µm
E
CeD
15 µm
F

Figure 1 (Continued). (G) Pattern Type G; (H) Pattern Type H; (I) Pattern Type I; (J) Pattern Type J with examples of the cellular disc (CeD) and cellular pits (CeP) indicated; (K) Pattern Type K with areas slightly smoother than other areas indicated by SmA; (L) Pattern Type L with examples of cellular discs (CeD) and cellular pits (CeP) indicated; and (M) Pattern Type M.

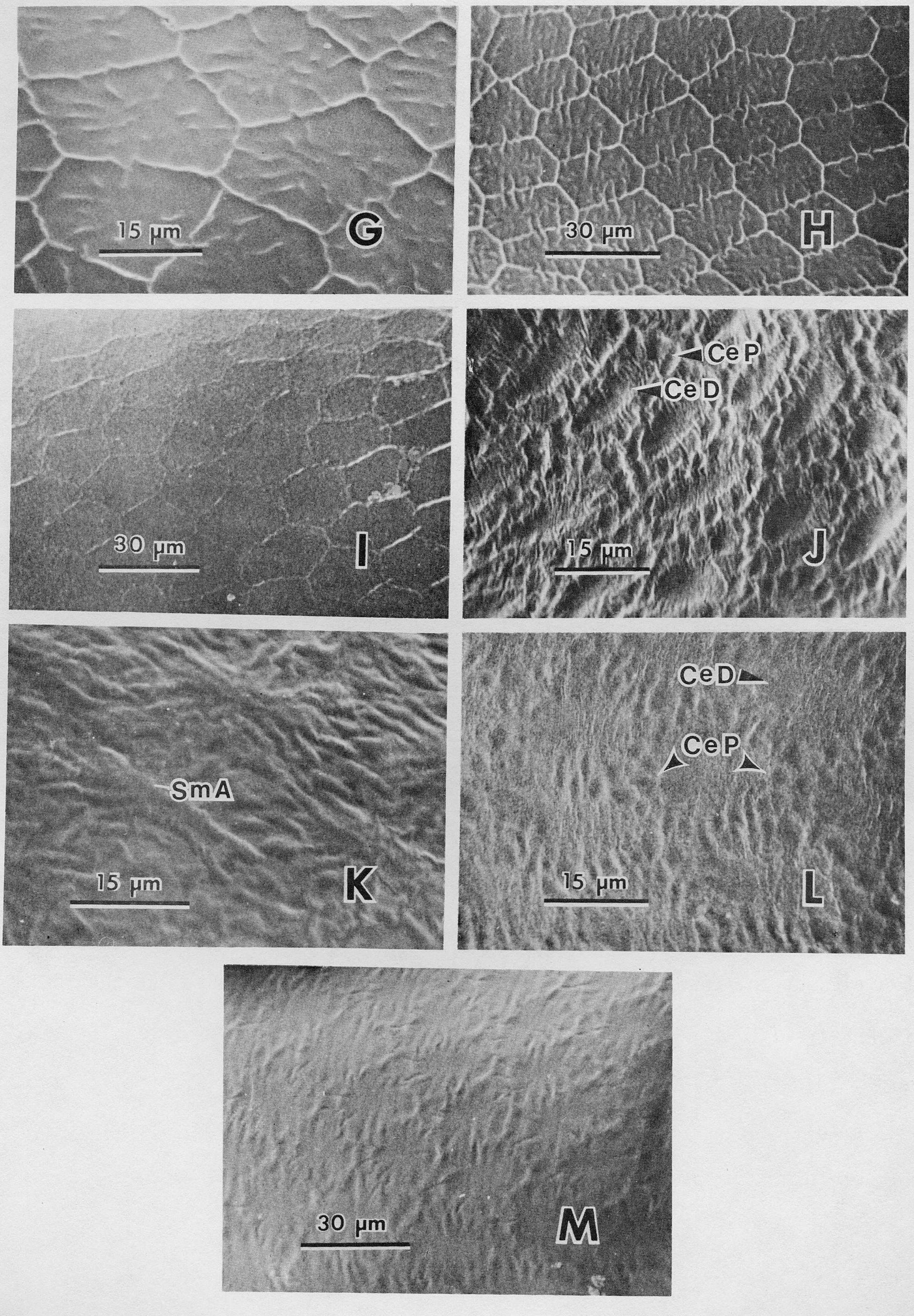
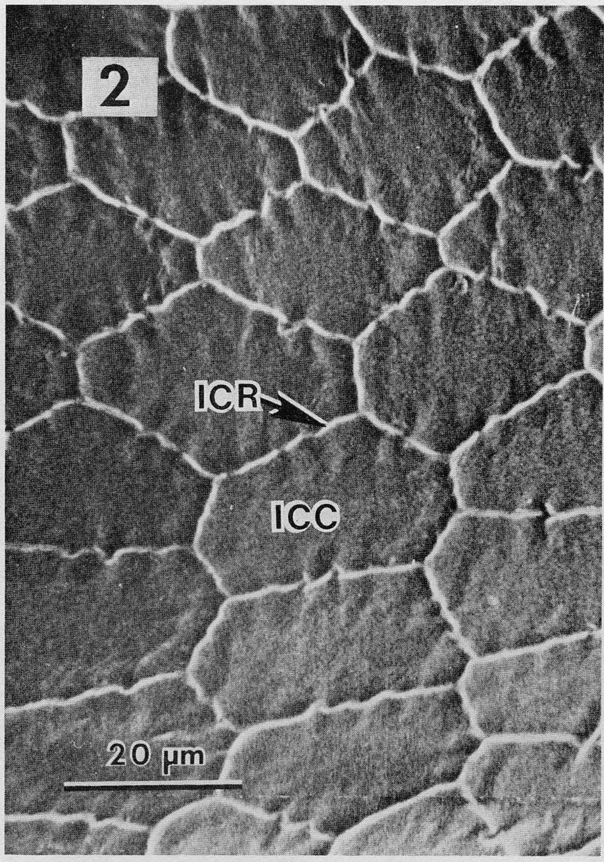
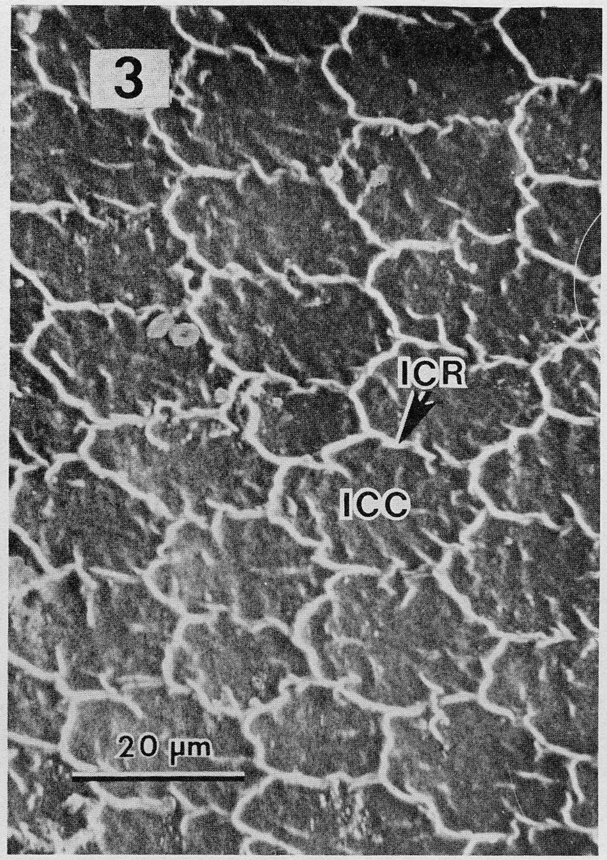

15 µm
G
30 µm
H
30 µm
I
CeP
CeD
15 µm
J
SmA
15 µm
K
CeD
CeP
15 µm
L
30 µm
M

Figure 2. Scanning electron micrograph of the inner chorionic sculpturing of the anterio-lateral region of an embryonated egg deposited by a *Psorophora columbiae* female collected in Brazos County, Texas, on 5 June 1979. Examples of the inner chorionic reticulum and cell are labeled ICR and ICC, respectively.

Figure 3. Scanning electron micrograph of the inner chorionic sculpturing of the anterio-lateral region of an unembryonated egg deposited by the same *Psorophora columbiae* female that deposited the egg pictured in Fig. 2. Examples of the inner chorionic reticulum and cell are labeled ICR and ICC, respectively.


2
ICR
ICC
20 µm


3
ICR
ICC
20 µm