

Identification of *Culex (Melanoconion)* Species of the United States Using Female Cibarial Armature (Diptera: Culicidae)

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ABSTRACT Species within the subgenus *Culex (Melanoconion)* Theobald are the primary enzootic vectors of viruses in the Venezuelan equine encephalitis complex including Everglades virus, and probable enzootic vectors of eastern equine encephalitis and West Nile viruses. Adult females of this subgenus are often difficult or impossible to identify to species based on external morphological characters. The use of female cibarial armature allows for the identification of field-collected adult female specimens of *Culex (Melanoconion)*. The cibarial armatures are described and illustrated for all species from the United States and a key to species using this character is presented.

KEY WORDS *Culex (Melanoconion)*, cibarial armature, mosquito identification

Species of the subgenus *Culex (Melanoconion)* Theobald are the primary enzootic vectors of viruses in the Venezuelan equine encephalitis complex including Everglades virus in the United States (Weaver et al. 2004, Coffey et al. 2006). *Culex (Melanoconion)* species have also been implicated as potential vectors of West Nile virus (Cupp et al. 2007) and eastern equine encephalitis virus (Cupp et al. 2003, 2004). There are nine known species of *Culex (Melanoconion)* in the United States: *Cx. abominator* Dyar and Knab, *Cx. anips* Dyar, *Cx. atratus* Theobald, *Cx. cedecei* Stone and Hair, *Cx. erraticus* (Dyar and Knab), *Cx. iolambdis* Dyar, *Cx. mulrennani* Basham, *Cx. peccator* Dyar and Knab, and *Cx. pilosus* (Dyar and Knab). In the United States, *Cx. (Melanoconion)* species are most diverse in the southeastern states and seven species occur in Florida.

Species of the subgenus *Cx. (Melanoconion)* in the United States are difficult to distinguish from one another based on external adult morphology, except for *Cx. erraticus*. This widespread species is easily identified by a patch of more than three scales on the mesepimeron (Fig. 1). Adult females of the remaining species are very difficult to identify, and the degree of difficulty is often exacerbated when mosquitoes are damaged by trapping or handling. However, as long as the head remains intact, the cibarial armature may be used to identify damaged specimens.

The cibarial armature is a part of the alimentary canal within the head of the mosquito. It is located at the base of the pharyngeal pump (Fig. 2). The cibarial armature was first used as a morphological character in mosquitoes by Sinton and Covell (1927). Michener (1944) and Sirivanakarn (1978) described this char-

acter from select species of *Cx. (Melanoconion)*, as well as representatives of several other subgenera of new world *Culex*. The cibarial armature has become a particularly important character in the taxonomy and identification of *Cx. (Melanoconion)* species because there is a large amount of morphological variation within the subgenus and many species display distinct morphological features (Sirivanakarn 1978, Forattini and Sallum 1992).

Materials and Methods

Specimens used in this study are deposited in collections of the Centers for Disease Control and Prevention (CDC), Fort Collins, CO, or the Walter Reed Biosystematics Unit, Smithsonian Institution, Washington, DC. Many specimens were individually reared from the larval stage; however, some specimens were collected as adults in light traps (Table 1). Reared specimens were identified based on morphological characters of the larval skins and adult characters in associated specimens (Belkin et al. 1970, Darsie and Morris 2000, Darsie and Ward 2005). Individually reared male and female adults were associated based on shared larval morphology, and species identification was confirmed by inspection of male genitalia (Rozeboom and Komp 1950, Sirivanakarn 1982, Pecor et al. 1992). Specimens collected in light traps were identified based on morphological characters of the adult female. All specimens except for *Cx. anips* were collected in the United States. In the United States, *Cx. anips* is only found in southern California, where it is rarely collected. The specimen of *Cx. anips* examined in this study was collected in Baja California, Mexico (Table 1).

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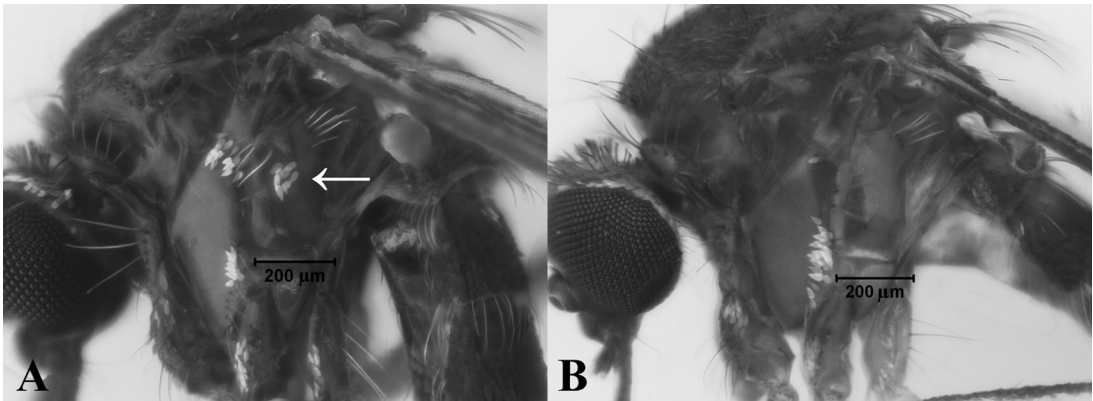


Fig. 1. (A) Pleurae of *Cx. (Mel.) erraticus* showing scale patch (arrow) on mesepimeron. (B) Pleurae of *Cx. (Mel.) pilosus* showing mesepimeron without scale patch.

Morphological terminology generally follows Sirivanakarn (1978) and Harbach and Knight (1980). The term cibarial crest is used following Harbach and Knight (1980) for the transverse ridge that supports the cibarial teeth. The base of the cibarial teeth may originate or insert near the apex of the cibarial crest (Figs. 3A and G and 4G and H) or insert basal to the apex of the cibarial crest (Figs. 3B and E and 4D and E).

Under light microscopy, the cibarial teeth of *Cx. (Melanoconion)* often appear to be composed of two portions. Typically, there is a basal or basomedial portion that appears hyaline or hollow, and a distal and sometimes lateral portion that appears solid and pigmented (Figs. 3B and 4F). Sirivanakarn (1978) described the basal portion as hollow, and this terminology is often followed in the literature. He described the distal portion as the distal cuticular portion, but this portion has subsequently been referred to as the distal margin (Forattini and Sallum 1992). However, scanning electron microscopy images (Forattini and Sallum 1992) clearly showed that the basal and distal portions appear different under light microscopy because they lie in different planes and that the basal portion is also solid. Herein, we refer to these portions of the teeth as the basal and the distal portions. In some taxa, e.g., *Cx. erraticus*, the distal portion is very narrow and may not be visible in all slide preparations (Figs. 4A–C).

The method used in preparation and dissection of female cibarial armatures was adapted from Sirivanakarn (1978). Pinned specimens were placed in a relaxing jar overnight, whereas fresh or frozen specimens and those in ethanol did not require this step. Heads were removed and macerated in a 10% potassium hydroxide solution for 1–2 h. They were rinsed briefly with distilled water and stained in Double Stain (BioQuip, Rancho Dominguez, CA) for 20 min. Heads were rinsed with distilled water and dehydrated first with 95% ethanol and then Cellosolve (BioQuip) for 5–10 min. The heads were placed on a microscope slide that had been scratched with identification information and immersed in a liquid solution of copal-

phenol. Minutepins affixed to wooden applicator sticks were used to carefully dissect the cibarial armature from the remainder of the head and associated structures, including the cibarial pump, clypeus and cibarial dome (Fig. 2).

The dissected cibarial armatures were photographed at $\times 400$ magnification on a compound microscope under bright field using the Automontage program (Syncroscopy, Frederick, MD).

Results

Descriptions of Female Cibarial Armatures

Culex (Melanoconion) abominator Dyar and Knab 1909. Teeth numerous, from 27 to 35 in number; base of teeth insert at or just below apex of cibarial crest; teeth narrow, long, and close-set; basal portion of tooth long; distal portion short, length of distal portion ≈ 0.09 total length of tooth, rectangular; lateral flange terminates before apex of teeth (Fig. 3A).

In the United States, only *Cx. abominator* and *Cx. peccator* (Fig. 4G) have >20 teeth. Based on the specimen we inspected (Table 1), *Cx. abominator* has 27–30 teeth. Sirivanakarn (1978) did not describe the cibarial armature of this species, but he did illustrate *Cx. abominator* with ≈ 35 teeth.

Culex (Melanoconion) anips Dyar 1916. Approximately nine teeth; base of teeth insert below apex of cibarial crest; basal gap between teeth rounded and broad; gap between basal portion of teeth ≈ 0.8 width of basal portion at midpoint base to apex of tooth; apex of basal portion rounded; distal portion of teeth prominent, length of distal portion ≈ 0.25 total length of tooth, apical margin weakly serrated; lateral flange terminating approximately at apex of teeth (Fig. 3B). Note: *Cx. anips* is known only from southern California and Mexico.

Culex (Melanoconion) atratus Theobald 1901. Usually with six teeth, occasionally five to nine; base of teeth insert at or below apex of cibarial crest; basal gap between teeth rounded or bilobed and broad; gap between basal portion of teeth ≥ 0.8 width of basal

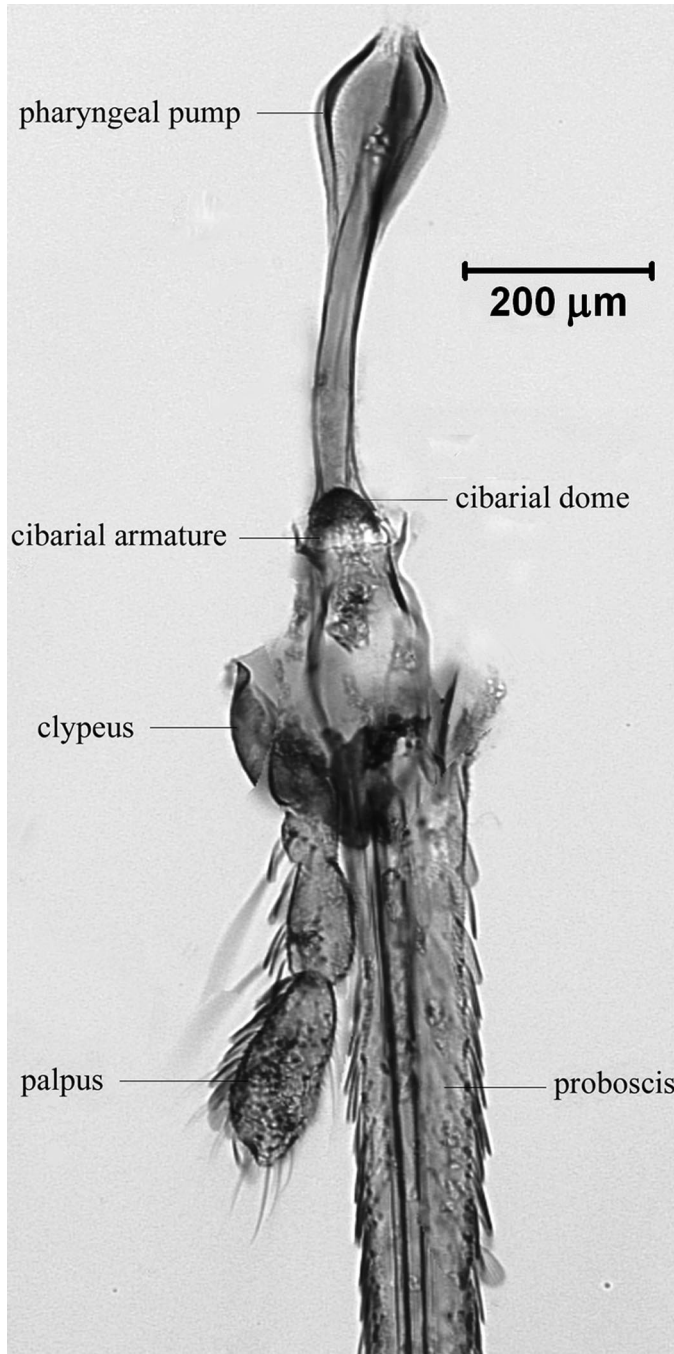


Fig. 2. Cibarial armature with attached structures shown.

portion at midpoint base to apex of tooth; basal portion of the teeth hourglass in shape, i.e., median area narrower than distal and basal areas, apex of basal portion flat; distal portion of teeth short but usually conspicuous, length of distal portion 0.05–0.16 total length of tooth, apex smooth or with small serrations; lateral flange terminating at or beyond apex of teeth (Fig. 3C and D).

The cibarial armature of *Cx. atratus* is morphologically similar to several other species from Florida, but usually can be easily distinguished from these species by the basal portion of the teeth, which is distinctly hourglass in shape. In addition, *Cx. atratus* can be distinguished from *Cx. atratus* B by the presence of wide gaps between the basal portion of the teeth, particularly in the median part of the tooth, and by the

Table 1. Collection data for specimens examined

Species	Locality	Collection method	Collectors	No. examined
<i>Cx. abominator</i>	Texas, Starr Co.	Larval rearing	K. Bennett, E. Gordon	1
<i>Cx. anips</i>	Mexico, Baja California	Unknown	B. Brookman, W. Reeves	1
<i>Cx. atratus</i>	Florida, Miami-Dade Co.	Larval rearing	M. Williams, E. Gordon	20
<i>Cx. atratus</i> B	Florida, Miami-Dade Co.	Larval rearing	M. Williams, E. Gordon	16
<i>Cx. cedecei</i>	Florida, Miami-Dade Co.	CDC light trap	M. Williams	35
<i>Cx. erraticus</i>	Florida, Miami-Dade Co.	Larval rearing	M. Williams, M. Godsey	20
	Minnesota, Dakota Co.	CDC light trap	S. Brogren	1
	North Carolina, Chatham Co.	CDC light trap	E. Powell	3
	North Carolina, Columbus Co.	CDC light trap	E. Powell	3
	North Carolina, Davidson Co.	CDC light trap	P. Whitt	14
	North Carolina, Duplin Co.	CDC light trap	E. Powell	17
	North Carolina, Harnett Co.	CDC light trap	E. Powell	3
	North Carolina, Sampson Co.	CDC light trap	E. Powell	3
	North Carolina, Wake Co.	CDC light trap	E. Powell	8
	North Carolina, Wayne Co.	CDC light trap	E. Powell	6
	Tennessee, Shelby Co.	CDC light trap	J. Grubaug	13
	Texas, Brewster Co.	Larval rearing	H. Savage, K. Bennett	4
<i>Cx. iolambdis</i>	Florida, Manatee Co.	Larval rearing	M. Williams, M. Godsey	5
<i>Cx. mulrennani</i>	Florida, Manatee Co.	Larval rearing	M. Williams, E. Gordon	7
<i>Cx. peccator</i>	North Carolina, Brunswick Co.	CDC light trap	R. Hickman, J. Brown	2
<i>Cx. pilosus</i>	Florida, Miami-Dade Co.	Larval rearing	M. Williams, E. Gordon	10

presence of a reduced distal portion with small serrations. *Cx. atratus* can be distinguished from *Cx. iolambdis* and *Cx. mulrennani* by the following combination of characters: the flattened shape of the apex of the basal portion of the teeth; the insertion of the teeth at or just below the apex of the cibarial crest; the reduced distal portion of the teeth, often with small serrations; and the lateral flange which often extends beyond the apex of the teeth.

Culex (Melanoconion) atratus B. Usually six teeth, occasionally five to eight; base of teeth insert below apex of cibarial crest; basal gap between teeth narrow and rounded; gap between basal portion of teeth usually 0.3–0.6 width of basal portion at midpoint base to apex of tooth, rarely 0.6–0.8 width of basal portion; basal portion of teeth usually taper evenly from base to apex, or teeth rectangular in shape, apex of basal portion rounded to flat; distal portion short to moderate in length, length of distal portion 0.10–0.20 total length of tooth, apex usually with moderate to large serrations; lateral flange terminating at or beyond apex of teeth (Fig. 3E and F).

Culex atratus B appears to be a new, undescribed species that is morphologically similar to *Cx. atratus*. We failed to detect consistent morphological differences in larval setae and male genitalia between *Cx. atratus* B and *Cx. atratus*. *Cx. atratus* B can be distinguished from *Cx. atratus* based on shape of the teeth of the cibarial armature and significant sequence differences in the internal transcribed spacers (ITS) of the ribosomal DNA gene array (unpublished data).

The cibarial armature of *Cx. atratus* B is morphologically similar to several species in Florida, e.g., *Cx. atratus*, *Cx. iolambdis*, and *Cx. mulrennani*, but can be distinguished by the following combination of characters: the narrow gap between the teeth; the shape of the teeth, which typically taper evenly to the apex or are rectangular; the short to moderate distal portions of the teeth that have large serrations; insertion of the

teeth well below the apex of the cibarial crest; and the lateral flange, which often extends beyond the apex of the teeth.

Culex (Melanoconion) cedecei Stone and Hair 1968. Teeth 11–15 in number; base of teeth insert at apex of cibarial crest; basal gap between teeth rounded, subequal to width of tooth; teeth strongly curved in basal half and appearance variable, typically basal portion appears hollow, and distal portion appears solid, occasionally entire tooth appears solid; typically basal portion triangular, short, 0.25–0.50 total length of tooth; distal portion of teeth long, length of distal portion 0.50–0.75 total length of tooth, apex with small serrations; lateral flange terminating beyond apex of teeth (Fig. 3G and H).

Culex (Melanoconion) erraticus (Dyar and Knab 1906). Teeth variable in number, from 6 to 13; base of teeth insert just below or at apex of cibarial crest; basal gap between teeth rounded, rarely bilobed, broad; gap between basal portion of teeth subequal to or greater than width of basal portion at midpoint base to apex of tooth; teeth shape variable, basal portion subrectangular to "bullet" shaped, i.e., tapering gradually with rounded apex; distal portion small, length of distal portion ≤ 0.2 total length of tooth, typically inconspicuous, occasionally well developed, apex typically smooth or with minute serrations, occasionally with moderate-sized serrations; lateral flange well developed, usually terminating beyond apex of teeth, occasionally terminating at apex of teeth (Fig. 4A–C).

Culex (Melanoconion) iolambdis Dyar 1918. Eight or nine teeth. Base of teeth insert below apex of cibarial crest; basal gap between teeth rounded, broad; gap between basal portion of teeth ≥ 0.6 width of basal portion at midpoint base to apex of tooth; basal portion of teeth rounded apically; distal portion small, length of distal portion ≤ 0.09 total length of tooth, inconspicuous, apex with minute serrations; lateral flange

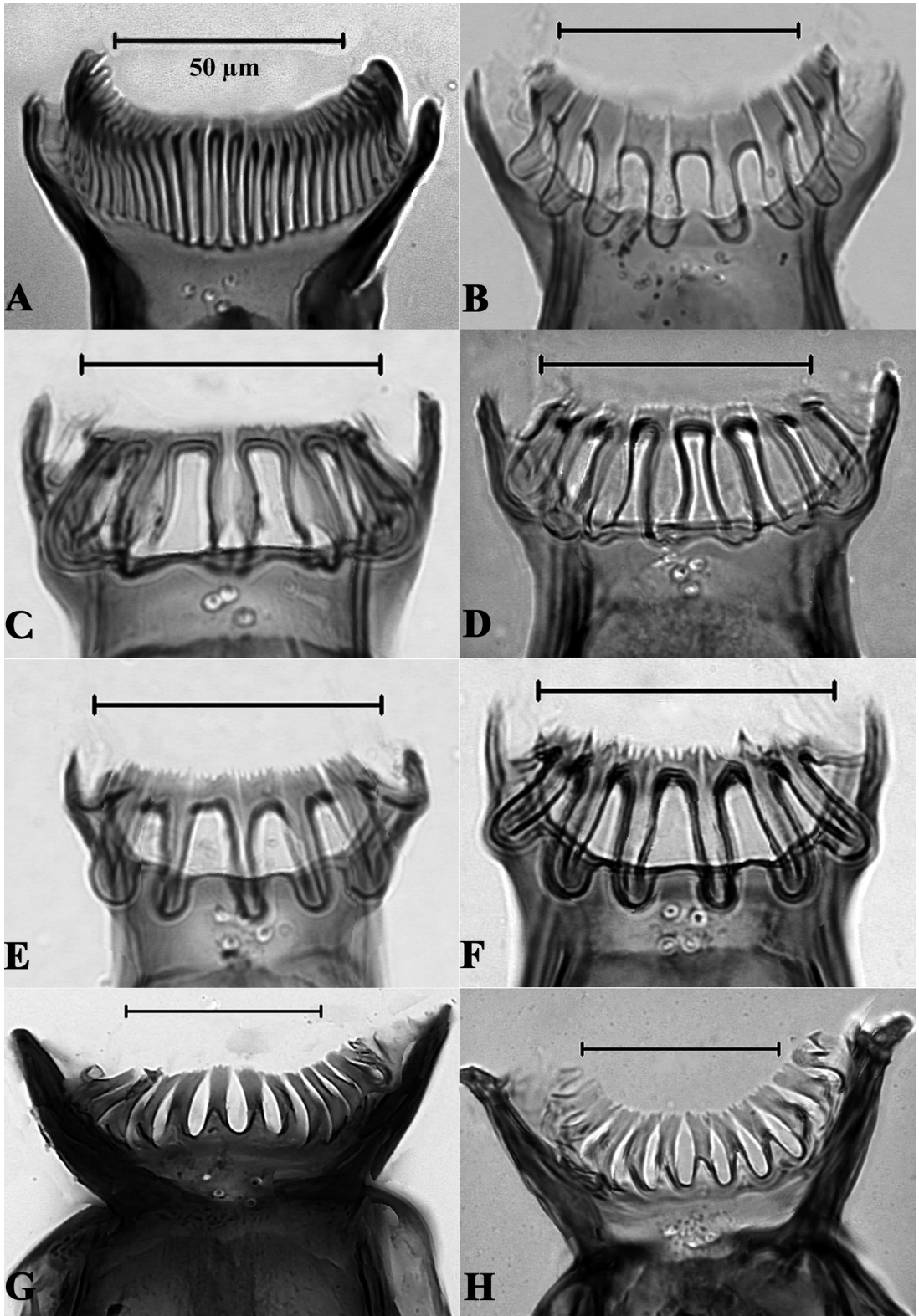


Fig. 3. Cibarial armatures of species of *Culex (Melanoconion)* in the United States. (A) *Cx. (Mel.) abominator*. (B) *Cx. (Mel.) anips*. (C and D) *Cx. (Mel.) atratus*. (E and F) *Cx. (Mel.) atratus* B: (E) typical and (F) variation. (G and H) *Cx. (Mel.) cedecei*.

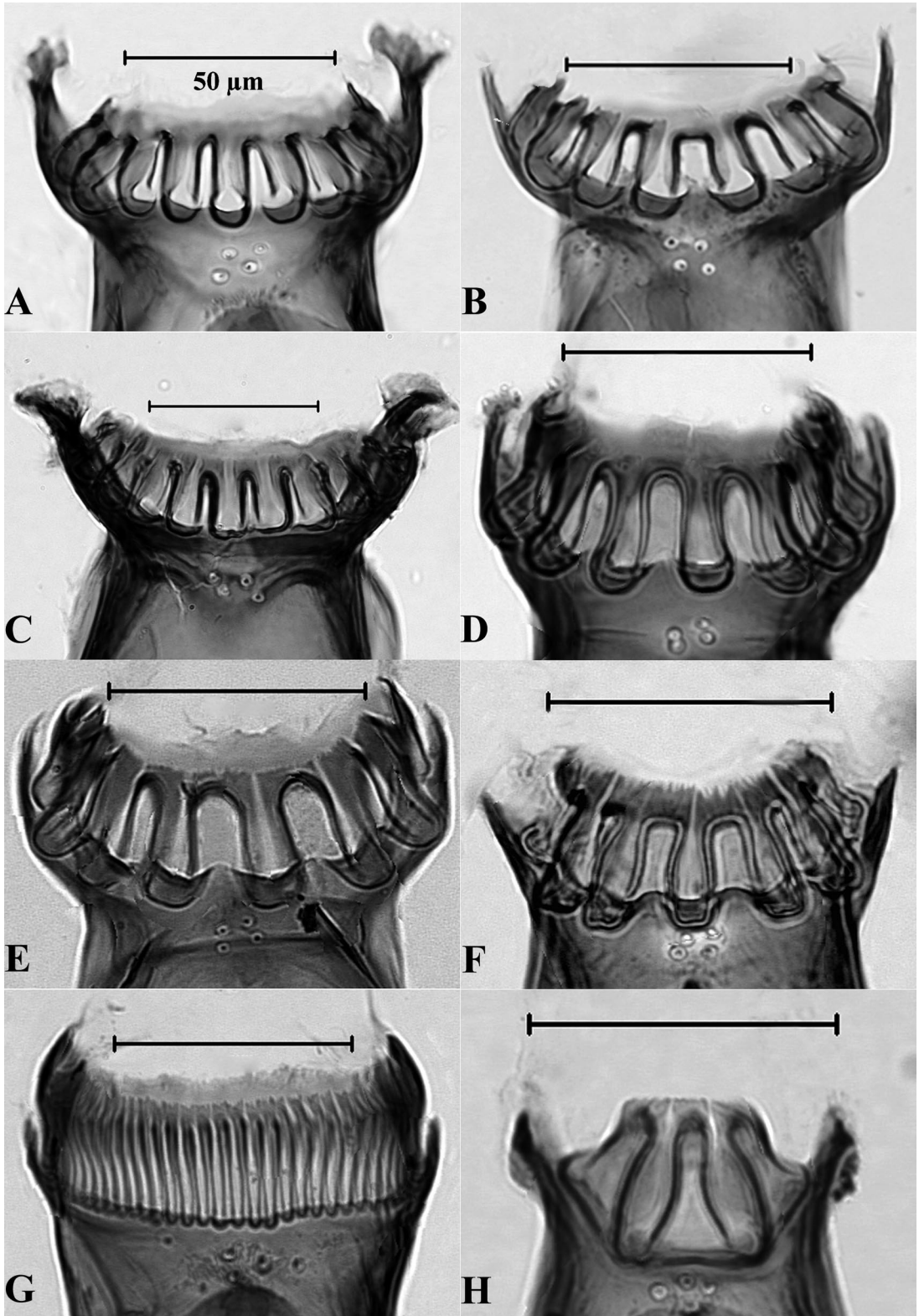


Fig. 4. (A–C) Cibarial armature of *Cx. (Mel.) erraticus*. (D and E) Cibarial armature of *Cx. (Mel.) iolambdis*. (F) Cibarial armature of *Cx. (Mel.) mulrenmani*. (G) Cibarial armature of *Cx. (Mel.) peccator*. (H) Cibarial armature of *Cx. (Mel.) pilosus*.

terminating before apex or at apex of teeth (Fig. 4D and E).

The cibarial armature of *Cx. iolambdis* is morphologically similar to several other species from Florida, e.g., *Cx. atratus*, *Cx. atratus* B, and *Cx. mulrennani*, but can be distinguished from these species by the following combination of characters: the rounded shape of the apex of the basal portion of the teeth; the rounded shape of the basal gap between the teeth; the insertion of the teeth below the apex of the cibarial crest; the reduced distal portion of the teeth, which may not be visible in all slide preparations; and the lateral flange, which usually terminates before the apex of the teeth.

***Culex* (*Melanoconion*) *mulrennani* Basham 1948.** Teeth usually eight, occasionally seven to nine in number; base of teeth insert below apex of cibarial crest; basal gap between teeth broadly rounded to bilobed, broad; gap between basal portion of teeth ≥ 0.7 width of basal portion at midpoint base to apex of tooth; basal portion of teeth subrectangular, tapering slightly to apex, or weakly hourglass in shape; apex of basal portion gently rounded to flat; distal portion distinct, moderate to large, length of distal portion 0.18–0.25 total length of tooth, apex with distinct serrations; lateral flange usually terminating before apex of teeth, rarely at apex of teeth (Fig. 4F).

The cibarial armature of *Cx. mulrennani* is morphologically similar to several other species in Florida, e.g., *Cx. atratus*, *Cx. atratus* B, and *Cx. iolambdis*, but can be distinguished from these species by the following combination of characters: the moderate to large distal portions of the teeth which have large serrations; insertion of the base of the teeth below the apex of the cibarial crest; the wide gap between the basal portion of teeth; the shape of the apex of the basal portion of the teeth, which is broadly rounded to flattened; and lateral flange, which usually terminates before the apex of teeth, or rarely at apex of teeth.

***Culex* (*Melanoconion*) *peccator* Dyar and Knab 1909.** Teeth numerous, 32–55 in number; base of teeth insert slightly below apex of cibarial crest; teeth narrow, long and close-set; basal portion of tooth long, distal portion short, length of distal portion ≈ 0.09 total length of tooth, rectangular, apex of distal portion weakly serrated; lateral flange terminates before apex of teeth (Fig. 4G).

Based on the limited number of specimens we inspected (Table 1), *Cx. peccator* has 32–40 teeth. Sirivanakarn (1978) did not describe the cibarial armature of this species, but he did illustrate *Cx. peccator* with ≈ 55 teeth.

***Culex* (*Melanoconion*) *pilosus* (Dyar and Knab 1906).** Teeth usually three, rarely two to four, in number, large; base of teeth insert at apex of cibarial crest; basal gap between teeth rounded, broad; basal portion of teeth taper apically; distal portion small, length of distal portion ≤ 0.10 total length of tooth, inconspicuous; lateral flange terminates subequal to apex of teeth (Fig. 4H).

Key to *Culex* (*Melanoconion*) Species of the United States Based on the Female Cibarial Armature and the Mesepimeron

1. Mesepimeron with distinct scale patch of three or more scales, scales directed dorsally (Fig. 1A) *erraticus*
Mesepimeron without scale patch (Fig. 1B) 2
2. Teeth of cibarial armature >20 in number (Fig. 3A) 3
Teeth <20 in number (Fig. 3B–H) 4
3. Teeth 32–55 in number (Fig. 4G) *peccator*
Teeth 27–35 in number (Fig. 3A) *abominator*
4. Cibarial armature usually with three large teeth, rarely with two to four teeth (Fig. 4H) *pilosus*
Cibarial armature with five or more teeth (Fig. 4A–F) 5
5. Teeth 10–15 in number; distal portion of teeth long, ≥ 0.5 length of tooth (Fig. 3G and H) *cedecei*
Teeth nine or less in number; distal portion of teeth short to moderate in length, ≤ 0.3 length of tooth (Fig. 3B–F) 6
6. Gap between basal portion of teeth narrow, gap usually <0.6 width of basal portion at midpoint base to apex of tooth (Fig. 3E and F); basal portion of teeth usually taper evenly from base to apex or rectangular *atratus* B
Gap between basal portion of teeth wide, gap ≥ 0.6 width of basal portion at midpoint base to apex of tooth (Figs. 3B–D and 4D–F); basal portion of teeth variable in shape 7
7. Basal portion of teeth with distinct hourglass shape, basal portion of teeth flat apically; lateral flange terminating at or beyond apex of teeth (Fig. 3C and D) *atratus*
Basal portion of teeth not distinctly hourglass in shape, basal portion of teeth rounded to flat apically; lateral flange terminating at or before apex of tooth (Figs. 3B and 4D–F) 8
8. Distal portion of teeth small, <0.10 length of tooth; apex of basal portion of teeth and basal gap between teeth smoothly rounded (Fig. 4D and E) *iolambdis*
Distal portion of teeth moderate to well developed, ≥ 0.10 length of tooth; apex of basal portion of teeth and basal gap between teeth flat to broadly rounded (Figs. 3B and 4F) 9
9. Apex of teeth with moderate to large serrations; basal gap between teeth broadly rounded to bilobed; distribution Florida (Fig. 4F) *mulrennani*
Apex of teeth with small serrations; basal gap between teeth rounded; distribution California (Fig. 3B) *anips*

Discussion

The cibarial armatures of some species are highly conserved from individual to individual, whereas other species display a great deal of variability. *Cx. erraticus* is an example of a species that displays a great

deal of variability in teeth number and shape. This species is widespread throughout a large area of the United States, occurring as far north as New York and Minnesota, and can be found throughout Central America, the Caribbean, and parts of South America. In the United States, the number of teeth may vary from 6 to 13, and shape may vary from square to rectangular, to bullet shaped, to long and thin. Comparisons of cibarial armatures in *Cx. erraticus* from southern Florida between mothers and their daughters reared from egg rafts shows that variation in teeth shape and number occurs within families. We were unable to correlate sequence differences in the ITS region with teeth-type in *Cx. erraticus* (unpublished data).

In contrast, we were able to associate ITS sequence differences with different teeth shapes among specimens identified as *Cx. atratus* based on larval characters and associated male genitalia. Although much future work is needed to define and differentiate the taxon *Cx. atratus* B from *Cx. atratus*, we believe that correlated sequence and cibarial armature teeth shape differences support the contention that *Cx. atratus* B represents a new species.

Examination of the cibarial armature is often the only way to identify an adult female *Culex* (*Melanoconion*) mosquito to species with a high degree of certainty. Cibarial armatures can be used for identification even when the specimen is severely damaged, as is sometimes the case with field-collected mosquitoes. The utility of the cibarial armature as a diagnostic character will be most useful in the southeastern United States, particularly in Florida, where *Cx.* (*Melanoconion*) are most diverse. Because of the time required to dissect and slide mount the cibarial armature, the use of this structure as a taxonomic character is tedious and may not be suitable for the routine identification of specimens collected during surveillance programs. Research to identify additional morphological characters and to develop molecular assays for species identification for members of the *Cx.* (*Melanoconion*) is needed.

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