STUDIES OF THE GENUS *CULEX* IN FLORIDA II. REDESCRIPTION OF THE FOURTH INSTAR OF *CULEX NIGRIPALPUS*

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ABSTRACT. The fourth instar of *Culex nigripalpus* is described in detail and completely illustrated for the 1st time as compared with previous descriptions of the larva of *Cx. nigripalpus*. Certain important features are shown in the figure.

KEY WORDS Culex nigripalpus, 4th instar, larva, description

INTRODUCTION

Of the 15 species of *Culex* that occur in Florida (Darsie and Morris 2003), Culex (Culex) nigripalpus Theobald stands out as the most important because it is a vector of human disease, as well as a major pest species (Darsie and Day 2002). Cx. nigripalpus is the principal vector of Florida's most serious mosquito-borne disease, St. Louis encephalitis, and also is an important vector of West Nile virus (Day and Curtis 1999, Day and Stark 1999, Rutledge et al. 2003). Because the larva is 1 of the main stages in the mosquito life cycle and 1 of 2 stages prominent in control programs, the more that is known of the larva's morphology the more accurately it can be identified. Such a detailed description of the morphology and a portrait of the whole larva (Fig. 1) of Cx. nigripalpus is presented here for the 1st time.

The 4th instar of Cx. nigripalpus has been variously described by Belkin et al. (1970), Bonne and Bonne-Wepster (1925), Bram (1967), Carpenter and LaCasse (1955), Carpenter et al. (1946), Dyar (1928), Howard et al. (1915, as Cx. caraibeus Howard, Dyar, and Knab), King et al. (1960), Lane (1953), Matheson (1944), Nayar (1982), and Yamaguti and LaCasse (1957). None have described the larva as detailed as below. In addition, Breeland and Loyless (1983, 1989), Breland (1953), Clark-Gil and Darsie (1983), Cova Garcia et al. (1966), Dyar and Knab (1906, as Cx. microsquamosus, Cx. mortificator Dyar and Knab, Cx. carmodyae Dyar and Knab, Cx. factor Dyar and Knab, and Cx. regulator, all synonyms of Cx. nigripalpus; see Knight and Stone [1977]), Forattini (1965), Georgia Department of Public Health (1970, 1971), King et al. (1944), Slaff and Apperson (1989), Stojanovich (1960) and the U.S. Department of Health, Education, and Welfare (1969) included the larva in identification keys, whereas Dodge (1945) partially described the larva as a means of separating it from other Nearctic Culex species. Bram (1967) stated that Culex azuayus Levi Castillo is a synonym of Cx. nigripalpus but the larval description by Levi Castillo (1954) definitely is not Cx. nigripalpus. In fact, we doubt that it belongs in the genus Culex.

MATERIALS AND METHODS

The specimens of Cx. nigripalpus used in this study were derived from progeny rearings. Females were collected by hand aspirator at Lockwood Hammock, Vero Beach, FL (Day and Curtis 1999) on March 29, 2002, given a blood meal, and isolated individually in 70 \times 25-mm vials containing 1 cm of water. After oviposition, the females were mounted on card points and eggs were transferred to rearing pans with a small amount of larval food consisting of 1:1 liver powder and lactoalbumin. Larvae were reared to the 4th instar and 25 were mounted in Canada balsam and 25 were individually reared to adults. Larval and pupal exuviae of the adults were mounted in balsam and the adults were placed on card points on pins. All specimens resulting from this rearing are deposited in the collection of the Florida Medical Entomology Laboratory. The characters included in Figs. 2-5 were selected because of their importance in identification.

DESCRIPTION OF THE FOURTH INSTAR

The range and modal number of branches of the setae are listed in Table 1. The position and size of the setae are given in Fig. 1. The mouthparts are not described here (see Yamaguti and LaCasse 1957). The morphological terminology follows that of Harbach and Knight (1980). The abbreviation "br" means branches. All measurements are ranges followed by means.

Head. Length 0.83–0.98 (\bar{x} 0.92 mm), width 1.42–1.63 (\bar{x} 1.51 mm); dorsomentum with 8 or 9 teeth on either side of apical tooth; hypostomal suture extending to posterior tentorial pit and onto occipital foramen; seta 1-C rather stout, attenuate apically; 3-C difficult to see, small, single; 4-C short, single; 5,6-C large, stout, with 3 or 4, rarely 5 br (see Fig. 2); 7-C long, stout, usually with 7,8 br; 8,9-C small, with 3–6 br; 10,11- and 13-C moderately long, thin, 10,11-C single to triple, 13-C with 3–5 br; 12, 14-C small, with 2–5, seldom 6 br; 15-C short, with 4–7 br.

Antenna. Length 0.55-0.70 (\bar{x} 0.63 mm), with notch at insertion with head, apical third constricted, darkly pigmented, basal two-thirds spiculate,



Fig. 1. Larva of *Culex nigripalpus*; (left) dorsal, (right) ventral. A, antenna; C, head; CS, comb scale; M, mesothorax; P, prothorax; PS, pecten spine; S, siphon; T, metathorax; I-VIII, X, abdominal segments.

		Thorax			Abdominal segments		
Seta no.	Head C	Р	М	Т	Ι	II	III
0		11-24 (16)				1	1
1	1	1	1-4 (2)	1-3 (2)	2-6 (3)	1, 2 (1)	2, 3 (3)
2		1	1-4(4)	1, 2 (2)	1, 2 (1)	1–3 (1)	1
3	1	1	1	2-6(4)	1, 2 (1)	1-2 (1)	1, 2 (1)
4	1	2	2, 3 (2)	4-7 (4)	7-15 (10)	4-8 (4)	2-4 (3)
5	3-5 (4)	1	1	1, 2 (1)	4-9 (6)	2, 3 (3)	2-4 (2)
6	3, 4 (3)	1	1	1	3-5 (3)	2-5 (3)	2, 3 (2)
7	5-11(8)	3, 4 (3)	1	5-8 (7)	1-3(2)	3-10 (6)	4-9 (6)
8	3-5 (4)	2-4(2)	4-9 (6)	6-14 (10)	_	1	1
9	3-6 (3)	1	3-8 (6)	4-8 (6)	1-4(2)	1	1
10	1-3(1)	1	1	1	1	1	1, 2(1)
11	1-3(2)	2-6(6)	1, 2 (2)	1-3(2)	2-8 (6)	1-3 (2)	2
12	36 (4)	1	1	1	1-3(1)	1-3(2)	1-3(2)
13	3-5 (4)	_	13-29 (?)	5-7 (6)	1, 2 (2)	10-25(10)	2-4(3)
14	2-5 (4)	1	13-24 (13)			_	1
15	4-7 (6)	_		_	_	_	

Table 1. Chaetotaxy of 4th instars of Culex nigripalpus. Values are ranges followed by the mode in parentheses."

¹ A, antenna; C, head; M, mesothorax; P, prothorax; S, siphon; T, metathorax; I-X, abdominal segments.

apical third sparsely spiculate, seta 1-A large, aciculate, with 12–28 br; 2,3-A moderately long, single; 4, 6-A short, single.

Thorax. Integument distinctly aculeate. Prothorax: Seta 0-P short with 11–24 br; 1-7-P very long, stout, 1-3-P and 5,6-P single, 4-P double and 7-P triple or rarely quadruple; 8,12-P very long, stout, 8-P double or triple, rarely quadruple; 12-P single; 9-10-P moderately long, single; 11,14-P very short, 11-P with 2-6 br, 14-P single. Mesothorax: Setae 1-2-M short, subequal in length, single to quadruple (see Fig. 3); 3,4-M moderately long, single or double, rarely triple; 5-7,10,12-M very long, stout, single; 8,9-M very long, stout, aciculate, usually 6branched (3–9); 11-M small, double, seldom single; 13-M short, with 13-29 br. Metathorax: Setae 1,4,5-T short, 1,5-T single to triple, 4-T with 4-7br; 2,3-T long, usually with 2-4 br, sometimes single; 7,9-T very long stout, usually 6- or 7-branched; 8-T short, with 6-14 br; 11-T short, single to triple; 13-T medium long, usually with 6 br.

Abdomen. Integument of segment VIII aculeate. Seta 0-II-VIII minute, single; seta 1-I,II short, usually double or triple; 1-III-VII long to very long,

1-III-V mostly triple, occasionally double or quadruple, 1-VI,VII single or double; seta 2-I-VII short, 2-I,II single or double, 2-III-VII single; seta 3-I-VII long, 3-I,II single or double, 3-III-VII single; seta 4-I-VII short, 4-I usually with 9-12 br, 4-II mostly quadruple, 4-IV,VII single or double, 4-V usually 4- to 6-branched, 4-VI with 2-4 br; setae 5-I-VII short, 5-I mostly with 4-8 br, 5-III-VI double or triple, seldom quadruple, 5-VII usually 5- or 6-branched; seta 6-I-VI long, stout, sometimes aciculate, 6-I,II usually triple, 6-III-VI double, seldom single or triple, 6-VII short, with 12-25 br; seta 7-I very long, aciculate, double, rarely single or triple, 7-II,VI,VII moderately long, 7-II mostly with 4-6 br, 7-VI,VII single, 7-III-V short, usually with 5-8 br; seta 8-II,III moderately long, single or double, 8-IV-VII short, 8-IV,V single or double, 8-VI,VII usually with 3-5 br; seta 9-I-VII short, 9-I,VII usually with 2-4 br, 9-II-VI single; seta 10-I-VII long, single; seta 11-I-VII short, 11-I usually with 3-6 br, 11-II-VII single to triple; seta 12-I,II and V-VII long to very long, 12-III,IV short, 12-I,II single to triple, 12-V-VII single, 12 III-IV short, single or double, rarely triple; seta 13-II,VI



Fig. 2. Branching of setae 5,6-C in larvae of Culex nigripalpus (n = 407).



Fig. 3. Length of setae 1-M and 2-M in larvae of Culex nigripalpus (n = 82).

Table 1. Extended.

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IV	V	VI	VII	VIII	X	Antenna and siphon
1	1	1	1	1	_	
2-4(3)	2-4(3)	2	1-3 (2)	4-9 (8)	1	1A 18–37 (?)
1	1	1	1	1	2-5 (2)	2A 1
1	1	1	1	6-10 (7)	1, 2 (2)	3A 1
$\hat{1} 2 (1)$	4-7(5)	2-4(3)	1	1	5-12 (?)	4A 1
2 3 (2)	1-4(2)	2-4(3)	4-6(5)	2-5 (4)	<u> </u>	5A 1
1, 2, (2)	2	2	12-25(14)		_	6A 1
3-9 (6)	5-10 (6)	1, 2(1)	1		4f 8-10 (9)	1a–S 1, 2 (1)
1, 2(2)	1-3(2)	2-4(3)	2-6(5)		4e 9~12 (9)	1b–S 1, 2 (2)
1	1	1	1-4 (4)		4d 9–11 (9)	1c-S 1-3 (2)
1	ĩ	1	1		4c 9-11 (9)	1d-S 1-3 (2)
$\hat{2}, 3(2)$	1-3 (3)	2, 3 (2)	1-4(1)		4b 8-10 (9)	6–S 1
1-3 (1)	1	1	1		4a 7–11 (9)	7–S 1
2, 3(3)	2-4(3)	13-31 (26)	3, 4 (3)			8S 1
1	1	1	1	1	_	9-S 1
—	—	—	_	_		

short, multibranched with 10-31 br, 13-III-V long, mostly triple, 13-VII moderately long, usually with 3,4 br; seta 14-III-VIII minute, single. Segment VIII: Aciculate, comb scales evenly fringed with short spicules on sides and apex, with 28-46 (\bar{x} 37.8) arranged in triangular patch; setae 1-5-VIII long, rather stout, 1,3,5-VIII aciculate, 1,3-VIII usually with 6-8 br, 5-VIII usually 4-branched, 2,4-VIII single. Siphon: Index 5.54-7.0 (x 6.2), subcylindrical, light brown, dark tracheoid area at base, prominent acus attached to base; pecten on basal 0.25 or less of siphon, with 8–15 (\bar{x} 11.6) (see Fig. 4), with 2 or 6 denticles on one side, of the 93 of each siphon seta counted, 1a-S very long with 89.5% single, 10.4% double, 1b-S long, with 80.4% single, 19.5% double, 1c-S moderately long, with 34.7% single, 65.2% double, 1d-S moderately long, with 22.7% single, 75% double, and 2.2% triple (see Fig. 5); 2-S short, about 0.22-0.26 length of apical siphon diameter, 6,7-S on anterior spiracular lobe short, single; 8,9-S on posterolateral spiracular lobe short, single. Segment X: Saddle complete, light brown, posterior margin with small spicules, length 0.35-0.48 (x 0.41 mm), siphon/saddle index



Fig. 4. Number of pecten spines on the siphon of larvae of *Culex nigripalpus* (n = 101).

4.18–5.70 (\bar{x} 4.69), no saddle acus. Seta 1-X long, thin, single, 0.60–0.89 (\bar{x} 0.71) length of dorsal length of saddle, 2,3-X very long, stout, 2-X double or triple, rarely single, usually with 1 br very long and 1,2 br shorter; 3-X single, seldom double; 4-X with 11–13 setae, all borne on grid, mean length in mm of the 6 pairs of setae as follows: 4f-X = 0.030, 4e-X = 0.066, 4d-X = 0.091, 4c-X = 0.080, 4b-X = 0.091, 4a-X = 0.099, usually with 9–12 br each; anal papillae light gray, elongate, tapered apically, dorsal pair 0.53–0.69 (\bar{x} 0.62 mm), ventral pair 0.41–0.63 (\bar{x} 0.52 mm), saddle/dorsal anal papilla index 0.53–1.21 (\bar{x} 0.73).

Of those who previously partially described the larva of *Cx. nigripalpus*, most are in general agreement with our findings. However, Belkin et al. (1970) recorded a siphon index "usually more than 8.0," but our measurements resulted in an index of less than 7.1, with a mean of 6.2. Carpenter and LaCasse (1955) reported seta 1a-S as usually double and as long or longer than the basal siphon diameter. In this study, only 14 (15%) of 93 of those counted were double and only 2 were shorter than the basal diameter.



Fig. 5. Number of branches of siphon setae on larvae of *Culex nigripalpus* (n = 382).

The larva of *Cx. nigripalpus* can be distinguished from that of all other Nearctic *Culex* by the following combination of characters: seta 6-C with 3,4 branches; thoracic integument aciculate; seta 1,2-M subequal in length; and siphon with index of 5.5-7.0, pecten confined to basal 0.33, with 4 pairs of setae placed lineally, with penultimate seta dorsally out of line, and without aciculae or apical spines (Darsie and Ward 1981).

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