

## **Taxonomy of the Genus *Aedes* (Insecta: Diptera: Culicidae) with the Microstructure of the Egg Surface**

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### **ABSTRACT**

The observations of the egg microstructures on the five Korean mosquito species showed some useful results to identify the species. There have appeared five classified groups with the characters of egg surface structures on 20 *Aedes* species and 3 *Culex* species in the cluster analysis. Among the 23 species, *A. desmotes* and *A. mediopunctatus perplexus* were completely separated from other *Aedes* species.

Key words: *Aedes*, eggs, SEM, microstructures, East Asia.

### **INTRODUCTION**

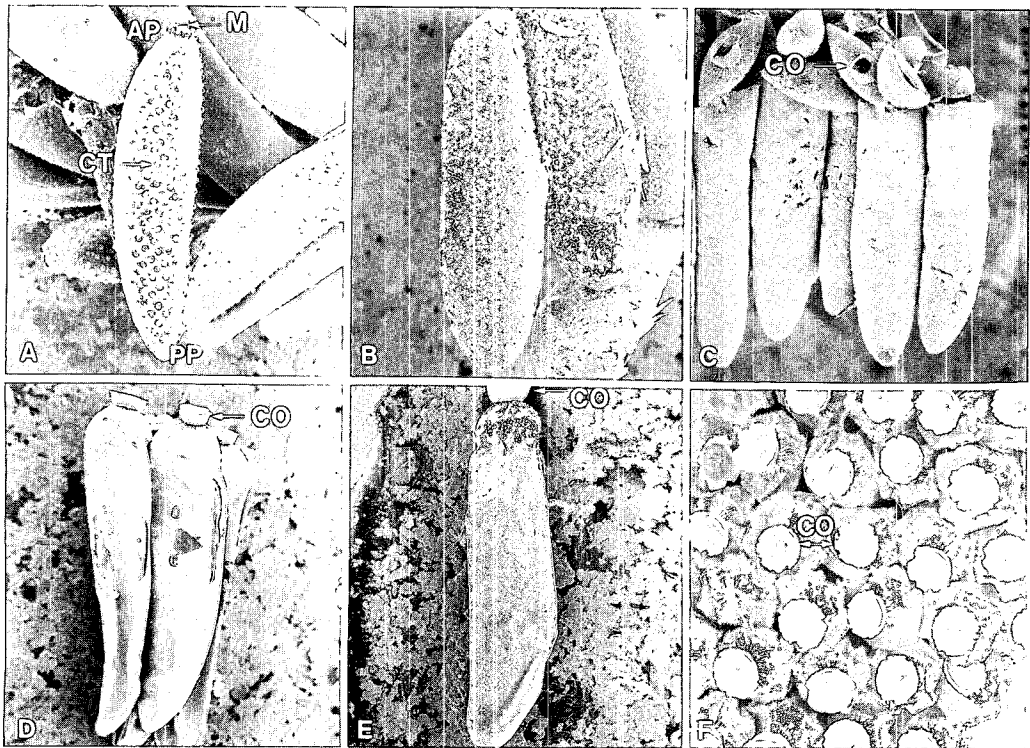
The microstructure on the egg of mosquitoes have been studied with the light microscope and the phase contrast microscope by observing egg size, morphology, color and chorionic sculpture in *Aedes* by Craig (1955, 1960). The scanning electron microscopic study on the egg surface microstructure and micropyle apparatus was done in many insect orders by Hinton (1969), Moriya *et al.* (1973), Harbach (1980), Sasa (1971) and Matsuo *et al.* (1972, 1974, 1975). They have reported the characteristics of mosquito egg surface on the genera *Aedes*, *Armigeres*, *Tripteroides*, and *Orthopodomyia* by SEM. On the Korean mosquitoes, Joo *et al.* (1987) have studied egg surface of *A. albopictus* and *C. pipiens* with the SEM. We attempted to describe and illustrate the egg microstructure of the five Korean mosquito species in this study. And also we investigated a similarity between 20 *Aedes* species and 3 *Culex* species.

**Table 1.** The 5 Korean mosquito species examined by SEM.

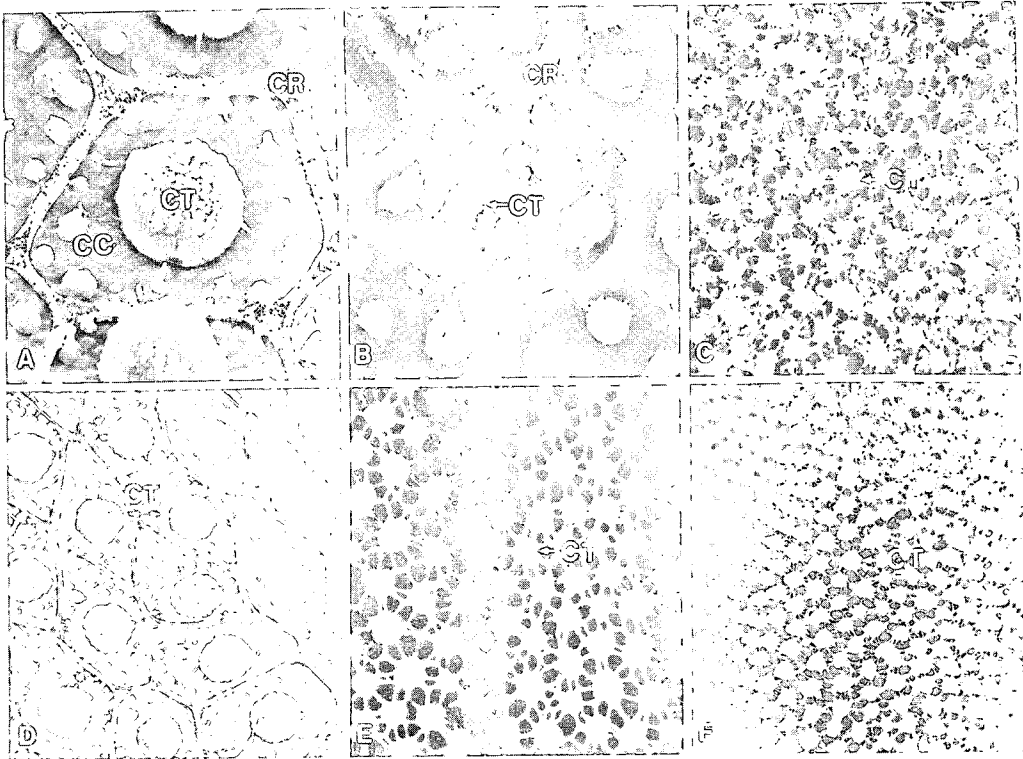
Species	Generation
<i>Aedes (Stegomyia) albopictus</i> (Skuse) 흰줄숲모기	F-17
<i>Aedes (Finlaya) togoi</i> (Theobald) 토고숲모기	F-14
<i>Culex (Culex) pipiens molestus</i> Forskal 지하집모기	F-60
<i>Culex (Culex) pipiens pallens</i> Coquillett 빨간집모기	F-170
<i>Cules (Culex) tritaeniorhynchus</i> Giles 작은빨간집모기	F-1

### MATERIALS AND METHODS

For scanning electron microscopic studies, eggs were fixed in 2.5% glutaraldehyde solution at 4°C for 2 hours and washed three or four times in phosphate buffer for 10-15 min., and post-fixed with 1% OsO<sub>4</sub> for 60-90 min. at 4°C, and rewashed twice for 10-15 min. with the phosphate buffer. They were dehydrated with a series of ethanol and for 1 hour with isoamyl acetate, and dried in CO<sub>2</sub> with Hitachi HCP-2 critical pointed drier. The dried specimens were coated with Pt-Pd using Eiko IB-



**Fig. 1.** Lateral views of eggs of 5 mosquito species from Korea: A, *A. albopictus* (x 170); B, *A. togoi* (x 200); C, *C. pipiens molestus* (x 150); D, *C. pipiens pallens* (x 150); E, *C. tritaeniorhynchus* (x 170); F, *C. pipiens pallens* (Anterior ends of eggs, x 200); AP, anterior pole; CO, corolla; CT, chorionic tubercle; M, micropyle; PP, posterior pole.



**Fig. 2.** Choric sculpturings of eggs of 5 mosquito species from Korea: A, *A. albopictus* (x 4,000); B, *A. togoi* (x 4,000); C, *C. pipiens molestus* (x 5,000); D, *A. albopictus* (Polygonal and rhombic reticulations with one large chorionic tubercle, x 1,500); E, *C. pipiens pallens* (x 5,000); F, *C. tritaeniorhynchus* (x 5,000); CC, chorionic cell; CR, chorionic reticulum; CT, chorionic tubercle.

3 ion coater. Specimens were examined with Hitachi S-120 SEM. The Korean mosquito specimens were supplied by National Institute of Health in Korea. Here, we treated 20 *Aedes* species and 3 *Culex* species for the comparison. And we used 21 microstructural characters to analyze a similarity of *Aedes* species by NTSYS.

## RESULTS AND DISCUSSIONS

The characteristics of the five Korean mosquitoes (Table 1 and Figs. 1-3).

### Genus *Aedes* Meigen

The species of genus *Aedes* had a micropyle without corolla on the anterior pole of egg and had generally regular polygonal, rhombic reticulations in the egg surface. *A. albopictus* had one or two large projected hemispherical papillae in every cell. *A. togoi* did not have a large papilla in the cell but had dispersed small papillae over the inside of reticulations. *A. albopictus* had a peculiar micropyle which was generally elevated and had a large projection surrounded by several big chorionic tubercles. *A. togoi* had a plane micropyle which was somewhat depressed and armed with

**Table 2.** Mosquito species from East Asia treated for the cluster analysis in this study.

Symbol	Species	Locality collected	References
A	<i>A. aegypti</i>	Taiwan	Hinton <i>et al.</i> (1969)
		Japan	Sasa <i>et al.</i> (1971)
		Malaysia, Taiwan	Matsuo <i>et al.</i> (1972, 1974)
		Japan	Moriya <i>et al.</i> (1973)
B	<i>A. albolateralis</i>	Taiwan	Matsuo <i>et al.</i> (1974)
C	<i>A. albopictus</i>	Korea	Joo <i>et al.</i> (1987), present study
D	<i>A. alcasidi</i>	Taiwan	Matsuo <i>et al.</i> (1974)
E	<i>A. amesii</i>	Taiwan	Matsuo <i>et al.</i> (1974)
F	<i>A. annandalei</i>	Taiwan	Matsuo <i>et al.</i> (1974)
G	<i>A. butleri</i>	Taiwan	Matsuo <i>et al.</i> (1974)
H	<i>A. desmotes</i>	Taiwan	Matsuo <i>et al.</i> (1974)
I	<i>A. excrucians</i>	Japan	Moriya <i>et al.</i> (1973)
		Japan	Matsuo <i>et al.</i> (1975)
J	<i>A. flavopictus</i>	Japan	Matsuo <i>et al.</i> (1975)
K	<i>A. formosensis</i>	Taiwan	Matsuo <i>et al.</i> (1974)
L	<i>A. gardnerii imitator</i>	Taiwan	Matsuo <i>et al.</i> (1974)
M	<i>A. hexodontus</i>	Japan	Matsuo <i>et al.</i> (1975)
N	<i>A. japonicus</i>	Malaysia, Japan	Matsuo <i>et al.</i> (1972, 1975)
		Japan	Moriya <i>et al.</i> (1973)
O	<i>A. mediopunctatus perplexus</i>	Taiwan	Matsuo <i>et al.</i> (1974)
P	<i>A. melanopterus</i>	Taiwan	Matsuo <i>et al.</i> (1974)
Q	<i>A. penghuensis</i>	Taiwan	Matsuo <i>et al.</i> (1974)
R	<i>A. pseudalbopictus</i>	Taiwan	Matsuo <i>et al.</i> (1974)
S	<i>A. togoi</i>	Korea	Joo <i>et al.</i> (1987), present study
T	<i>A. vexans nipponii</i>	Japan	Matsuo <i>et al.</i> (1975)
X	<i>C. pipiens molestus</i>	Korea	present study
Y	<i>C. pipiens pallens</i>	Korea	Joo <i>et al.</i> (1987), present study
Z	<i>C. tritaeniorhynchus</i>	Korea	present study

a rugged, broad, roundish and necklace shaped plate.

### Genus *Culex* Linnaeus

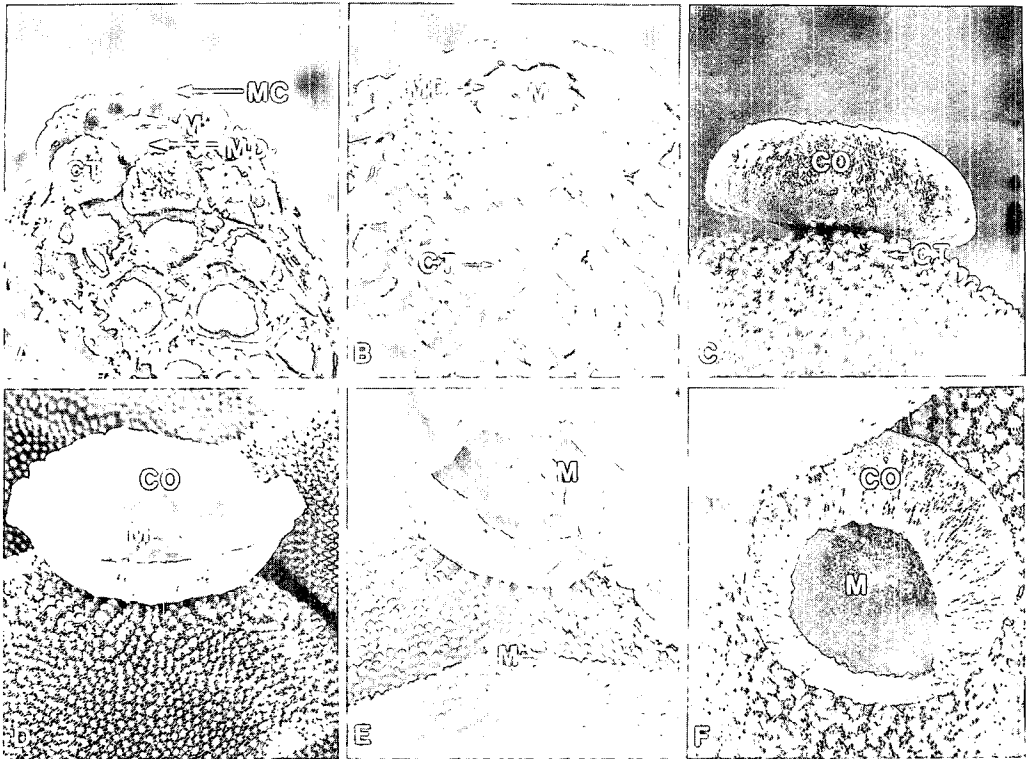
The eggs of the species belonging to the genus *Culex* had a micropyle with a broad concaved corolla. Generally, they have had rather a compact and pointed papillae in the egg surface and not showed a regular polygonal reticulations, there appeared a small sharp pointed projection when corolla was gotten rid of. With the corolla shape, we could identify one after another between three *Culex* spp. *C. pipiens molestus* had a narrow and hollowed corolla, *C. pipiens pallens* had a broad mouth corolla, and *C. tritaeniorhynchus* had a corolla with an long and bulged protrusion in it.

**Table 3.** Characters extracted from egg microstructure for the cluster analysis of 23 mosquito species.

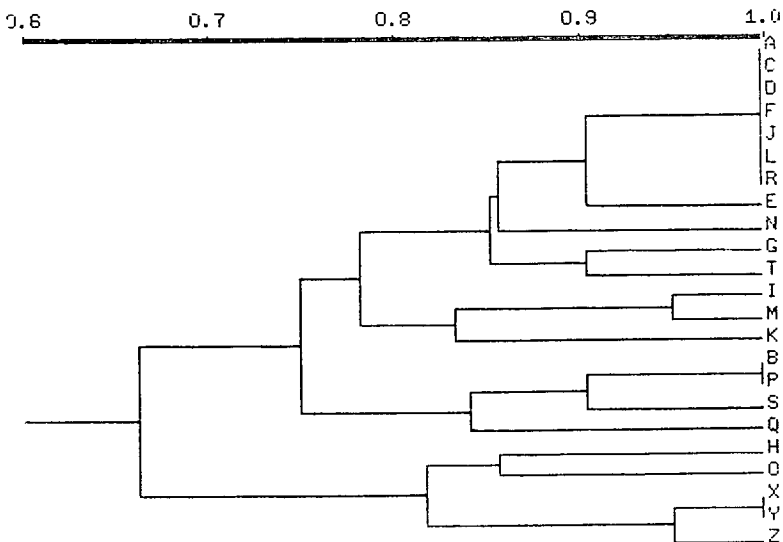
No.	Diagnostic characters
1	With reticulation
2	With papillae of irregular shape on background of fine corrugations
3	With 1 large hemispherical papilla in each indistinct cell formed by corrugations and small papillae
4	Reticulation with transversely elongated cells
5	Reticulation with transversely elongated rhombic cells only
6	Reticulation with anterioposteriorly elongated cells
7	Conspicuous ridge of reticulation encompassing from 6 to 12 cells
8	With 1 large hemispherical papilla in each cell
9	With 1 large flatish topped papilla at center of each cell
10	With 2 to 4 large flatish topped papillae in each cell
11	With 1 papilla having several lines radiating toward ridge in each cell
12	Ridge of reticulation high, hence cells appearing concave
13	Ridge of reticulation low, hence cells appearing plane
14	Reticulation with polygonal and rhombic cells
15	With small papillae of more or less uniform size in each cell
16	With small and large papillae in each cell
17	With 1 to 3 large papillae of irregular shape in each cell
18	With 1 or 2 large hemispherical papillae at center of each cell
19	Numerous globular tubercles connected with peculiar networks
20	With cup shaped corolla surrounding the micropyle
21	With pin-head shaped tubercles at center of corolla

### The cluster analysis of the genus *Aedes* (Tables 2-4, and Fig. 4)

In the cluster analysis of 20 *Aedes* species and 3 *Culex* species as a out group, with the microstructural character of the egg, these were divided into five groups: *A. aegypti*, *A. albopictus*, *A. alcasidi*, *A. amesii*, *A. annandalei*, *A. flavopictus*, *A. gardnerii imitator*, *A. pseudoalbopictus*, *A. japonicus*, *A. butleri*, *A. vexans nipponii* belonged to group I, group II included 3 species with *A. excrucians*, *A. formosensis*, *A. hexodontus*, and group III had *A. albolateralis*, *A. melanopterus*, *A. penghuensis*, *A. togoi*, and group IV had *A. desmotes*, *A. mediopunctatus perplexus*, and group V had *C. pipiens molestus*, *C. pipiens pallens*, *C. tritaeniorhynchus*. The first group including 11 *Aedes* species was the biggest group. The fourth group composed of two *Aedes* species, *A. desmotes* and *A. mediopunctatus perplexus*, was completely separated from other *Aedes* species groups on account of not having chorionic reticulation which seemed to be a common characteristics of *Aedes* species. Here we have just used egg's microstructures for the taxonomic analysis in this study. However, about the separation of *A. desmotes*, *A. mediopunctatus perplexus* from other *Aedes* species, it needs more detailed investigation on these two species in the future.



**Fig. 3.** The anterior pole and micropyle apparatus of eggs of 5 mosquito species from Korea: A, *A. albopictus* (x 1,500); B, *A. togoi* (x 1,700); C, *C. pipiens molestus* (x 1,300); D, *C. pipiens pallens* (x 1,100); E, *C. tritaeniorhynchus* (x 1,300); F, *C. pipiens molestus* (Inside of corolla, x 1,500); CO, corolla; CT, chorionic tubercle; MC, micropylar collar; MD, micropylar disc; M, micropyle.



**Fig. 4.** Dendrogram of 23 mosquito species by UPGMA method.

**Table 4.** Character state matrix of 23 mosquito species.

No.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	X	Y	Z
1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	0	0
2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
6	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
7	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	1	0	1	1	1	1	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	0	0
9	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
12	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
13	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	0	0	0
14	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	0	0	0	0
15	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0
16	1	0	1	1	1	1	1	0	0	1	1	1	0	1	0	0	0	1	0	1	0	0	0
17	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
18	1	0	1	1	0	1	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

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RECEIVED: 19 August 1996

ACCEPTED: 2 December 1996

## 난표면 미세구조에 의한 숲모기속(곤충강: 파리목: 모기과)의 분류

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### 적 요

한국산 모기 5종의 난표면을 주사전자현미경으로 관찰한 결과 속과 종에 따라 난각의 특이한 형태를 보여 모기의 동정에 상당히 유용함이 확인되었으므로, 난각의 미세구조 형질을 이용하여 *Aedes*속 20종, *Culex*속 3종을 군분석 한 결과 5개 군으로 분리되었으며, 이 중 *A. desmotes*와 *A. mediopuntatus perplexus*는 다른 *Aedes*종들로부터 확연히 분리되었다.