Original Research Paper

Distribution of sensory sensilla in the labral food canal and cibarium of *Chrysops exitans* (Diptera: Tabanidae)

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Abstract: Sensilla, beginning at the distal-most tip of the labrum and extending proximally through the cibarium to the stomodaeum, were examined in females of *Chrysops exitans* Walker. Totals of 328 setiform sensilla (range, 14 to 21; mean = 18.2; ±1 SD = 1.67), and 36 basiconic sensilla (mean = 2.0; ±1 SD = 0) were observed in the food canal of n = 18 sample individuals. Both types of sensilla were aggregated distally in the canal. Additionally, a group of five to 10 sensilla was observed in each lateral wall of the epipharynx, with a single median group, consisting of both setiform and basiconic sensilla, positioned immediately distal to the mouth opening into the cibarium. Two pairs of basiconic sensilla were consistently observed in the stomodaeum of every fly.

Keywords: *Chrysops exitans*, cibarium, food canal, sensilla

Introduction

Tabanid flies (e.g., deer flies, horse flies) have received relatively little attention when compared with other blood-feeding Diptera (Baldacchino et al. 2014), an inexplicable gap in our understanding of the tabanid feeding apparatus given that these flies are known for their persistent, painful, biting activity (Snodgrass 1944, Foil and Hogsett 1994, Mullens 2009), and their capacity for vectoring, either biologically or mechanically, pathogenic agents (Krinsky 1976, Mullens 2009). The lack of published reports on mouth parts of deer flies (i.e., *Chrysops* spp.) is especially notable with only Buerger (1967) and Joy and Stephens (2016) providing information on sensory structures in the food canal of *C. nigripes* Zetterstedt, and *C. callidus* Osten Sacken, respectively. This study was designed to map sensilla associated with feeding structures of *C. exitans* Walker, with the added goal of assessing type, numbers, and position of sensory sensilla throughout the labrum and cibarium.

We observed two basic types of sensory sensilla – setiform and basiconic – residing within the food canal and more proximal regions (e.g., the epipharynx and cibarium) of the *Chrysops exitans* feeding complex. The goal of this study was not, however, to describe the morphology and function of these sensilla, but rather to map their location in the food canal and test for aggregation patterns. This approach was taken because classification of morphological sensilla types and their putative functions have received considerable attention (Snodgrass 1935, Chapman 1998, Romoser and Stoffolano 1998), whereas little attention has been given to the dynamics of blood flow through the food canal into the cibarium. Given that the velocity profile of a fluid through a "pipe" changes as distance along the pipe increases (Waite and Fine, 2007), one might posit that sensilla would be positioned in the feeding complex to signal changes in blood flow to the feeding fly. Buerger (1967) was the first to suggest that food canal sensilla were not evenly spaced, noting for *Hybomitra rupestris* that spacing between sensilla increased towards the basal end of the labrum. More recently, Joy (2017)

demonstrated that setiform sensilla were significantly aggregated in the distal regions of the *Tabanus atratus* food canal, a finding that compliments Waite and Fine's (2007) discussion that fluid in the entrance region of a "pipe" has a relatively flat velocity profile flow which develops into an increasingly parabolic flow profile as distance along the pipe increases. Eventually, flow profile becomes constant and no longer changes with increasing distance from the point of entry, which coincided with significantly fewer than expected setiform sensilla reported in the *T. atratus* example.

We believe that establishing sensilla location throughout the feeding complex of other dipteran species is necessary to determine if aggregation is a common characteristic of these flies, leading, eventually, to the development of fluid dynamics models for blood flow in hematophagous dipterans.

Materials and Methods

Study Area

This study was carried out at the Clifton F. McClintic State Wildlife Station in Mason County, West Virginia (Camp Conley, WV; Google Maps 2016) The 3,655 acre station, originally a Federal installation for the production of TNT explosives during WW II, is owned by the state and managed by the West Virginia Division of Natural Resources. The station has 40 relatively small (< 15 acres) shallow (2-4 meters deep) ponds. Flies were collected from two areas: (1) between ponds 13 and 14 along a trail that intersects at (38º 54' 42" N; 82º 04' 32" W) with Fairground Rd; and (2) along Park Forest Rd 803, west of the ponds, that intersects at (38º 54' 48" N; 82º 04' 30" W) with Fairground Road.

Specimen Collection and Preperation

Twenty eight *Chrysops exitans* individuals (all females), measuring 7.9 to 8.9 mm in body length, were collected on 12, 19, and 28 May 2016 by sweep netting as they "visited" the authors (MIR, JEJ). Flies were placed in zip-lock bags containing 70% ethanol for killing and preservation. The head of each specimen was severed from the body using a #15 surgical scalpel blade. A sagittal section of each head was then made by cutting through the head, dorsal to ventral, beginning at the outer edge of the antennal bases (Fig. 1A) to reveal the mouth parts and cibarium in dorsal (Fig. 1B) and lateral (Fig. 1C) aspects. This sagittal section, with attached mouth parts, was then placed in a 5% solution of KOH for 24 h to desclerotize the cuticle and degrade cibarial dilator musculature. After KOH treatment, specimens were passed through three washes of distilled water. Cuticular elements of the head and remaining muscle tissue were carefully teased away from the cibarium with the aid of Jewelers forceps while being viewed with a steromicroscope. This microdissection procedure yielded a "clean" head capsule containing the cibarium (i.e., cibarial"pump") and attending mouth parts (hypopharynx, labium, labrum, mandibles, maxillae). Sagittal sections of the head capsule were mounted in glycerol and photographed with a Leica M420 Macroscope (1:6 Apozoom objective with a JVC KY-F75U digital camera mounted on a phototube).

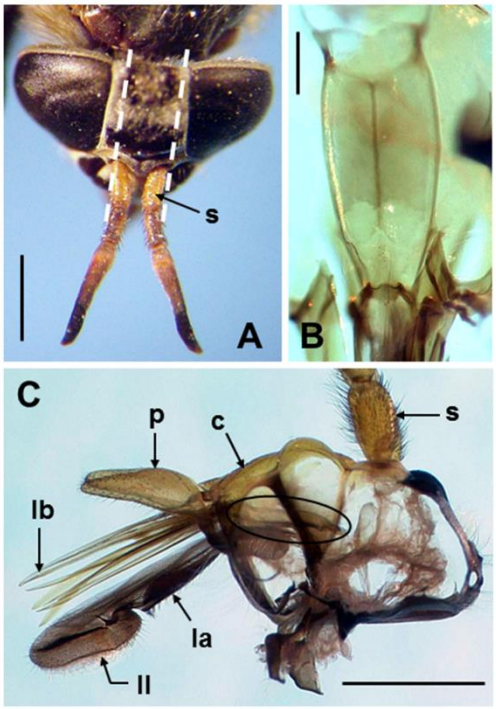

Figure 1. *Chrysops exitans*: **(A)** dorsal view of head showing plane of cuts (dashed lines) to obtain sagittal section; **(B)** dorsal view with top of head removed to reveal cibarium; **(C)** lateral view (sagittal section) of head capsule showing position of cibarium (oval) and mouth parts. Legend: c, clypeus; la, labium; lb, labrum, ll, labella; p, palp, s, scape of antenna. Scale bars A & C = 1.0 mm; B = 200 *u*m.

Since the focus of this study dealt primarily with sensilla lining the food canal, all mouth parts except the labrum (holding the canal) were removed, leaving only the labral / cibarium complex extending from the distal tip of the labrum, and running proximally through the cibarium to the stomodaeum (Fig. 2A). Dissections of six specimens were unsuccessful, and these were excluded from the data set. Of the remaining 22 sample individuals, 18 were prepared for study by dehydrating in an ethanol series (85%, 95%, 99%), clearing in methyl salicylate in two stages (1:1 solution of 99% ethanol and methyl salicylate, followed by 100% methyl salicylate), and then mounting, ventral side up, on glass slides in Permount®. Positions of both setiform and basiconic sensilla along right and left food canal walls were determined in each specimen of the sample population (n = 18) by measuring sensilla distance, to the nearest 1.0 *u*m, from the vestibule/food canal junction using a Zeiss compound microscope equipped with a calibrated ocular micrometer. Photomicrographs were made with Permount® mounted specimens with a ProgRes C5 digital camera (Jenoptik, Jena, Germany) mounted to the Zeiss microscope.

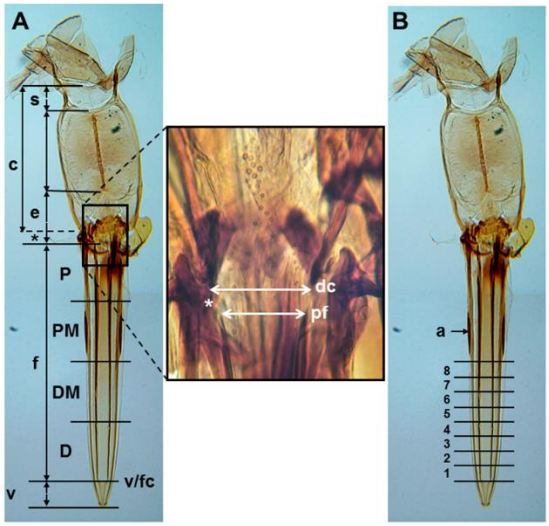

Figure 2. *Chrysops exitans*, ventral views of cibarium / labrum complex. **(A)** cibarium and four equidistant regions of food canal as; D, distal; DM, distal median, PM, proximal median; and P, proximal. Box in A enlarged (center) to show gap (*) between lateral walls of cibarium and food canal. **(B)** distal half of labrum / food canal showing eight equidistant sections (arabic numerals). Legend: a, labral apodeme; c, cibarium; dc, distal extent of lateral cibarium wall; e, epipharynx; f, food canal; pf, proximal extent of food canal; s, stomodaeum; v, vestibule, v/fc, vestibule / food canal junction.

The four remaining specimens were prepared for examination of the food canal and cibarium in cross section. Two of these preparations were transferred to glycerol, and two to 50% ethanol, and the labral / cibarium complex dissected from all four. Sections 0.3 to 0.5 mm in length were cut with a #15 scalpel blade from the labrum and cibarium at seven selected points (Fig. 3A). Sections in glycerol were transferred by micropipette directly to lactophenol and photographed with the Zeiss ProgRes C5 unit. Sections in ethanol were micropipetted through an ethanol series, cleared in methyl salicylate, and mounted in Permount® before photographing. Mounted cross-sectional material was measured with a calibrated ocular micrometer in the Zeiss unit. Both cross-section methods produced similar results, but the former may be preferred because the single transfer from glycerol to lactophenol reduces risk of losing, or damaging, specimens.

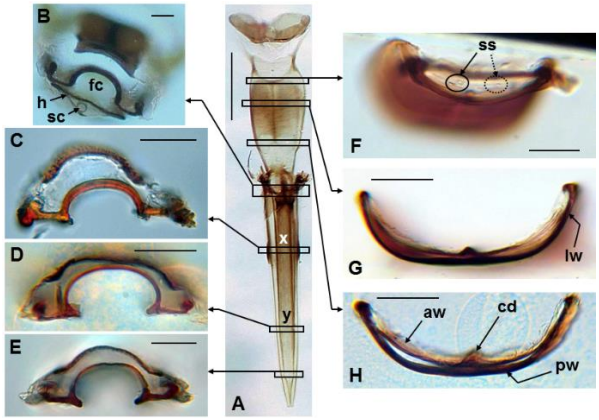

Figure 3. *Chrysops exitans*: **(A)** foregut, entire (x, food canal width between apodemes; y, food canal width distally); **(B)** through **(E)** cross-section views of labrum / food canal (fc, food canal; h, hypopharynx; sc, salivary canal); **(F)** through **(H)** cross-section views of cibarium (aw, anterior wall; cd, cibarial dilator muscle attachment; lw, lateral wall; pw, posterior wall; ss, stomodaeum sensilla). Scale bars: A, 0.5 mm; B – E, 50 *u*m; F – H, 100 *u*m. Note: A, B, F & G, glycerol mounts for photomicography; C & E in lactophenol; D & H in Permount®.

Statistical Analyses

We established two null hypotheses: (Ho1) that setiform sensilla were equally distributed throughout the food canal; and (Ho2) that basiconic sensilla were equally distributed throughout the distal half of the canal. To test Ho1 we divided the food canal into four equidistant regions: D, distal; DM, distal median; PM, proximal median; and P, proximal (Fig. 2A) and used a Chi-square goodness-

of-fit test (http://www.vassarstats.net/csfit.html) to assess the distribution of sensilla within each canal region. To test Ho2 we subdivided the distal half of the food canal into eight equidistant sections (Fig. 2B) and, again, used the Chi-square goodness-of-fit test for aggregation. Levels of significance for Ho1 and Ho2 are given in the appropriate tables.

Results

Putative sensory structures associated with the mouth parts and cibarium of *C. exitans* are described in terms of their type (i.e., setiform or basiconic), numbers, and position (Fig. 2A).

The Vestibule

Mean vestibule length in n = 18 *Chrysops exitans* specimens was 149.7 *u*m (±1 SD = 10.64). There were two pairs of sensilla in the vestibule of every specimen; a distal pair of basiconic sensilla and a proximal pair of setiform design located near the vestibule food canal junction. Number and location of these sensilla types was consistent throughout the sample population.

The Food Canal

Mean length of the food canal, extending proximally from the vestibule / food canal junction to the proximal end of the canal near the head (Fig. 2A), was 1334.4 *u*m (SD = 69.14). Food canal width and depth appeared relatively consistent, as seen in cross-sectional views (Fig. 3A – E), but minor variations were observed. For example, that portion of the canal midway between the lateral (labral) apodemes and vestibule / food canal junction was somewhat wider than canal width between the apodemes in five individuals, whereas the reverse was true in seven specimens. Food canal width at these two points was the same in four individuals (Fig. 3A). Walls of the food canal between the lateral apodemes, and extending proximally to the head were, however, noticeably thicker (Fig. 2A and B, Fig. 3A, D, and E) in every specimen. The food canal widened as it approached the head, reaching its maximum width at the proximal-most extent where it emptied into the epipharynx (Fig. 2A).

There were 328 setiform sensilla observed in the food canal of n = 18 sample individuals; 162 on the left canal wall and 166 on the right wall. Total setiforms varied in number from one individual to another, ranging from 14 to 21 (mean = 18.2; SD = 1.67). The null hypothesis (Ho1) that setiform sensilla were evenly distributed throughout the food canal was rejected (X^2 = 129.80, df = 3, P <0.0001), because a significantly greater number of setiform sensilla than expected aggregated in the distal-most region of the canal, with significantly fewer setiforms than expected in the proximal-most region (Table 1; Fig. 2A).

Table 1. Setiform sensilla distribution in four equidistant regions of the *Chrysops exitans* food canal (see Fig. 2A). Obs. f and Exp. f are observed and expected frequencies; Exp. p, expected proportion; % dev., percentage deviation; Std. res., standardized residuals. Total observed frequencies = 328.

Region	Obs. f	Exp. f	Exp. p	% dev.	Std. res.
P	19	82	0.25	-76.83	-6.96
PM	57	82	0.25	-30.49	-2.76
DM	93	82	0.25	+13.41	+1.21
D	159	82	0.25	+93.90	+8.50

X^2 = 129.80; df = 3; P < 0.0001

There were 36 basiconic sensilla in the food canal of n = 18 individuals; two such sensilla (one in each lateral canal wall) in every individual. All basiconic sensilla were located in the distal half of the canal. We divided this canal region into eight equidistant regions (Fig. 2B), and established the null hypothesis (Ho2) that basiconic sensilla were evenly distributed throughout the distal half of the canal. The null hypothesis (Ho2) was rejected (X^2 = 37.33; df = 7; P < 0.0001), and we concluded that basiconic sensilla were significantly aggregated in sections 3 and 4 (Table 2; Fig. 2B).

Table 2. Basiconic sensilla distribution in eight equidistant sections of the *Chrysops exitans* distal food canal region(see Fig. 2B). Obs. f and Exp. f are observed and expected frequencies; Exp. p, expected proportion; % dev., percent deviation; Std. res., standardized residuals. Total observed frequencies = 36.

Section	Obs. f	Exp. f	Exp. p	% dev.	Std. res.
8	0	4.5	0.125	-100	-2.12
7	0	4.5	0.125	-100	-2.12
6	2	4.5	0.125	-55.56	-1.18
5	6	4.5	0.125	+33.33	+0.71
4	12	4.5	0.125	+166.67	+3.54
3	11	4.5	0.125	+144.44	+3.06
2	5	4.5	0.125	+11.11	+0.24
1	0	4.5	0.125	-100	-2.12

X^2 = 37.33; df = 7; P < 0.0001

The Epipharynx and Cibarium

Even with careful dissection these regions were damaged in some members of the n = 18 sample population, so we give n values of only those individuals that had complete sets of epipharyngeal and cibarial sensilla. The epipharynx begins at the proximal end of the food canal (Fig. 2A) and ends, proximally, at the point of the cibarial opening (i.e., the "functional mouth aperture" of Snodgrass 1935, p. 320). There is a slight gap between the sclerotized proximal food canal walls and distal lateral walls of the cibarium (Fig. 2A) which, presumably, allows for the dorso-ventral movement of the labrum.

Two lateral rows of setiform sensilla were observed in the epipharynx of n = 14 individuals (Fig. 4A and B); the right row with a mean of 7.00 (SD = 1.75) sensilla, and left row with a mean of 6.93 (SD = 1.49). No basiconic sensilla were observed in these lateral rows. A median group of sensilla, somewhat ventral, and proximal, to the lateral rows, was positioned immediately distal to the functional mouth opening (Fig. 4A and B). With but two exceptions, in n = 17 individuals, there were six basiconic sensilla in this median group (mean = 5.82; SD = 0.53), and a variable number, ranging from 12 to 21 (mean = 15.76; SD = 2.86), of setiform sensilla. Finally, at the proximal end of the cibiarium (i.e., the stomodaeum), there were (in n = 18 individuals) always two pairs of basiconic sensilla (Fig. 4A and C).

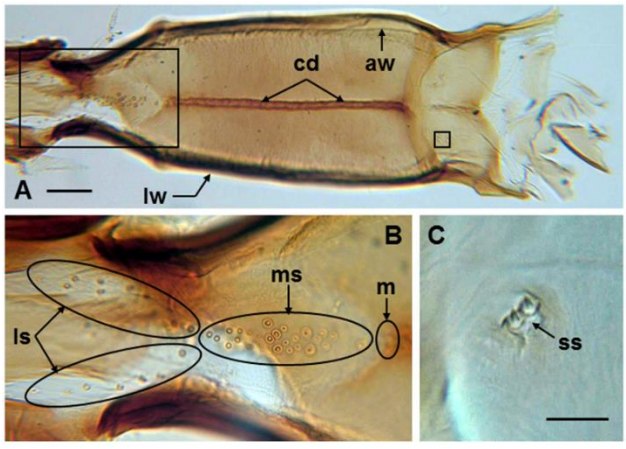

Figure 4. *Chrysops exitans*: **(A)** cibarium, dorsal view. Boxes in A enlarged in **(B)** and **(C)**. Legend: aw, anterior wall (lateral edge); cd, cibarial dilator muscle attachment to mid-line of aw (see Fig. 4E for cross sectional view); ls, lateral sensilla; lw, lateral wall; m, functional mouth; ms, median sensilla; ss, sensilla of stomodaeum. Scale bars: A, 100 *u*m; C, 20 *u*m.

Discussion

Very little published information on sensilla numbers and location in the food canal and epipharynx of *Chrysops* spp. is available. Lall and Davies (1971) reported paired trichoid hairs in the food canal of *C. vittatus* (n = 5 or 6), but provided no information on number, or position, of these structures. The two pairs of vestibular sensilla, a distal pair of setiforms and proximal pair of basiconic design, in the present study was the same as reported for *C. nigripes* (Buerger 1967), and *C. callidus* (Joy and Stephens 2016). There were, however, somewhat fewer sensilla in the food canal of *C. exitans* (mean = 18.2; range 14 to 20) than reported for *C. nigripes* (mean = 24; range 20 to 29) and *C callidus* (mean 20.5; range 17 to 24). The aggregation of sensilla in the distal region of the food canal was common to all three of these *Chrysops* species, strengthening the contention of Joy and Stephens (2016) that food canal sensilla are likely positioned to monitor blood flow through the canal. Few descriptions of sensilla proximal to the food canal (i.e., in the epipharynx and cibarium) in tabanids are available. Joy and Stephens (2016) made no mention of epipharyngeal or cibarial sensilla, and Buerger (1967) merely referred to "groups of sensilla in the membranous region" between the labrum and cibarium. Buerger (1967) also noted the presence of four basicone sensilla on the posterior part of the labrum. We found no evidence of basiconic sensilla in the posterior region of the labrum, but there were two pairs of such sensilla at the posterior end of the cibarium (i.e., the stomodaeum, one pair shown in Fig. 4C). In *C. exitans*, sensilla in each lateral wall of the epipharynx were all of setiform design (Fig. 4A and B), whereas a median group of sensilla, consisting of both basiconic and setiform types, was observed immediately in front of the opening into the cibarium (the "functional mouth" of Snodgrass, 1935). Six basiconic sensilla were characteristic for this median group, but the number of setiforms varied from 12 to 21. Basiconic sensilla so close to the cibarial opening, in addition to those in the stomodaeum, may be chemoreceptors.

Our reporting of 4 basiconic sensilla in the stomodaeum of *C. exitans* appears new for this genus, but Buerger (1967) did note the presence of four basiconic sensilla at the "posterior end" of the cibarium in *Hybomitra rupestris* McDunnough. The

lack of reporting basiconic sensilla in the stomodaeum of tabanids appears to be an oversight because we have casually observed (admittedly in small samples) four basiconic sensilla associated with the stomodaeum of *C. vittatus*, *C. nigribimbo*, *Tabanus atratus*, and *Hybomitra difficilis*, in our lab.

Future work on design of tabanid mouthparts, and the pattern of sensory structures associated with these mouthparts and foregut, would seem desirable because feeding strategies of these insects may yield important information relative to the transmission of pathogens to humans and livestock (Gouteux et al. 1989). This seems supported by Lall and Davies (1971) who posited that the longer, more slender feeding apparatus possessed by chrysopines (e.g., *Chrysops* spp.) is well suited for feeding on a wider range of hosts, whereas larger tabanines (e.g., *Tabanus*, *Hybomitra*), may be restricted by their short, broad proboscis, to feeding on animals with sparse or short hair. Of course, host availability will guide feeding behavior of those tabanids seeking suitable hosts, but feeding strategies could also be affected by type and arrangement of sensory structures in the food canal of the "probing" fly that detect compounds in the blood that stimulate feeding activities. For example, ATP and ADP have been reported as significant phagostimulants for tsetse fly feeding (Galun and Margalit 1969, Friend and Stoffolano 1983). More recently, Emami et al (2017) found that humans infected with a malaria parasite were more attractive to the mosquito vector than uninfected people. This is brought about by a key *Plasmodium falciparum* metabolite, which indirectly triggered human red blood cells of infected individuals to increase the release of CO_2, aldehydes, and monoterpenes which in combination, enhanced attraction and feeding behavior of the *Anopheles gambiae* s.l. vector

There may be a variety of compounds in the blood of vertebrates that play a role in modulating feeding behavior of hematophagous dipterans, and for that reason it is all the more important to have a better understanding of the sensory structures – their morphological types, and their distribution – found throughout the labral food canal and foregut of tabanid flies.

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Literature Cited

Baldacchino, F., M. Desquesnes, S. Mihok, L. D. Foil, G. Duvallet, and S. Jittapalapong.2014. Tabanids: Neglected subjects of research, but important vectors of disease agents. Infect. Genet. Evol. 28: 596-615.

Buerger, G. 1967. Sense organs on the labra of some blood-feeding Diptera. Quaest. Entomol. 3: 283-290.

Camp Conley, WV Map. *Google Maps*. Google, 2016. Web. 11 Nov.2016.

Chapman, R. F. 1998. The Insects: Structure and Function, 4th ed., Cambridge University Press, Cambridge, UK

Foil, L. D., and J. A. Hogsette. 1994. Biology and control of tabanids, stable flies and horn flies. Rev. Sci. Technol. 13: 1125-1158.

Gouteux, J. P., F. Noireau, and C. Staak. 1989. *The host preferences of Chrysopssilacea and C. dimidiata (Diptera: Tabanidae) in an endemic area of Loa loa in the Congo*. Ann. Trop. Med. Parasitol. **83**: 167-172.

Joy, J. E., and C. R. Stephens. 2016. Sensory trichites associated with the food canal of *Chrysops callidus* (Diptera: Tabanidae). J. Med. Entomol. 53: 961-964.

Joy, J. E. 2017. Putative sensory structures associated with the food canal of *Tabanus atratus* (Diptera: Tabanidae). J. Med. Entomol. 54:471-475.

Krinsky, W. L. 1976. Animal-disease agents transmitted by horse flies and deer flies (Diptera: Tabanidae). J. Med. Entomol. 13: 225-275.

Lall, S. B., and D. M. Davies. 1971. An intergeneric comparison of cephalic structure in tabanids (Diptera) in relation to feeding habits. J. Med. Entomol. 8: 700-706.

Mullens, B. A. 2009. Horse flies and deer flies (Tabanidae), pp. 261-274. In G. Mullen and L. Durden (eds.), Medical and veterinary entomology, 2nd ed., Academic Press, San Diego, CA.

Snodgrass, R. E. 1935. Principles of insect morphology. McGraw-Hill, Inc. New York and London.

Snodgrass, R. E. 1944. The feeding apparatus of biting and sucking insects affecting man and animals. Smithsonian Misc. Coll. 104: 1-113.

Romoser, W. S., and J. G. Stoffolano. 1998. The Science of Entomology, 4th ed., McGraw-Hill, Inc. Boston, MA

Waite, L., and J. Fine. 2007. Applied Biofluid Mechanics. McGraw Hill, New York, NY