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DIFFERENTIATING THE LARVAE OF ANOPHELES GEORGIANUS KING, A. BRADLEYI KING, AND A. PUNCTIPENNIS (SAY)

VIRGIL I. MILES*
 Fourth Service Command Medical Laboratory
 Fort McPherson, Georgia

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While the larvae of *Anopheles georgianus* King and *A. bradleyi* King are usually distinguishable on the presence or absence of functional palmate hairs on abdominal segments 3 and 7, these are sometimes missing or otherwise confusing and a separation of these species is dependent upon other differential characters. Those set forth by King (1939) are as follows:

| | <i>georgianus</i> | <i>bradleyi</i> |
|--|--|---|
| Inner clypeal hairs | closely approximated basally, the tubercles separated by less than the diameter of one of the basal tubercles. | usually closely approximated but sometimes separated by more than the diameter of one of the basal tubercles. |
| Posterior clypeal hairs | simple or forked at middle or apical third, occasionally 3-branched at tip. | usually simple, occasionally split on apical half. |
| Palmate hairs | well developed only on segments 4, 5 and 6, those on segments 3 and 7 slightly differentiated but apparently not functional. | developed on segments 3 to 7 inclusive, those on 3 and 7 somewhat smaller than the others and most of the leaflets with smooth margins. |
| Antepalmate hairs (hair 2) on segments 4 and 5 | with 2 to 6 branches (about 50 per cent with 3 branches, 24 per cent with 4 or more). | usually simple or double, very rarely triple. |
| Lateral hair on segment 4 | with 3 to 6 branches, usually 4 or 5, and usually with comparatively long basal stalk or irregular branching. | usually branched from near base, sometimes with secondary branching farther out. |
| Lateral hair on segment 5 | with 2 to 4 branches, usually arising from the same point near the base. | same as for segment 4. |

These characters are helpful in separating *georgianus* and *bradleyi* but due to the variations and overlapping of these differences there is a need for more consistent differential characters for separating them.

The larvae of *A. bradleyi* and *A. punctipennis* (Say) are often difficult to separate due to their close similarity. King, Bradley and McNeel (1944) give the following distinguishing characters:

| <i>bradleyi</i> | <i>punctipennis</i> |
|--|--|
| Leaflets of palmate hairs on segments 3 and 7 slender, usually somewhat smaller than those on segments 4 to 6 and mostly with smooth margins. Antepalmate hairs on segments 4 and 5 single or double; distance between clypeal hairs variable. | Palmate hairs on segment 3 with broad leaflets, usually notched or serrated on outer half and about equal in size to those on segments 4 to 6. Antepalmate hairs on segments 4 and 5 usually double, except in specimens from central Florida, in which they are usually single. |

*Captain, S.P.C., A.U.S., Entomologist. Fourth Service Command Medical Laboratory.

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For distinguishing *bradleyi* from *punctipennis*, Matheson (1944) states "the only differentiating characters are that the palmate tufts of abdominal segments 3 and 7 are smaller than the others" and, for distinguishing *georgianus*, "palmate tufts are well developed only on segments 4 to 6; antepalmate hairs (hair No. 2) have only 2 to 5 branches."

It is evident that additional differential characters are needed for distinguishing the larvae of these anophelines.

In the present study of differences and character variations in the three species, a series of mounted 4th instar larvae were examined consisting of:

(1) 55 *A. georgianus* from 19 localities in the Atlantic and Gulf coastal states from North Carolina to Louisiana inclusive, collected during the period March 1942 through March 1945 with dates representing each month of the year.

(2) 53 *A. bradleyi* from 10 localities in the coastal states from Virginia to Texas inclusive, excepting North Carolina and Louisiana, collected during the period February 1938 through February 1945 and representing all months except January, August and November.

(3) 58 *A. punctipennis* from 17 localities in 7 southeastern states and Washington, D. C., and representing the months May through November.

The following characters were found to be a useful supplement to the palmate hair character in separating *georgianus* and *bradleyi*:

| | <i>georgianus</i> | <i>bradleyi</i> |
|---|---|--|
| Antennal spicules | fine and slender with little thickening at base and not pigmented. | course and stout, thickened at base and darkened by pigmentation or sclerotization. |
| Head hair 9 or outer occipital hair | rarely reaching beyond bases of frontal row of hairs and little if any longer than inner occipital hairs. | usually reaching beyond bases of frontal hairs and distinctly longer than inner occipital hairs. |
| Hair 5 of abdominal segment I, or first hair dorsal to the lateral hair | 5 to 7 branches of variable length arising irregularly and with secondary branching outward from base. | 4 or 5 long branches arising from near the base, occasionally with the central branch divided or with a single branch arising outwardly. |
| Prothoracic hair 1 on the inner submedian prothoracic hair | usually with 3 to 5 branches arising variously along central portion. | simple (about 30 per cent) or weakly branched at the tip. |

These are listed in the order of their dependability as differential characters. The antennal spicules of all *georgianus* were definitely more delicate (Fig. 1,c) than were those of *bradleyi*. In all specimen of *bradleyi* examined the predominating spicules along the inner surface of the antennae were stout and darkened by sclerotization or by pigmentation (Fig. 2,c). The darkening was somewhat less pronounced in specimens from Myrtle Beach, S. C., and from Virginia Beach, Va., but the spicules were coarse as compared

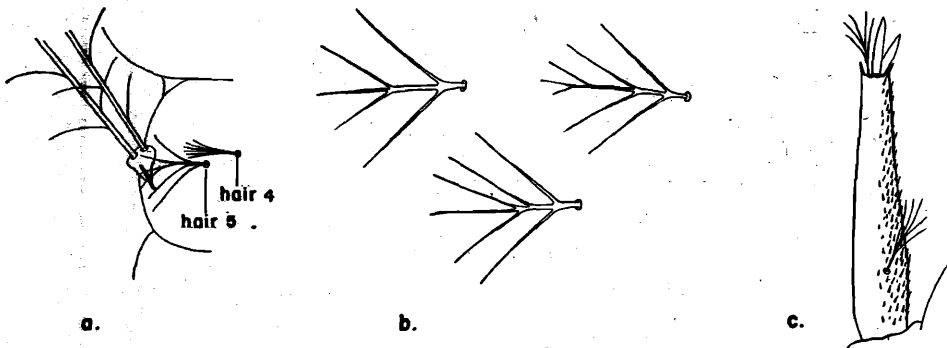


Fig. 1. *A. georgianus*

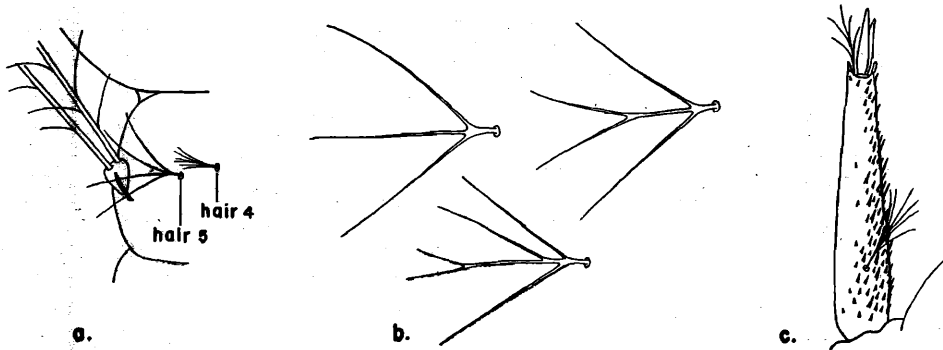


Fig. 2. *A. bradleyi*

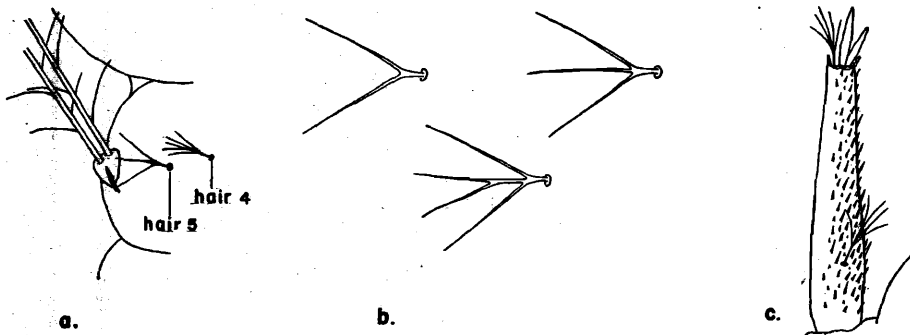


Fig. 3. *A. punctipennis*

Plate 1.

- (a) The left side of abdominal segment I showing typical branching of hair 5 and its size relative with that of hair 4.
- (b) Common variations in the branching of hair 5 of abdominal segment I (drawn to the same scale for the three species).
- (c) Characteristic texture of spinulation of the antennae.

with those typical of *georgianus*. With little study of the antennal spicules this character should become a useful diagnostic difference.

The outer occipital hairs reached beyond the bases of the frontal hairs in only one specimen of *georgianus* examined (from Camp Shelby, Mississippi). In five specimens of *bradleyi* these hairs fell slightly short of reaching the frontals but they were longer than those typical of *georgianus* in which species they usually fall far short of reaching the frontals.

The number of branches in hair 5 of abdominal segment I is less distinctive than the arrangement of the branches of this hair. It may be described as a tree-shaped hair not much longer than the branched hair No. 4 above it in *georgianus*, (Fig 1,a) and as a crow-foot shaped hair about twice as long as hair 4 in *bradleyi* (Fig. 2,a). The comparative length with that of hair 4 results both from an increased length of the branches of hair 5 in *bradleyi* and a reduced length of hair 4 in this species.

The inner submedian prothoracic hair, while less consistent than the other characters, in *georgianus* is more likely to be branched and to have more branches, and the branches may arise from the middle or below the middle. In no specimen of *bradleyi* examined did the branches, when present, involve as much as the outer half of this hair.

For distinguishing between larvae of *punctipennis* and *bradleyi* the following supplemental characters may be used:

| | <i>punctipennis</i> | <i>bradleyi</i> |
|--|---|---|
| Hair 5 of abdominal segment I | about equal in size to hair 4, and 3-branched, or may have 4 branches by a splitting of the central branch. | about twice as long as hair 4 and usually with 4 or 5 branches. |
| Prothoracic hair I | usually with 3 to 5 strong branches arising along central portion. | simple or weakly branched at tip. |
| Head hair 9 | usually with 6 to 10 branches and about equal in size to hair 8. | usually with 3 to 5 branches and longer than hair 8. |
| Prothoracic hair 1 or the inner submedian prothoracic hair | usually with 3 to 5 branches arising variously along central portion. | simple (about 30 per cent or weakly branched at the tip. |

The relative size of hair 5 as compared with that of hair 4 immediately above it on segment I is more characteristic than is the number of branches. The branches of hair 5 are of moderate length and not much longer than hair 4 in *punctipennis* (Fig 3,a) while in *bradleyi* their length is considerably increased (see discussion above and Fig. 2,a).

The inner submedian prothoracic hair is somewhat variable and may appear simple or branched only on its outer end in *punctipennis*, but strong side branches usually present along its center or basal half in this species distinguish it from *bradleyi*.

The outer occipital hair (head hair 9), while involving considerable variation in the number of its branches in both species, its relative size as compared with that of the inner occipital hair (head hair 8), together with the extent of branching, should make this a useful differential character. Furthermore, the failure of head hair 9 to reach beyond the bases of the frontals would distinguish the majority of *punctipennis* from *bradleyi*.

The character of the antennal spicules is less distinctive for *punctipennis* than for *georgianus* in that they are not as fine and delicate in *punctipennis*. In all *punctipennis* examined, however, the spicules were clear and unpigmented (Fig 3,c) as distinguished from the pigmented, coarse spicules present on the antennae of *bradleyi* (Fig. 2,c).

For separating the three species the following key may be found useful:

1. With 3 pairs of functional palmate hairs only (on segments 4 and 6); hair 5 of abdominal segment I usually with 5 to 7 branches of variable length arising irregularly and with secondary branching outward from the base (Fig 1,a and b) *georgianus*.

With 5 pairs of functional palmate hairs (on segments 3 to 7 inclusive); hair 5 of abdominal segment I usually with 3 to long branches arising from near the base (Figs. 2a & b and 3,a & b). 2.

2. Palmates on segments 3 and 7 usually smaller than those on segments 4, 5 and 6, and the leaflets mostly slender and without notches or serrations on the margins; hair 5 of abdominal segment I about twice as long as hair 4 and usually 4 or 5 branched (Fig. 2,a); head hair 9 usually 3 to 5 branched; prothoracic hair I simple or weakly branched at the tip *bradleyi*.

Palmates on segments 3 and 7 about equal in size to the others, and with broad leaflets notched or serrated on the margins; hair 5 of abdominal segment I not distinctly longer than hair 4 and usually 3 branched, (Fig. 3,a), frequently 4 branched, (Fig. 3,b); head hair 9 usually 6 to 10 branched; prothoracic hair I usually with 3 to 5 strong branches arising along the central portion *punctipennis*.

Variations in the character differences in the three species, calculated as percentages of the total number of each character present and visible in this series of larvae, are as follows:

| | <i>georgianus</i> | <i>bradleyi</i> | <i>punctipennis</i> |
|--|-------------------|-----------------|---------------------|
| Palmate hairs on segments III and VII: | | | |
| Well developed and considered functional | 0.0 | 100.0 | 100.0 |
| Poorly developed and apparently not functional | 100.0 | 0.0 | 0.0 |
| Antennal spicules: | | | |
| Coarse, spinelike, and pigmented | 0.0 | 100.0 | 0.0 |
| Fine, slender, and not pigmented | 100.0 | 0.0 | 100.0 |
| Head hair 9 or outer occipital hair: | | | |
| 2 - branched | 7.1 | 3.3 | 0.0 |
| 3 - branched | 25.9 | 31.5 | 1.0 |
| 4 - branched | 43.4 | 35.8 | 1.0 |
| 5 - branched | 16.4 | 21.7 | 12.5 |
| 6 - branched | 7.1 | 7.6 | 29.9 |
| 7 - branched | 0.0 | 0.0 | 30.8 |
| 8 - branched | 0.0 | 0.0 | 16.3 |
| 9 - branched | 0.0 | 0.0 | 6.7 |
| 10 - branched | 0.0 | 0.0 | 1.9 |
| Reaching beyond bases of frontal hairs | 2.3 | 87.0 | 18.6 |
| Not reaching bases of frontal hairs | 97.7 | 12.0 | 54.0 |
| Reaching about to bases of frontals but not beyond | 0.0 | 1.0 | 27.4 |
| Hair 5 of abdominal segment I: | | | |
| 2 - branched | 0.0 | 0.0 | 2.1 |
| 3 - branched | 0.0 | 8.0 | 70.0 |
| 4 - branched | 4.3 | 40.0 | 26.9 |
| 5 - branched | 37.2 | 46.0 | 1.0 |
| 6 - branched | 42.0 | 4.0 | 0.0 |
| 7 - branched | 14.3 | 2.0 | 0.0 |
| 8 - branched | 1.1 | 0.0 | 0.0 |
| 9 - branched | 1.1 | 0.0 | 0.0 |
| All branches arising from near base | 1.1 | 74.3 | 88.7 |
| One or more branches arising farther out | 98.9 | 25.7 | 11.3 |
| Prothoracic hair 1 or submedian prothoracic hair: | | | |
| Simple | 4.0 | 29.7 | 8.4 |
| 2 - branched | 5.0 | 34.0 | 13.1 |
| 3 - branched | 28.4 | 20.4 | 30.8 |
| 4 - branched | 29.3 | 13.6 | 21.5 |
| 5 - branched | 25.2 | 2.3 | 21.5 |
| 6 - branched | 8.1 | 0.0 | 4.7 |
| Of those branched, the branches arising from: | | | |
| The tip only | 5.3 | 51.6 | 4.2 |
| The apical fourth | 17.8 | 25.7 | 5.3 |
| The apical third | 42.1 | 22.7 | 19.0 |
| The apical half | 27.4 | 0.0 | 16.0 |
| Below the apical half | 7.4 | 0.0 | 55.5 |
| Tubercles of inner clypeal hairs separated by: | | | |
| Less than half the tubercle width or touching | 14.8 | 0.0 | 50.0 |
| Approximately half a tubercle width | 51.8 | 9.6 | 45.0 |
| Slightly less than a full tubercle width | 24.2 | 40.4 | 3.3 |
| Fully the width of one tubercle | 9.2 | 27.0 | 1.7 |
| Distinctly more than the width of one tubercle | 0.0 | 23.0 | 0.0 |
| Posterior clypeal hairs: | | | |
| Simple | 43.3 | 89.6 | 28.5 |
| 2 - branched | 52.2 | 9.2 | 50.9 |
| 3 - branched | 1.1 | 0.0 | 15.2 |
| 4 - branched | 3.3 | 1.2 | 5.2 |
| With branches arising from | | | |
| The apical half | 31.0 | 4.6 | 51.0 |
| The basal half | 26.0 | 5.8 | 20.5 |
| Antepalmate hair or hair No. 2: | | | |
| On abdominal segment IV: | | | |
| Simple | 4.0 | 49.0 | 9.1 |

| | | | |
|---|------|------|-------|
| 2 - branched | 9.0 | 33.6 | 65.4 |
| 3 - branched | 58.7 | 17.4 | 23.7 |
| 4 - branched | 14.2 | 0.0 | 1.8 |
| 5 - branched | 11.1 | 0.0 | 0.0 |
| 6 - branched | 2.0 | 0.0 | 0.0 |
| On abdominal segment V: | | | |
| Simple | 0.0 | 47.9 | 15.5 |
| 2 - branched | 2.2 | 34.1 | 60.0 |
| 3 - branched | 68.1 | 15.9 | 24.5 |
| 4 - branched | 19.8 | 2.1 | 0.0 |
| 5 - branched | 8.8 | 0.0 | 0.0 |
| 6 - branched | 1.1 | 0.0 | 0.0 |
| Lateral hairs on abdominal segment IV: | | | |
| Branches arising from the same point | 42.0 | 86.0 | 100.0 |
| With irregular branching | 58.0 | 14.0 | 0.0 |
| Lateral hairs on abdominal segment V: | | | |
| Branches arising from the same point | 77.0 | 94.5 | 100.0 |
| With irregular branching | 23.0 | 5.5 | 0.0 |
| Leaflets of palmate hairs on segment III: | | | |
| Mostly with notched or serrated margins | | 31.1 | 100.0 |
| Mostly with smooth margins | | 68.9 | 0.0 |
| Leaflets of palmates on segment VII: | | | |
| Mostly with notched or serrated margins | | 7.9 | 100.0 |
| Mostly with smooth margins | | 92.1 | 0.0 |

This tabulation shows agreement with the difference as noted by King and others with the following exceptions:

- (1) The branching of the posterior clypeal hairs may involve more than the apical half of the hair in both *georgianus* and *bradleyi*. It is also noted that this hair may rarely have as many as four branches in both these species as well as in *punctipennis*.
- (2) The antepalmate hairs on segments 4 and 5 may be simple or two-branched in *georgianus* and they are frequently triple and may rarely be four-branched in both *bradleyi* and *punctipennis*.
- (3) The leaflets of the palmates on segment 3 of *bradleyi* are often notched or serrated on the margins, causing confusion of this species with *punctipennis*.

As a characteristic of *georgianus* it is worthy of note that in this species there is a tendency toward more branching of all the hairs studied, both in number of branches and in the extent of sub-branching of branches outwardly.

No distinguishing differences were found in the antennal hairs nor in the tergal plates. The pecten and respiratory apparatus of the eighth abdominal segment were not studied in this series.

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