

## MORPHOLOGICAL ABNORMALITIES IN SPECIES OF THE QUADRIMACULATUS COMPLEX OF *ANOPHELES* (DIPTERA: CULICIDAE)

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**ABSTRACT.** This report provides results of the first detailed examination of morphological abnormalities occurring in adults, pupae, and 4th-instar larvae of a sibling species complex within the family Culicidae. Aberrant and/or anomalous setae or structures are reported, along with their frequency of occurrence, for the 5 sibling species (*Anopheles diluvialis*, *Anopheles inundatus*, *Anopheles maverlius*, *Anopheles quadrimaculatus*, and *Anopheles smaragdinus*) of the Quadrimaculatus Complex of *Anopheles*. A total of 21,684 specimens were examined. In the pupal stage, the presence of seta 11-II was the most common anomaly, whereas in the 4th-instar larva, branching of seta 2-C occurred most frequently. Abnormalities in the male genitalia usually involved the claspette or tergum IX. Other abnormalities in females, male genitalia, pupae, and 4th-instar larvae occurred infrequently.

**KEY WORDS** *Anopheles diluvialis*, *An. inundatus*, *An. maverlius*, *An. quadrimaculatus*, *An. smaragdinus*, mosquito

### INTRODUCTION

While developing the homology of the chaetotaxy of immature mosquitoes and a revised nomenclature for the chaetotaxy of the pupa, Belkin (1952) indicated, "Anomalies, particularly those produced through developmental arrest or excess, furnish some of the most convincing evidence for the homology of structures and are widely used in the field of comparative anatomy." Belkin (1953) further stated, "Anomalies have been particularly useful in establishing homologies but unfortunately their occurrence is limited and unpredictable, and it is hoped that other workers will report them as they come to light."

Aberrant and/or anomalous setae or structures have been reported for several species within the Anophelinae, but usually the published information represented the examination of a limited number of specimens. Most of these reports have dealt with setae of the larval and pupal stages, and as Puri (1931) indicated, they concern suppression or duplication of a seta, displacement of 1 or 2 setae, appearance of a profusely branched seta in place of a frayed seta, and a frayed or branched seta appearing in place of a simple seta.

Literature references of anomalous/aberrant conditions in the Anophelinae include Swellengrebel and Swellengrebel-de Graaf (1920), Root (1922), Iyengar (1922), La Face (1926, 1931), Puri (1931), Martini (1933), Dampf (1936), Yao and Wu (1936), Crawford (1938), Roth (1944, 1945), Belkin et al. (1945, 1970), Gaud (1947), de Burca and Forshaw (1947), Gaud and Laurent (1950), Downs (1950), Belkin (1953), Haider (1961), Abul-Hab (1963), Denisova (1964), Gad (1965), Reid (1968), Harrison and Scanlon (1975), Floore et al. (1976), Marks (1976), Tenorio (1977), Wood et al. (1979), Reinert (1980), Faran (1980), Harrison (1980), Mason

(1980), Nagpal and Sharma (1983), Harrison and Peyton (1984), and Hribar (1996). Kitzmiller and Mason (1967) provided a review of morphological mutants and "heritable" variations for which a genetic basis had been determined. The following are published articles dealing specifically with anomalous/aberrant condition in *Anopheles quadrimaculatus* s.l.: Root (1922), Russell (1925), Matheson (1945), Mitchener (1947), Burton (1953) (seta 2-C, larva); Roth (1944) (claspette and tergum IX, ♂ genitalia), (1945) (setae 2-C, 3-C, 6-C, and 1-IV,V, larva); Buren (1944) (setae 2-C and 9-C, larva); Jones (1955) (aberrant trachea, larva); and Reinert et al. (1997) (seta 2-C, larva; setae 8-II, 10,11-I,II, 13-III,V-VIII, pupa). In some articles, it is difficult to determine if the character condition mentioned was aberrant or part of the normal variation of the feature. Also, in some reports, conditions of a feature (especially setal branching) considered by the authors to be variable or abnormal may need to be reconsidered in the light of cryptic species of a sibling species complex.

Variation in structures of the male genitalia may be affected by larval rearing temperature, as shown by Hribar (1996) for *Anopheles albimanus* Wiedemann. Le Sueur et al. (1992) found winter-summer (temperature dependent) variation in dark-scaled patches on wings of species in the Gambiae Complex. For specimens of the Quadrimaculatus Complex evaluated in this study, the isofemale progeny broods (IPBs) and field-collected immatures were reared by the author in the same laboratory and under nearly identical conditions (e.g., temperature, relative humidity, light-darkness regime, larval food, etc.); therefore, the anomalous/aberrant conditions reported here should not have been affected by the rearing conditions of the immature stages. Also, none of the genitalic characters affected by

larval rearing temperature reported by Hribar (1996) was used as a key character for the separation of the 5 sibling species of the *Quadrimalaculatus* Complex (Reinert et al. 1997).

During an analysis of morphological characters of the 5 sibling species (*Anopheles diluvialis* Reinert, *Anopheles inundatus* Reinert, *Anopheles maverlii* Reinert, *Anopheles quadrimaculatus* Say, and *Anopheles smaragdinus* Reinert) in the *Quadrimalaculatus* Complex of *Anopheles* Meigen, several anomalous and/or aberrant features were observed and are listed below. Numerous specimens (females, males, male genitalia, pupae, 4th-instar larvae, and pupal and 4th-instar larval exuviae), most of which were from IPBs, were examined to determine the forms of abnormalities occurring in each species. Many of the 141 IPBs consisted of long series of individually reared adults with their associated 4th-instar larval and pupal exuviae. The following data represent the first detailed examination of morphological abnormalities occurring in adults, pupae, and 4th-instar larvae of a sibling species complex of the family Culicidae.

## MATERIALS AND METHODS

A total of 21,684 specimens were examined as follows (letters used in parentheses represent the following species, D = *An. diluvialis*, I = *An. inundatus*, M = *An. maverlii*, Q = *An. quadrimaculatus*, and S = *An. smaragdinus*): 3,482 females (D = 553, I = 838, M = 417, Q = 1,083, and S = 591); 2,629 males (D = 336, I = 505, M = 435, Q = 817, and S = 536); 169 male genitalia (D = 31, I = 35, M = 33, Q = 34, and S = 36); 5,871 pupal exuviae (D = 846, I = 1,363, M = 850, Q = 1,741, and S = 1,071); 331 pupae (D = 37, I = 140, M = 21, Q = 79, and S = 54); 6,228 4th-instar larval exuviae (D = 883, I = 1,537, M = 875, Q = 1,845, and S = 1,088); and 2,974 4th-instar larvae (D = 309, I = 781, M = 366, Q = 877, and S = 641). Because of the large numbers of specimens, stages, and long specimen series of IPBs, an indication of frequency (f) for the conditions could be calculated with the above numbers. Frequency was determined by taking the number of times a character occurred and dividing by the total number of specimens of that stage for the species. A detailed study of larval seta 2-C and pupal setae 10,11-I,II was conducted on all specimens; other abnormalities were noted only when observed. Generally, the following criteria were used to determine the condition of a feature: anomalous = condition resulting from an extra seta or structure, a duplication of a seta or structure, or absence of a normally occurring seta or structure (e.g., occurrence of 2 seta 3-C on 1 side of the larval cranium); aberrant = a seta or a structure modified from the normal condition (e.g., seta 2-C, a normally single, simple seta, is a 2,3-branched seta); and variation = an increase or decrease in range of a structure

(e.g., increase or decrease in the number of branches of a branched seta). In the following descriptions, setalike cuticular filaments, when located in the position of a seta in the pupal or larval stages, are referred to as a seta without an alveolus.

The following format is used for specimens examined: stage/sex; species name; description of anomaly/aberration; frequency (f); and number of specimens with their state(s) (i.e., FL = Florida, GA = Georgia, LA = Louisiana, MN = Minnesota, and MS = Mississippi) of origin. Abbreviations used are ♀ = female, g = genitalia, L = 4th-instar larva, Le = 4th-instar larval exuviae, and Pe = pupal exuviae.

## ANOMALIES/ABERRATIONS NOTED IN THE QUADRIMACULATUS COMPLEX

### Females

*Anopheles diluvialis*: One long brown scale on left side of prespiracular area (f = 0.0036) (2 ♀, FL). One long flattened seta, without ridges, on left side of subspiracular area (f = 0.0018) (1 ♀, FL). One long brown scale on left side of hypostigmal area (f = 0.0018) (1 ♀, FL). One long brown scale on upper area dorsal to setae on left side of lower mesokatepisternal area (f = 0.0018) (1 ♀, FL).

*Anopheles inundatus*: Parascutellar area without setae on both sides (f = 0.0036), without seta on 1 side (f = 0.0143) (15 ♀, FL and LA). One brown scale on both sides of prealar area (f = 0.0012) (1 ♀, GA).

*Anopheles maverlii*: Two long moderately broad brown setae on left side of postspiracular area (f = 0.0024) (1 ♀, MS). One long brown scale dorsal to setae on left side of lower mesokatepisternum (f = 0.0024) (1 ♀, MS). One long brown scale ventral to setae on left side of upper mesokatepisternum (f = 0.0024) (1 ♀, MS).

*Anopheles quadrimaculatus*: One long brown scale on left side of upper mesokatepisternum (f = 0.0028) (3 ♀, MS). One moderately broad brown scale on left side of postspiracular area (f = 0.0009) (1 ♀, MN).

*Anopheles smaragdinus*: Five setae shorter (ca. 0.6–0.7 normal length), flattened, with distal 0.25–0.30 sharply curved, on left side of supraalar area (f = 0.0017) (1 ♀, FL). One long, moderately broad, brown scale on left side of prespiracular area (f = 0.0017) (1 ♀, LA).

### Male genitalia

Relatively small numbers of male genitalia preparations were examined for each of the 5 sibling species; therefore, the frequencies for the abnormal conditions are not given. For these species, Reinert et al. (1997) reported that the development and number of stems of seta 5 of the claspette were quite variable and that the apex of the posterolateral lobes of tergum IX varied from round to truncate.

*Anopheles diluvialis*: Seta 3 on left side of claspette, bluntly rounded, clublike (1 g, FL). Seta 4 on left side of claspette with apical 0.5 broader, flattened (1 g, FL).

*Anopheles inundatus*: Two seta 4 on left side of claspette, equally developed, situated side by side (1 g, FL). Extra seta on right side of claspette, long, curved, lanceolate, situated between seta 4 and seta 5, developed similarly to seta 3 except without sheathlike basal structure (1 g, FL).

*Anopheles maverlius*: Two seta 1 on right side of claspette, extra seta mesally (2 g, MS). Two seta 1 on left side of claspette, extra seta mesally (1 g, MS). Two seta 3 on right side of claspette, extra seta laterally (2 g, GA and MS).

*Anopheles quadrimaculatus*: Posterolateral lobe on right side of tergum IX with long, narrow, pointed projection mesally resembling beak of bird's head (1 g, FL). Setae 1 and 5 absent on left side, seta 4 absent on right side of claspette (1 g, LA). Two seta 1 on right side of claspette, extra seta laterally (1 g, FL). Internal seta forked apically on left gonocoxite (1 g, LA).

*Anopheles smaragdinus*: Posterolateral lobes acuminate, long, on both sides of tergum IX (1 g, MS).

### Pupae

*Anopheles diluvialis*: Two seta 2-VI on right side, both 4-branched, each with alveolus, normally developed, 1 seta slightly posterolateral to other ( $f = 0.0011$ ) (1 Pe, FL). Two seta 6-II on right side, 1 seta 6-forked, extra seta very slightly anterolateral, 2-branched at base but each branch 3-forked ( $f = 0.0011$ ) (1 Pe, FL). Two seta 9-III on right side, extra seta slightly shorter, anterolateral to other seta ( $f = 0.0011$ ) (1 Pe, FL). Seta 9-VI on right side reduced in size, ca. 0.6 normal length and 0.3 normal thickness ( $f = 0.0011$ ) (1 Pe, FL). Seta 10-II moderately long, thin, present only on left side ( $f = 0.0102$ ), present only on right side ( $f = 0.0079$ ), present on both sides ( $f = 0.0034$ ), present on 1 or both sides (2.4% of specimens), simple (7.7%), 2,3-branched (65.4%), 4-6-branched (26.9%), 70.8% with alveolus (remainder without) (21 Pe, FL). Seta 11-II short, thin, present only on left side ( $f = 0.0045$ ), present only on right side ( $f = 0.0045$ ), present on both sides ( $f = 0.0011$ ), present on 1 or both sides (1.0% of specimens), simple (70.0%), 2,3-branched (30.0%), 100% without alveolus (9 Pe, FL).

*Anopheles inundatus*: Seta 9-VI on right side consisting of very short, somewhat broader knob ( $f = 0.0007$ ) (1 Pe, FL). Two seta 9-VIII on left side, each normally branched and developed, with alveolus ( $f = 0.0007$ ) (1 Pe, FL). Seta 10-II moderately long, thin, present only on left side ( $f = 0.0120$ ), present only on right side ( $f = 0.0160$ ), present on both sides ( $f = 0.0193$ ), present on 1 or both sides (4.7% of specimens), simple (21.0%), 2,3-branched

(56.0%), 4-6-branched (23.0%), 43.0% with alveolus (remainder without) (71 Pe, FL and GA). Two seta 10-IV on right side, each 3-branched with alveolus, normally developed, extra seta posterolateral to other seta ( $f = 0.0007$ ) (1 Pe, FL). Seta 11-II short, thin, present only on left side ( $f = 0.0053$ ), present only on right side ( $f = 0.0033$ ), present on both sides ( $f = 0.0033$ ), present on 1 or both sides (1.2% of specimens), simple (34.8%), 2,3-branched (56.5%), 4-branched (8.7%), 13.0% with alveolus (remainder without) (18 Pe, FL and GA). Seta 14-VIII on left side ca. 4 $\times$  normal length, 5-branched ( $f = 0.0007$ ) (1 Pe, FL).

*Anopheles maverlius*: Seta 9-III on left side simple, long (ca. 4 $\times$  normal length), relatively thin ( $f = 0.0012$ ) (1 Pe, MS). Seta 9-III on left and right sides simple, long, relatively thin ( $f = 0.0012$ ) (1 Pe, MS). Seta 9-VIII on left side with broad central stem, apex brush-tipped with 4 very short spines ( $f = 0.0012$ ) (1 Pe, MS). Seta 10-II moderately long, thin, present only on left side ( $f = 0.0356$ ), present only on right side ( $f = 0.0459$ ), present on both sides ( $f = 0.0494$ ), present on 1 or both sides (13.1% of specimens), 33.3% with alveolus (remainder without), simple (13.0%), 2,3-branched (73.4%), 4,5-branched (13.6%) (114 Pe, FL and MS). Seta 11-II short, thin, present only on left side ( $f = 0.0057$ ), present only on right side ( $f = 0.0080$ ), present on both sides ( $f = 0.0034$ ), present on 1 or both sides (1.7% of specimens), simple (44.4%), 2,3-branched (55.6%), 100% without alveolus (15 Pe, MS). Seta 1-Pa on right paddle simple, very thin, ca. 0.3 normal length ( $f = 0.0012$ ) (1 Pe, MS). Left paddle developed as small lobe, setae 1,2-Pa and midrib absent, right paddle and setae normally developed except mesal 0.4 deformed, 3rd deformed, elongated paddle situated mesally to right of left paddle, seta 1-Pa normally developed ( $f = 0.0012$ ) (1 Pe, MS). Seta 1-Pa on left paddle, moderately long, thin ( $f = 0.0012$ ) (1 Pe, MS).

*Anopheles quadrimaculatus*: Two seta 5-CT on right side, 1 seta 4-branched, normally developed, extra seta 5-branched, situated mesal and very slightly posterior to other seta, each with alveolus ( $f = 0.0006$ ) (1 Pe, MS). Seta 8-II on left side 3-branched, short, thin, with small alveolus, situated in similar location as 8-III ( $f = 0.0006$ ), right side without seta or alveolus (1 Pe, MN). Seta 8-II represented on both sides by small alveolus ( $f = 0.0006$ ) (1 Pe, MN). Two seta 8-VII on right side, 1 seta 3-branched, short, thin, extra seta simple, situated slightly lateral to other seta, each with alveolus ( $f = 0.0006$ ) (1 Pe, MN). Seta 10-I on left side 2-branched, short, right side simple, each without alveolus ( $f = 0.0006$ ) (1 Pe, FL). Seta 10-I on left side single, short, without alveolus, right side without seta ( $f = 0.0006$ ) (1 Pe, FL). Seta 10-II moderately long, thin, present only on left side ( $f = 0.0039$ ), present only on right side ( $f = 0.0028$ ), present on both sides ( $f = 0.0055$ ), present on 1 or

both sides (1.1% of specimens), 25.0% with alveolus (remainder without), simple (18.8%), 2,3-branched (75.0%), 4-branched (6.3%) (22 Pe, FL, LA, and MS). Seta 11-I on left and right sides simple, very short, without alveolus ( $f = 0.0006$ ) (1 Pe, FL). Seta 11-I on left side single, very short, right side with seta 2-branched, short, each without alveolus ( $f = 0.0006$ ) (1 Pe, FL). Seta 11-II short, thin, present only on left side ( $f = 0.0330$ ), present only on right side ( $f = 0.0467$ ), present on both sides ( $f = 0.1324$ ), present on 1 or both sides (20.3% of specimens), simple (20.9%), 2,3-branched (73.7%), 4,5-branched (5.4%), 3.9% with alveolus (remainder without), represented by short stout spicule on left side ( $f = 0.0022$ ) (390 Pe, FL, GA, LA, MN, and MS). Seta 13-V on left side 3-branched, short, thin, without alveolus, situated posterior and slightly lateral to seta 11-V ( $f = 0.0006$ ) (1 Pe, MN). Seta 13-VI on left side 6-branched, short, thin, right side with seta 4-branched, each with alveolus, situated slightly anterior and mesal to seta 11-VI ( $f = 0.0006$ ) (1 Pe, MN). Seta 13-VI on both sides 5-branched, short, thin, without alveolus, situated anterior and slightly mesal to seta 11-VI ( $f = 0.0006$ ) (1 Pe, MN). Seta 13-VI on left side 4-branched, short, thin, without alveolus, situated anterior and slightly mesal to seta 11-VI ( $f = 0.0006$ ), right side with small scar (1 Pe, MN). Seta 13-VII on left and right sides simple, moderately long, thin, without alveolus, situated lateral and slightly anterior to seta 11-VII ( $f = 0.0006$ ) (1 Pe, MN). Seta 13-VII on left and right sides simple, moderately long, thin, without alveolus, situated lateral and slightly posterior to seta 11-VII ( $f = 0.0006$ ) (1 Pe, MN). Seta 13-VIII on both sides, left side with seta 3-branched, right side with seta simple, each moderately long, thin, without alveolus, situated in similar location as seta 13-VII ( $f = 0.0006$ ) (1 Pe, MN).

*Anopheles smaragdinus*: Cephalothorax on left side with 3-branched seta, moderately thick, moderately long, with alveolus (encircled by narrow darkly pigmented area), situated anteriorly ca. 0.5 distance between setae 6,7-CT and ca. 0.5 distance between setae 4,7-CT ( $f = 0.0009$ ) (1 Pe, FL). Seta 10-II moderately long, thin, present only on left side ( $f = 0.0036$ ), present only on right side ( $f = 0.0036$ ), present on both sides ( $f = 0.0080$ ), present on 1 or both sides (1.5% of specimens), simple (11.5%), 2,3-branched (84.6%), 4-branched (3.8%), 15.4% with alveolus (remainder without) (17 Pe, FL and GA). Seta 11-I on right side simple or 3-branched, without alveolus ( $f = 0.0018$ ) (2 Pe, FL). Seta 11-II short, thin, present only on left side ( $f = 0.0213$ ), present only on right side ( $f = 0.0044$ ), present on both sides ( $f = 0.0293$ ), present on 1 or both sides (5.5% of specimens), simple (20.0%), 2,3-branched (76.8%), 4,5-branched (3.2%), 4.2% with alveolus (remainder without), represented by short, stout spicule on both sides ( $f = 0.0160$ ) (80 Pe, FL, GA, and MS). Seta 1-Pa on right paddle

short (ca. 0.25 of normal length), spiniform ( $f = 0.0009$ ) (1 Pe, FL). Two seta 1-Pa on right paddle, situated side by side, stout, 1 simple and other 2-branched at base, each branch long, stout ( $f = 0.0009$ ) (1 Pe, FL). Seta 2-Pa on left paddle simple, longer, stout, similar to seta 1-Pa ( $f = 0.0009$ ) (1 Pe, GA).

### Larvae

*Anopheles diluvialis*: Pair of seta 2-C with 1 seta simple and other 2-branched on distal 0.25–0.60, only on left side ( $f = 0.0050$ ), only on right side ( $f = 0.0008$ ) (7 Le, FL). Seta 2-C 3-branched on distal 0.4–0.5, only on left side ( $f = 0.0017$ ), on both sides ( $f = 0.0008$ ) (3 Le, FL). Only 1 seta 2-C anteromesally, simple ( $f = 0.0034$ ) (4 Le, FL). Seta 2-C on left side shorter (0.78 length of other seta), both simple ( $f = 0.0008$ ) (1 Le, FL). Alveoli of pair of seta 2-C narrowly separated, 0.3–0.9 width of 1 alveolus ( $f = 0.0403$ ) (48 Le, FL). Alveoli of pair of seta 2-C very widely separated, ca. 4.5× width of 1 alveolus ( $f = 0.0008$ ) (1 Le, FL). Two seta 3-C on left side, situated side by side, both normally developed ( $f = 0.0017$ ) (2 Le, FL). Seta 1-P on left side displaced mesally 2.5× normal distance from seta 2-P ( $f = 0.0008$ ) (1 L, FL). Two seta 1-P on left side, extra seta lateral to and ca. equal distance between setae 1-P and 2-P, equal in length, 4-branched on distal 0.6, each with alveolus ( $f = 0.0008$ ) (1 L, FL). Two seta 9-III on left side, side by side, equal in length, each with alveolus, 1 seta 8-branched, other 9-branched ( $f = 0.0008$ ) (1 Le, FL). Terga I-VII with large irregularly shaped sclerotized and heavily pigmented patches on mesal and posterior areas, patches separated from and of different consistency than tergal plates ( $f = 0.0016$ ) (2 Le, FL).

*Anopheles inundatus*: Pair of seta 2-C with 1 seta simple and other 2-branched on distal 0.2–0.7, only on left side ( $f = 0.0035$ ), only on right side ( $f = 0.0048$ ), on both sides ( $f = 0.0004$ ) (20 Le, FL and GA). Only 1 seta 2-C anteromesally, simple ( $f = 0.0013$ ) (2 L and 1 Le, FL). Three seta 2-C, simple, equal in length, alveoli equal distance apart ( $f = 0.0004$ ) (1 Le, LA). Left seta 2-C of pair shorter (0.84 length of other seta), both simple ( $f = 0.0004$ ) (1 Le, GA). Seta 2-C on right side long, broad and flattened on distal 0.4 (without ridges) ( $f = 0.0004$ ) (1 Le, GA). Alveoli of pair of seta 2-C narrowly separated, 0.5–0.9 width of 1 alveolus ( $f = 0.0073$ ) (17 Le, FL). Alveoli of pair of seta 2-C very widely separated, 4.5× width of 1 alveolus ( $f = 0.0004$ ) (1 L, FL). Pro-, meso-, and metathorax and abdominal terga I-VIII with 1 or more segments having irregularly shaped, sclerotized, and heavily pigmented patches on dorsal surface ( $f = 0.0030$ ) (7 Le, FL). Two seta 12-I on right side, each 3-branched, with alveolus, extra seta posterolateral, shorter ( $f = 0.0004$ ) (1 Le, FL).

*Anopheles maverlius*: Pair of seta 2-C with 1

seta simple and other 2-branched at ca. 0.5, only on left side ( $f = 0.0008$ ), only on right side ( $f = 0.0016$ ) (3 Le, MS). Three seta 2-C, simple, equal in length, alveoli equal distance apart ( $f = 0.0016$ ) (2 Le, MS). Three seta 2-C, equal in length, alveoli equal distance apart, lateral 2 setae simple, middle seta 2-branched ( $f = 0.0008$ ) (1 Le, MS). Seta 2-C on right side short (ca. 0.45 length of other seta), broad with brush tip (7 branches) ( $f = 0.0002$ ) (1 Le, MS). Alveoli of pair of seta 2-C narrowly separated, 0.6–0.9 width of 1 alveolus ( $f = 0.0113$ ) (14 Le, MS). Alveoli of pair of seta 2-C very widely separated, 4.0–6.0 $\times$  width of 1 alveolus ( $f = 0.0089$ ) (11 Le, MS). Two seta 9-C on right side, 1 seta posterior and slightly thinner than other seta, each 5-branched, with alveolus ( $f = 0.0008$ ) (1 Le, FL). Meso- and metathorax and/or terga I-VII with irregularly shaped patches of sclerotized, heavily pigmented areas ( $f = 0.0089$ ) (11 Le, MS). Seta 2-I on left side plumose with long thick stem having 20 long branches, 9.2 $\times$  length of 2-I on right side ( $f = 0.0008$ ) (1 Le, MS). Seta 2-I on right side pectinate, with long branches, 4.6 $\times$  length of 2-I on left side which is short, 5-branched ( $f = 0.0008$ ) (1 Le, MS). Seta 2-I on right side lightly plumose, with 6 long thin branches, 7.8 $\times$  length of 2-I on left side ( $f = 0.0008$ ) (1 Le, MS).

*Anopheles quadrimaculatus*: Pair of seta 2-C with 1 seta simple and other 2-branched on distal 0.3–0.7 (once at base), only on left side ( $f = 0.0151$ ), only on right side ( $f = 0.0136$ ), seta on left side 3-branched ( $f = 0.0007$ ), seta on right side 3-branched ( $f = 0.0007$ ), seta on right side plumose with 14 branches on distal 0.67 ( $f = 0.0004$ ), both seta 2-C 2-branched ( $f = 0.0029$ ), 1 or both seta 2-C branched (3.34% of specimens) (14 L and 77 Le, FL, GA, LA, MN, and MS). Only 1 seta 2-C anteromesally, simple ( $f = 0.0026$ ) (2 L and 5 Le, FL and LA). Three seta 2-C, simple, alveoli equal distance apart, middle seta 0.33 length of 2 lateral setae ( $f = 0.0004$ ) (1 L, FL). Alveoli of pair of seta 2-C very widely separated, 2.8–4.0 $\times$  width of 1 alveolus ( $f = 0.0037$ ) (5 L and 5 Le, FL). Two seta 1-A on left antenna, nearly side by side, 1 seta 4-branched, other 3-branched, each with alveolus ( $f = 0.0004$ ) (1 L, LA). Metathorax with 1 ( $f = 0.0018$ ) or 2 ( $f = 0.0018$ ) small tergal plates on anteromedian area (2 L and 8 Le, FL). Seta 1-I on right side well developed (1.9 $\times$  length of 1-I on left side), palmate with leaflets spread ( $f = 0.0004$ ) (1 Le, FL). Seta 1-III-VI smaller, leaflets narrow, pale (except 1-III on left side normally developed, dark) ( $f = 0.0004$ ) (1 Le, LA). Seta 1-III-VII on both sides with proximal 0.5 pale, remainder darkly pigmented ( $f = 0.0007$ ) (2 Le, GA and MN). Two seta 9-III on left side, nearly side by side, 1 seta 8-branched, other 10-branched, equal in length, each with alveolus ( $f = 0.0004$ ) (1 Le, FL). Sternum VII with sternal plate located posterior to seta 14-VII ( $f = 0.0007$ ) (2 Le, LA).

*Anopheles smaragdinus*: Pair of seta 2-C with 1

seta simple, other 2-branched on distal 0.3–0.6 (once at base), only on left side ( $f = 0.0041$ ), only on right side ( $f = 0.0046$ ), 1 of seta 2-C branched (0.9% of specimens) (15 Le, FL and GA). Three seta 2-C, simple, equal in length, alveoli equal distance apart ( $f = 0.0006$ ) (1 Le, FL). Seta 2-C on left side spiniform, short, broad ( $f = 0.0006$ ) (1 Le, GA). Alveoli of pair of seta 2-C narrowly separated, 0.7–0.9 width of 1 alveolus ( $f = 0.0064$ ) (11 Le, FL and MS). Alveoli of pair of seta 2-C widely separated, 3.0 $\times$  width of 1 alveolus ( $f = 0.0012$ ) (2 Le, FL). Seta 9-P on right side simple, deformed, twisted, broader, with distal ca. 0.6 more or less recurved ( $f = 0.0006$ ) (1 Le, FL). Two seta 4-T on left side, 1 seta 4-branched, extra seta 3-branched, displaced mesally, each with alveolus ( $f = 0.0006$ ) (1 Le, FL). Meso- and metathorax and terga I-VIII having 2 or more segments dorsally with irregularly shaped, sclerotized, and heavily pigmented patches ( $f = 0.0018$ ) (3 Le, FL). Two seta 9-III on left side, 1 seta 9-branched, extra seta 7-branched, slightly shorter, situated slightly posteromesal to other seta ( $f = 0.0006$ ) (1 Le, FL).

## DISCUSSION

For the 4 anomalous pupal setae, 10-I,II and 11-I,II, examined in all specimens, the following was observed: seta 10-I was found in 0.1% of *An. quadrimaculatus* (0 with an alveolus); seta 11-I was found in 0.2% of *An. smaragdinus* and 0.1% of *An. quadrimaculatus* (0 with an alveolus); seta 10-II was found in 13.1% of *An. maverlius* (33.3% with an alveolus), 4.7% of *An. inundatus* (43.0% with an alveolus), 2.4% of *An. diluvialis* (70.8% with an alveolus), 1.5% of *An. smaragdinus* (15.4% with an alveolus), and 1.1% of *An. quadrimaculatus* (25.0% with an alveolus); and seta 11-II was found in 20.3% of *An. quadrimaculatus* (3.9% with an alveolus), 5.5% of *An. smaragdinus* (4.2% with an alveolus), 1.7% of *An. maverlius* (0 with an alveolus), 1.2% of *An. inundatus* (13.0% with an alveolus), and 1.0% of *An. diluvialis* (0 with an alveolus). These data indicate that for the 5 sibling species of the Quadrimaculatus Complex seta 10-II occurs at the greatest frequency in *An. maverlius* and seta 11-II occurs most frequently in *An. quadrimaculatus*.

In 4th-instar larvae, branching of 1 or both seta 2-C of the pair was found in 3.34% of *An. quadrimaculatus*, 0.87% of *An. smaragdinus*, 0.76% of *An. diluvialis*, and 0.24% of both *An. inundatus* and *An. maverlius*. For larval seta 2-C, the separation between the pair of setae by the width of 1 alveolus or greater has been used in several keys to North American *Anopheles* (e.g., Carpenter and LaCasse 1955, King et al. 1960, Darsie and Ward 1981, Darsie and Morris 1998) to distinguish *An. quadrimaculatus* s.l. from several other species of *Anopheles* (including *Anopheles freeborni* Aitken). During the present study, 4.03% of *An. diluvialis*, 1.13% of

both *An. inundatus* and *An. maverlius*, and 0.64% of *An. smaragdinus* were found to have the alveoli of the pair of seta 2-C separated by less than the width of 1 alveolus. Abul-Hab (1963) stated that in *An. freeborni* 3–20% of the larvae, from reared IPBs, had the alveoli of the pair of seta 2-C separated by a distance greater than the width of 1 alveolus. These data indicate that some larval specimens of the *Quadrimalaculatus* Complex could be misidentified with existing keys if the features of seta 2-C being simple and separated by at least the width of 1 alveolus were used.

Other abnormalities in the females, male genitalia, pupae, and 4th-instar larvae occurred infrequently, usually appearing in only 1 or 2 specimens (e.g., females = scales present on following structures: prespiracular area, hypostigmal area, postspiracular area, prealar area, and upper and lower mesokatepisternal areas; male genitalia = claspette without setae 1, 4, or 5, or with an extra seta 1, 3, or 4, and tergum IX modified [see Reinert et al. 1997 for male genitalia terminology]; pupae = 2 seta 5-CT on 1 side, 2 seta 6-II on 1 side, 2 seta 9-III on 1 side, and sterna V-VIII with seta 13; and 4th-instar larvae = 3 seta 2-C evenly spaced, only 1 seta 2-C anteromesally, 2 seta 1-A situated side by side, and 2 seta 1-P on one side).

No gynandromorphs or intersexes were noted in the 3,482 females and 2,629 males examined of the 5 sibling species of the *Quadrimalaculatus* Complex. Gynandromorphs and intersexes are apparently very rare in *Anopheles* because the only published account of a gynandromorph in the genus is by Mason (1980) for *Anopheles gambiae* (as species A).

In individually reared adults with their associated immature pupal and 4th-instar larval exuviae, the occurrence of an anomaly in 1 stage did not correlate with any anomaly in the other stages.

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