Separation of Variable *Culex territans* Specimens from other *Culex (Neoculex)* in North America

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ABSTRACT. The siphon indices, branching of setae 5-C and 6-C, lengths of 5-C compared to the lengths of 6-C, length of the most proximal seta 1-S compared to the siphon length, and the occurrence of a paler abdominal segment IV were compared among *Culex apicalis, Cx. boharti* and *Cx. territans* larvae. These characters in *Cx. territans* were found to be much more variable than previously recognized, and often overlapping completely with these characters in *Cx. apicalis* and *Cx. boharti*. The use of male genitalia characters on reared adults from larval collections is the best means of separating these species, particularly where they are sympatric or in close proximity.

INTRODUCTION AND BACKGROUND

This is a report of a study of certain morphological variations found in *Culex (Neoculex) territans* Walker, and of characters used to separate this species from several other species with which it can be confused: *Cx. apicalis* Adams, *Cx. boharti* Brookman and Reeves, and *Cx. reevesi* Wirth. The study of natural variations occurring in *Culex territans* is important as this species is the type by original designation (Dyar 1905) for subgenus *Neoculex* Dyar. This subgenus was reclassified by Sirivanakarn (1971) and reduced from approximately 70 to 25 species (6 North and Central America, 13 Australasian, 5 European-Mediterranean and 1 Japan-Korea-USSR). Since then at least one new species has been described, from Venezuela. Also, *Cx. territans* is the only species in *Neoculex* with an exceptionally wide distribution, i.e., Nearctic and Palearctic. The type of *Cx. territans* is non-extant (Belkin 1968, Knight and Stone 1977) and the type locality is listed as "United States" in Knight and Stone (1977). However, Belkin et al. (1966: 3, 19) restricted the type locality to the vicinity of Charleston (Charleston County), South Carolina.

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This study was initiated because of *Cx. territans* larvae submitted to the Walter Reed Biosystematics Unit (WRBU), or previously seen by the authors, which exhibited *apicalis*-like or *boharti/reevesi*-like characters.

Two types of larval variations were observed in *Cx. territans* that seem to cause the majority of identification problems: (1) larvae with an exceptionally long siphon more like that classically attributed to *Cx. apicalis*; and (2) larvae with abdominal segment IV paler than segments III and V (banded larvae) that would key to *Cx. boharti* or *Cx. reevesi* in Bohart and Washino (1978) and *Cx. boharti* in Darsie and Ward (1981). Variation in the siphon index of *Cx. territans* has not been defined previously, but has been listed as about 7 (Carpenter et al. 1946), 6 to 7 (Bohart 1948), usually 6-7 (Mattingly 1953), 6 to 7 (Carpenter and LaCasse 1955), about 6 (King et al. 1960), less than 7 (Chapman 1966), about 6 1/2 (Gjullin and Eddy 1972), not given (Bohart and Washino 1978), 5 to 6 (Wood et al. 1979) and usually less than 7 (Darsie and Ward 1981).

MATERIALS AND METHODS

We assume that previous authors were all using the siphon index in the classical sense, i.e., the length of the siphon (minus the siphon acus at the base and the spiracular apparatus at the apex) divided by the width at the base, not that used by Belkin (1962) and Harbach and Knight (1980). In this study we used the classical index described above and as used by Harbach (1988).

Specimens examined include larvae and adults identified by the junior author from the mid-Atlantic and southern states over the last 24 years, and larvae and adults currently deposited in the mosquito collection of the National Museum of Natural History, Smithsonian Institution, Museum Support Center, Suitland, Maryland. Over 370 larvae, 200 adults and 150 male genitalia in this collection were examined from 25 states.

RESULTS AND DISCUSSION

Table 1 clearly shows that the siphon indices for *Cx. territans* listed by the above authors are gross underestimates. Based on our measurements of the siphons of 221 *Cx. territans* from 23 states (Table 2), *Cx. territans* not only has a wider range in its index than *Cx. apicalis*, but also can have a higher index. The mean index for *Cx. territans*, however, is lower than that of *Cx. apicalis*. Based on our measurements of *Cx. territans* throughout its distribution, we conclude that the siphon index is of no value in separating *Cx. territans* from *Cx. apicalis*, except possibly in areas where they are sympatric, or nearly so (Arizona, California, Nevada, Oregon and Texas) (see Darsie and Ward 1981). Chapman (1966) very astutely reported, "While larvae of *apicalis* and *territans* are easily separated by eye in the West, the characters used in keys to differentiate these species, i.e., the length and shape of the siphon and relative length of siphonal hairs to length of siphon, are not tenable on a nationwide basis. The writer has collected *territans* larvae from Florida that possess the above mentioned characters supposedly indigenous to *apicalis*." A comparison of the siphon indices of these two species is needed from areas of sympatry. Only four specimens of *Cx. territans* were available from such areas in this study (all from California) and they all exhibit a low siphon index (Table 2), lower than most *Cx. apicalis* (Table 1).

Other characters that have been used to differentiate larvae of *Cx. apicalis* from *Cx. territans* are: (1) branching of setae 5-C and 6-C; (2) length of 5-C in relation to 6-C; and (3) length of the most proximal seta 1-S in relation to length of siphon. Our study of the branching of 5-C and 6-C in *Cx. territans* revealed 5-C highly variable. Of 217 *Cx. territans* larvae examined, 132 (61%) had 5-C single, 56 (26%) with 5-C double, 1 (0.5%) with 5-C triple; and 28 (13%) were mixed (left and right not the same). Seta 6-C was single in 186 (86%), double in 18 (8%) and the remaining 13 (6%) were mixed. Only 20 *Cx. apicalis* larvae were available for examination and 5-C was double in 5 (25%), mixed double and triple in 9 (45%), triple in 5 (25%) and 4-branched in 1 (5%). Seta 6-C on *Cx. apicalis* was single on one side on only 1 larvae (5%) and bifid on the remaining 19 (95%). The furcation of 6-C in *Cx. apicalis* in the Smithsonian collection does not occur at the immediate base of the seta, but a short distance from the base, as noted by Bohart and Washino (1978).

The length of 5-C relative to that of 6-C may also be of value. Typically 5-C in *Cx. apicalis* is about 0.50 the length of 6-C while 5-C in *Cx. territans* is normally 0.67 the length of 6-C. Several *Cx. territans* were found with 5-C only 0.50-0.60 the length of 6-C while several were 0.70-0.80 the length of 6-C.

The length of the most proximal seta 1-S relative to the length of the siphon was used by Bohart and Washino (1978) and Darsie and Ward (1981) to separate *Cx. apicalis* and *Cx. territans* larvae. Actually, the lengths of 1-S in *Cx. territans* are variable in the eastern U.S., with a few specimens having short 1-S like those in *Cx. apicalis*. However, nearly all of the western U.S. (including Alaskan specimens) of *Cx. territans* had the most proximal seta 1-S long and distinctly different from those of *Cx. apicalis*.

The other major variation causing infrequent identification problems with *Cx. territans* concerns larvae with abdominal segment IV pale in comparison with segments III and V, giving a banded appearance. The pale abdominal segment IV is a primary character of *Cx. boharti* and *Cx. reevesi*. Chapman (1966) apparently was the first to publish a record of this variation, noting *Cx. territans* larvae from Georgia and Michigan with segment IV pale. Gjullin and Eddy (1972) also detected this variation in *Cx. territans* larvae from the northwestern states and included it in a key couplet. In *Cx. territans*, this variation is more common than the long siphon variation, and can be very common in local populations to the extent that an entire collection of larvae may be banded. Confirmation that the banded larvae studied here are indeed *Cx. territans* was made using male genitalia from adults reared from pure collections of banded larvae from North Carolina and Pennsylvania. In all cases the aedeagus and dorsal aedeagal bridge were typical of *Cx. territans*. There are larvae of *Cx. territans* in the Smithsonian collection from Arkansas, Kansas, New York, North Carolina, Pennsylvania and Virginia with abdominal segment IV paler than III and V. In addition, the junior author has seen specimens with this character from Georgia. Thus, this variation occurs widely in the distribution of *Cx. territans*, and should be expected infrequently in specimens occurring in the western states (as pointed out by Gjullin and Eddy 1972). Since the length of 5-C in relation to that of 6-C in *Cx. boharti*, *Cx. reevesi* and *Cx.*

territans widely overlaps and the number of branches on setae 5-C broadly overlap between *Cx. boharti* and *Cx. territans (Cx. boharti* 85% with 2-3 branches, *Cx. territans* 36% with 2-3 branches), and because 6-C for these two species is usually single, we suggest that banded larvae collected in the western and northwestern states should be identified by association with adults, which exhibit better characters for separating these species. Thus, the spotty distribution of *Cx. boharti* based on larval collections in Nevada (Richards et al. 1956, Chapman 1966), as well as in Idaho, Oregon and Washington (Linam and Nielsen 1970), should be confirmed based on adult characters.

The basis for the paler pigmentation of abdominal segment IV in larvae of *Cx. boharti, Cx. reevesi* and *Cx. territans* has not been studied. Benedict and Seawright (1987) have demonstrated that some larval color changes may be due to homochromy, i.e., a response to the color of the environment. However, Bohart and Washino (1957) found that the second and third instars of *Cx. boharti* and *Cx. reevesi* possess the banding pattern found in the fourth instar, which lends credence to a fixed character in these two species. Michener (1945) found that the siphon length of *Cx. territans* in Mississippi was associated with different seasons, i.e., the "summer form" of *Cx. territans* (as *Cx. apicalis*) had a longer siphon while the "winter form" had a shorter, more robust siphon. Regardless of the basis for this banding, transverse banding (cf. longitudinal stripes) is not uncommon in mosquito larvae. Figure 1 depicts various banding patterns published and/or illustrated for certain other species besides *Cx. boharti, Cx. reevesi* and *Cx. territans: Aedes bimaculatus* (Coquillett) and *Ae. tormentor* Dyar and Knab (Breland 1948), *Anopheles aberrans* Harrison and Scanlon and *An. palmatus* (Rodenwaldt) (Harrison and Scanlon 1975), an undescribed *Bironella* species (Tenorio 1977) and *Culex antennatus* (Becker), *Cx. poicilipes* (Theobald) and *Cx. sinaiticus* Kirkpatrick (Harbach 1988). Several members of the Lindesayi Complex of *Anopheles*, not figured here, also have banded larvae. Ross (1943) and Breland (1948) reported that *Ae. bimaculatus* larvae have abdominal segments VI and VII darkly pigmented, however, the two larvae in the Smithsonian collection (Brownsville, Texas, March 2 and 21, 1945, C. R. Joyce) have segments V and VI dark. The *Ae. bimaculatus* in Figure 1 represents these two specimens. These patterns may be constant in certain species (*Cx. boharti* and *Cx. reevesi*), however in *An. aberrans* they are not. In Thailand, of 173 *An. aberrans* larvae examined 110 (54%) had bands as depicted in Figure 1 and the remainder were unicolorous, while 5 larvae of *An. palmatus* (all that were available) were banded (Harrison and Scanlon 1975). Adults of both sexes of these two species also exhibit abdominal banding on the same segments as the larvae. Killed pupae were not available to determine if this color pattern persisted in that stage.

The male genitalia possess the best structures for separating the five species of *Neoculex* in North America (north of Mexico). Carpenter and LaCasse (1955) described and illustrated these differences quite clearly: (1) *Cx. apicalis* recognized by the absence of a sclerotized dorsal aedeagal bridge (it is membranous and very faint) between the aedeagal sclerites ; (2) *Cx. arizonenis* with long setae on the gonocoxite immediately proximal to the subapical lobe; (3) *Cx. boharti* with apices of the aedeagal sclerites strongly narrowed and sclerotized, and dorsal aedeagal bridge thick (broad); (4) *Cx. reevesi* with apices of the aedeagal sclerites rounded, without knobs, denticles or teeth; and (5) *Cx. territans* with apices of the aedeagal sclerites broadly rounded, with knobs, and dorsal aedeagal bridge narrow. In this study we examined 56 male genitalia of *Cx. territans* from 20 states and one Canadian province and found very little variation. Invariably, these could be identified as *Cx. territans* using Bohart (1948) and Carpenter and LaCasse (1955). Bohart (1948) reported male palpomere 3 of *Cx. territans* with many more long lateral setae at the apex than those of *Cx. apicalis* and *Cx. boharti* (as *Cx. reevesi*). In the adult female, Bohart and Washino (1978) reported the maxillary palpus of *Cx. apicalis* to be about twice the length of antennal flagellomere 2, while the maxillary palpi of *Cx. boharti* and *Cx. territans* were only slightly longer than that flagellomere. Bohart (1948) and Darsie and Ward (1981), however, compared the palpus lengths to the length of flagellomere 4. In addition, Carpenter and LaCasse (1955) and Darsie and Ward (1981) stressed the presence of pale scales on the maxillary palpus of female *Cx. apicalis*, while the maxillary palpi of the other species are entirely dark. Carpenter and LaCasse (1955) also noted that the hindfemur of *Cx. apicalis* does not have a complete posterior pale stripe, while *Cx. territans* has this pale stripe along the entire length of the hindfemur. Various authors have reported the dorsoapical pale bands on the abdominal terga of *Cx. apicalis* and *Cx. territans* to be wider than those of *Cx. boharti*. However, these bands are highly variable in *Cx. territans*, and specimens were seen with the terga entirely black from dorsal view as described for *Cx. reevesi* and as noted in the "summer form" by Michener (1945). The pale scales on the vertex and tergal pale scales of *Cx. apicalis* and *Cx. territans* are white, while those of *Cx. boharti* are yellow. Darsie and Ward (1981) also used the length of wing cell R_2 compared to the length of vein R_{2+3} to separate *Cx. boharti* from *Cx. territans*. Although *Cx. boharti* typically has a shorter cell R_2 than *Cx. territans* and can be separated from most *Cx. territans* by this character, a few *Cx. territans*, particularly from the eastern states, have cell R_2 similar to that of *Cx. boharti*.

Our study demonstrates the excessive variation that occurs in the siphon shape and index, the branching of setae 5-C and 6-C and their lengths, length of siphonal seta 1-S and pigmentation in larvae of North American *Cx. territans*. These variations can cause considerable confusion in separating the larvae of *Cx. apicalis*, *Cx. boharti* and *Cx. territans*, particularly in the western states. Also, *Cx. territans*, *Cx. boharti* and/or *Cx. apicalis* are sympatric in several areas of western North America (Darsie and Ward 1981). For these reasons, it is obvious that records based exclusively on larval collections in the western states should be augmented by collections of larvae and reared adults and these records should be confirmed based on adult characters. This study suggests that *Cx. territans* is more closely related to *Cx. boharti* than to *Cx. apicalis*.

ACKNOWLEDGMENTS

We are very grateful to William Irby and Charles Apperson, Department of Entomology, N.C. State University for providing a long series of banded larvae with reared adults, Ralph Harbach and E.L. Peyton, WRBU, Department of Entomology, Walter Reed Army Institute of Research for critically reviewing the manuscript and James Pecor, WRBU, for preparing the manuscript for photo-offset reproduction.

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Table 1. Siphon indices for *Culex apicalis*, *Cx. arizonensis*, *Cx. boharti* and *Cx. territans**.

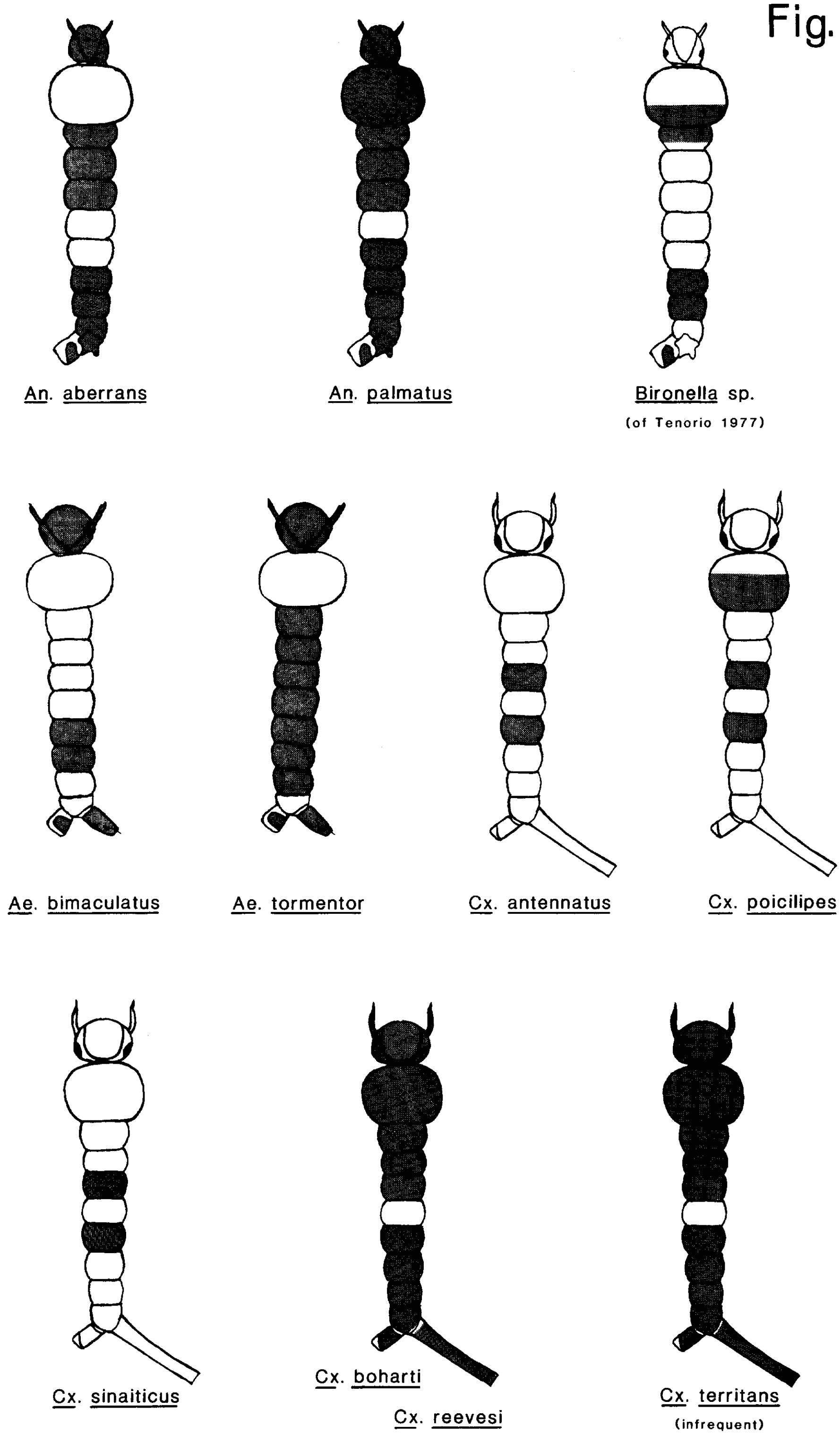
Species	Specimens examined (n)	Siphon index Range	Mean
apicalis	20	5.69 - 9.52	7.57
arizonensis	4	7.18 - 8.10	7.64
boharti	14	5.00 - 6.60	5.63
territans	214	4.67 - 9.90	7.06

*There are no *Cx. reevesi* larvae in the Smithsonian collection.

Table 2. Siphon indices for *Cx. territans* from 23 states.

State	Specimens examined (n)	Range	Siphon index Mean	Mean > 7
Alaska	20	5.00 - 7.50	6.12	
Alabama	2	5.33 - 6.30	5.82	
Arkansas	11	6.10 - 8.56	7.28	+
California	4	5.20 - 6.27	5.94	
Florida	6	5.67 - 6.62	6.15	
Georgia	20	4.67 - 9.50	7.12	+
Illinois	1		5.14	
Kansas	1		7.40	+
Louisiana	21	4.89 - 8.21	6.37	
Maryland	22	6.08 - 9.90	7.68	+
Massachusetts	17	5.75 - 9.33	7.39	+
Minnesota	1		6.72	
Missouri	4	6.60 - 8.50	7.58	+
Nebraska	1		7.36	+
New Hampshire	10	5.85 - 8.00	7.18	+
New Jersey	3	5.58 - 6.36	5.95	
New York	5	5.62 - 7.80	6.19	
North Carolina	25	5.92 - 9.40	8.08	+
Pennsylvania	22	6.70 - 8.60	7.39	+
South Carolina	4	5.14 - 6.67	6.10	
Utah	1		7.82	+
Vermont	9	6.18 - 7.20	6.77	
Virginia	4	7.30 - 7.80	7.46	+

Fig.1


An. aberrans
An. palmatus
Bironella sp.
(of Tenorio 1977)
Ae. bimaculatus
Ae. tormentor
Cx. antennatus
Cx. poicilipes
Cx. sinaiticus
Cx. boharti
Cx. reevesi
Cx. territans
(infrequent)