Proceedings of the West Virginia Academy of Science

Original Research Paper

Aggregation Patterns of Sensory Sensillae in the Food Canal and Cibarium of *Glossina morsitans morsitans* (Diptera: Glossinidae)

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Article history
Received: 17 January 2019
Revised: 20 February 2019
Accepted: 25 March 2019

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Abstract: Mouthparts of hematophagous vectors serve as intermediaries, enabling the transfer of blood and pathogens from and to their hosts. We describe aggregation patterns of basiconic and setiform sensillae in the food canal and cibarium of the medically significant tsetse fly, *Glossina morsitans morsitans* Westwood. Mean body length of females was significantly greater than males (n = 20 for each sex). Mean lengths of food canal and cibarium were also significantly greater in females, even when correcting for the greater body lengths of females, but there was no significant difference in total number of sensillae in the food canal or cibarium between the sexes. A pair of basiconic (campaniform) sensillae was consistently present in the food canal of every individual, but numbers of setiform sensillae in the canal of both females and males varied from 53 to 74. No basiconic sensillae were observed in the cibarium proper of any individual, but four minute conical basicones embedded in a sclerotized plate at the posterior edge of the cibarial wall were observed. Number of setiform sensillae in the cibarium varied from 5 to 12 in females and 7 to 11 in males. Setiform and basiconic sensillae were significantly aggregated in the proximal-most (i.e., nearest the head) food canal region of both sexes, whereas setiforms were significantly aggregated in the mid regions of the cibarium. Sensilla aggregation patterns in tsetse flies are very different from those documented for tabanid flies indicating potential differences in monitoring blood flow between these two groups of hematophagous feeders.

Keywords: *Glossina*; tsetse flies; labrum; cibarium; sensilla

Introduction

Hematophagous insects often play a significant role in affecting economic and social development over wide geographic regions. For example, nagana, a trypanosome disease of many domestic and wildlife mammals, has been responsible for suppressing a viable animal agriculture industry on the African continent for decades (Krinsky 2009). Since the mouthparts of blood-feeding arthropods are the main organs involved in the transfer of pathogens and parasites to vertebrates, it is not surprising that the feeding apparatus and attending sensory structures have received considerable attention (Krenn and Aspok 2012).

The head and mouthparts of tsetse flies have long piqued the interest of investigators since the original brief description of the genus *Glossina* by Wiedmann in 1830. The presence of a labrum and hypopharynx were subsequently identified in tsetse flies (MacQuart 1835), along with observations that the proboscis of *Glossina* was similar to that of *Stomoxys* (MacQuart 1835, Hansen 1903, Giles 1906, Jobling 1933). Stuhlmann (1907) published a thorough narrative of *Glossina* anatomy, but his figures of the head and mouthparts were too small to be helpful. Stephens et al. (1906) were the first to provide a

detailed account, accompanied by helpful figures, of sensory sensillae (i.e., their "stalked hairs") in the food canal of *Glossina palpalis*. Jobling (1933) published a detailed narrative, and excellent figures clearly depicting setiform sensilla in the food canal and cibarium of *G. palpalis*. While it is clear from his figures that such sensillae are concentrated in the bulb region of the labrum-epipharynx, there was no attempt to characterize aggregation patterns. A new approach, focusing on the labrocibarial sensillae in terms of general positioning and innervation, was provided by Rice et al. (1973), but precise sensillar patterns (i.e., aggregation) in the food canal/cibarium complex still received little attention.

Although much literature is devoted to the characterization of different sensilla types and their putative functions (e.g., mechanoreception, chemoreception) in insects (Snodgrass 1935, Slifer 1970, Stoffolano and Yin 1983, Romoser and Stoffolano 1998), relatively little attention has been given to aggregation patterns of sensory sensillae within the feeding complex of hematophagous flies. We sought to address this oversight by critically mapping positions of sensory sensillae in the food canal and cibarium of tsetse flies. This approach seems especially relevant, given that reports of aggregation patterns of sensory sensillae in the food canal of certain tabanid flies (Buerger 1967, Joy and Stephens 2016, Joy 2017), suggests that the monitoring of blood flow is more critical in certain regions of the tabanid feeding complex. Thus the focus of this study was to identify general sensilla types in the food canal and cibarium of *Glossina m. morsitans*, and determine if evidence of aggregation patterns of these sensory structures exists, and further, to determine if there are differences in any such patterns between female and male tsetse. Since the ability to receive and process information about the environment is a basic characteristic of life, new approaches to how tsetse may interpret environmental cues for feeding adds to our understanding of this important assemblage of vector species.

Materials and Methods

Methods

Dead *Glossina morsitans morsitans* Westwood adults (n = 20 females; n = 20 males) were obtained from an insect colony at the Department of Biology (Rio lab), West Virginia University. Tsetse flies are maintained in a 12 h light:12 h dark photoperiod at 24+/-1°C with 50-55% relative humidity. All flies received defibrinated bovine blood (Hemostat) every 48 hours through an artificial membrane feeding system (Moloo 1971). Three to four day old adults (both sexes) that had been fed one blood meal were sacrificed via ethyl acetate and individually stored in 75% ethanol within microcentrifuge tubes.

Each individual adult fly was removed from its container and measured from the tip of the head to the tip of the abdomen to the nearest 0.1 mm while viewed under a Zeiss stereoscope equipped with a calibrated ocular micrometer. Sexes were separated for all subsequent measurements and sensory sensilla counts. The head of each fly was removed from the body and lateral cephalic extremities (i.e., portions of the compound eyes) were cut off to expose the internal head region in sagittal section after a procedure described for *Chrysops exitans* (Ranavaya and Joy 2017). These sagittal sections were then placed in a 5% KOH solution for 24 to 48 hours on a warming tray at 40 ºC to soften and clear sclerotized elements (e.g., the head capsule, rostrum), and to remove muscle tissue surrounding the cibarium. After KOH treatment each sagittal section was washed in distilled water, then the labium/labrum/cibarium complex (Figs. 1A, B, and C) was gently pulled from the head capsule with microforceps while viewed under a stereomicroscope. The complex of each fly was then mounted separately in lactophenol, for clearing, on glass microscope slides under #1 glass coverslips for critical examination of labral and cibarial sensillae. Food canal sensillae were measured with a Zeiss compound microscope equipped with a calibrated ocular micrometer as the distance (in μm) from the proximal-most point of the food canal and continuing to the distal-most point of the canal. This procedure provided: (1) sensilla counts for each of four equidistant regions – P, proximal; PM, proximal median; DM, distal median; D, distal – of the food canal (Fig. 1A); (2) the distance of each sensilla on both right and left walls of the canal from the proximal-most to distal-most point of the canal; and (3) the total food canal length for each sample individual (n = 20 females; n = 20 males). A similar procedure was employed for sensilla counts and position in the cibarium, except that cibarial sensilla

Fig. 1

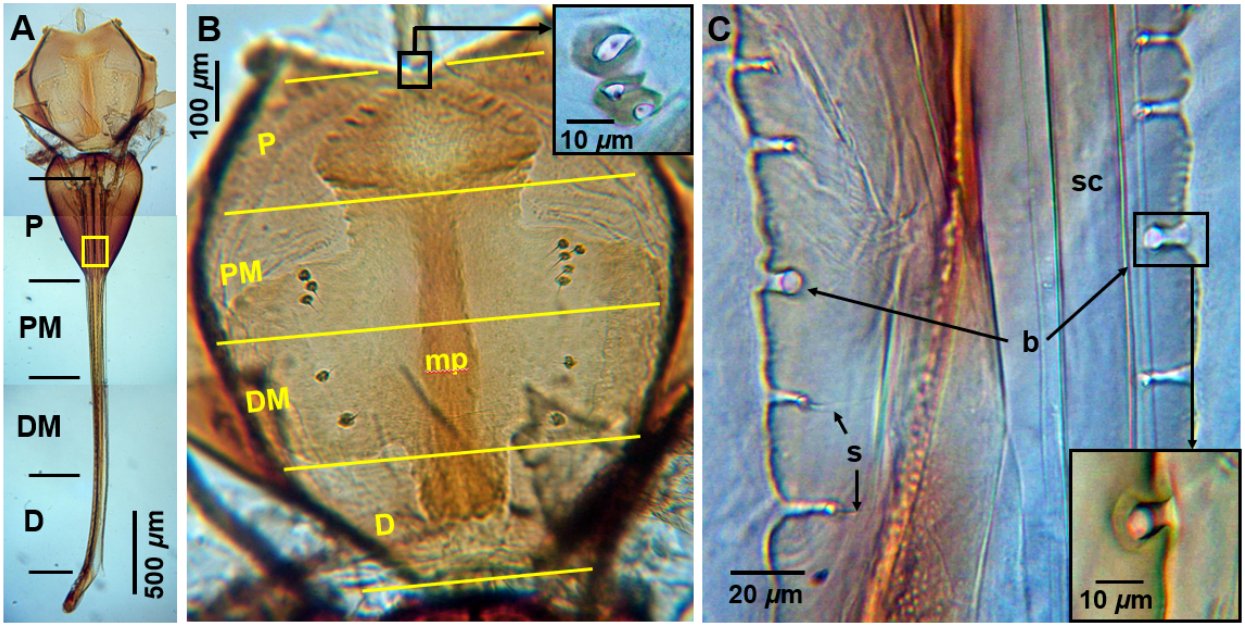

Figure 1. Composite view of labrum (food canal)/cibarium complex of *Glossina m. morsitans*. A. Entire, with food canal divided into four equidistant regions, as: D, distal; DM, distal median; PM, proximal median; and P, proximal. **B**. Cibarium enlarged to show four equidistant regions (as defined in A); insert (upper right), sensory plate of the pharynx (i.e., cibarium) with peg-like basiconic sensillae (after Jobling 1933). Note that setae of sensillae in PM region are directed toward the cibarium/food canal junction, whereas setae of sensillae in the DM region are directed otherwise (corroborating observations of Rice et al. 1973). **C**. Enlarged section of food canal (box in A) showing setiform and basiconic sensillae. Legend: b, basiconic sensilla; mp median plate (insertion of cibarial dilator muscles); s, setiform sensilla; sc, salivary canal.

positions were recorded from the distal to proximal ends of the cibarium (Fig. 1B).

Statistical Analyses and Hypotheses

We compared mean lengths (adult body size, food canal and cibarium) and mean sensilla numbers in the food canals and cibaria of females v. males using independent groups t-tests. Relationships between food canal and cibarium length as a function of body length, and sensilla numbers as a function of food canal and cibarium lengths were determined using linear regression (SPSS Inc., version 24, and Microsoft Excel). Controlling for differences in body length between females and males as a factor in observed differences of food canal and cibarium lengths by sex was analyzed by ANCOVA (SPSS Inc. version 24) after applying Levene's Test of Equality of
Error Variances to satisfy the ANCOVA assumption that error variance of the dependent variable (i.e., food canal length, or cibarium) is equal across groups (i.e., females and males). This assumption was satisfied for the food canal (Levene's $F = 0.319$; df1 = 1; df2 = 38; $P = 0.576$), and the cibarium (Levene's $F = 0.240$; df1 = 1; df2 = 35; $P = 0.627$). The Chi-square goodness-of-fit test (http://vassarstats.net/csfit.html) was used to test for aggregation of sensillae in different regions of the food canal and cibarium. We established ten null hypotheses for testing, with test values and probability levels given, as appropriate, in the narrative, tables, or figures.

Hypothesis 1 ($H_0$1). Mean body length of females = mean body length of males. Test statistic: t-test.

Hypothesis 2 ($H_0$2). Mean food canal length of females = mean food canal length of males. Test statistic: t-test. Controlling for greater body length in females, ANCOVA, test statistic: F-test.

Hypothesis 3 ($H_0$3). Mean number of setiform sensillae in females = mean number of setiform sensillae in males. Test statistic: t-test.

Hypothesis 4 ($H_0$4). Total numbers of setiform sensillae in the food canal of both females and males was a function of food canal length. Linear regression, R^2, ANOVA, test statistic: F-test.

Hypothesis 5 ($H_0$5). Numbers of setiform sensillae were equal in each equidistant region of the food canal. Test statistic: Chi-square goodness-of-fit.

Hypothesis 6 (H_06). Numbers of basiconic sensillae were equal in each equidistant region of the food canal. Test stastic: Chi-square goodness-of-fit.

Hypothesis 7 (H_07). Mean cibarium length of females = mean cibarium length of males. Test statistic: t-test. Controlling for greater body length in females, ANCOVA, test statistic: F-test.

Hypothesis 8 (H_08). Mean number of setiform sensillae in the cibarium of females = mean number of setiform sensillae in cibarium of males. Test statistic: t-test.

Hypothesis 9 (H_09). Total numbers of setiform sensillae in the cibarium of both females and males was a function of cibarium length. Linear regression, R^2, ANOVA, test statistic: F-test.

Hypothesis 10 (H_010). Numbers of setiform sensillae were equal in each equidistant region of the cibarium. Test statistic: Chi-square goodness-of-fit.

Results

Adult Body Length

Mean body length of *G. m. morsitans* females (n = 20; 8.65 mm; ±1 SD = 0.353) was significantly greater ($t_{.05,38}$ = 4.535; $P < 0.000$; one-tailed test) than that of males (n = 20; 8.09 mm; ± 1 SD = 0.425) (Fig. 2). Reject H_01.

Food Canal Lengths, Sensilla Numbers and Aggregation

Mean food canal length of females was significantly greater than mean canal length of males (Table 1; Reject H_02). Controlling for body length as a factor, the mean food canal length of females was still significantly greater (ANCOVA: F = 1.474; P = 0.022; Reject H_02) than the mean canal length of males. There was no relationship between food canal length and body length in either sex (Figs. 2A and B).

Table 1. Range and mean food canal lengths (FCL, in μm), and mean numbers of setiform sensillae in the food canal (FCS) of *Glossina m.morsitans* by sex (f = female; m = male).

	sex	n	range	mean	± 1 SD	$t_{05,38}$	*P*-value
FCL	f	20	2484 - 3073	2830.75	141.82	3.826	0.0005
	m	20	2217 - 2873	2633.90	181.17		
FCS	f	20	53 - 74	61.85	4.65	1.272	0.2113
	m	20	53 - 74	63.80	5.04		

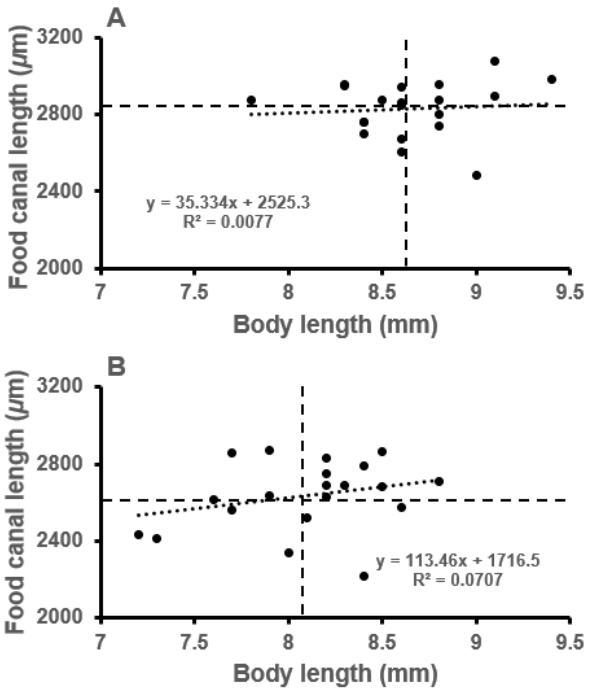

Figure 2. Food canal length as a function of body length in *Glossina m. morsitans* females (A), and males (B). R^2, ANOVA (females): F = 0.140; P = 0.712. R^2, ANOVA (males): F = 1.370; P = 0.257. Vertical dashed lines indicate mean body lengths; horizontal dashed lines, mean food canal lengths.

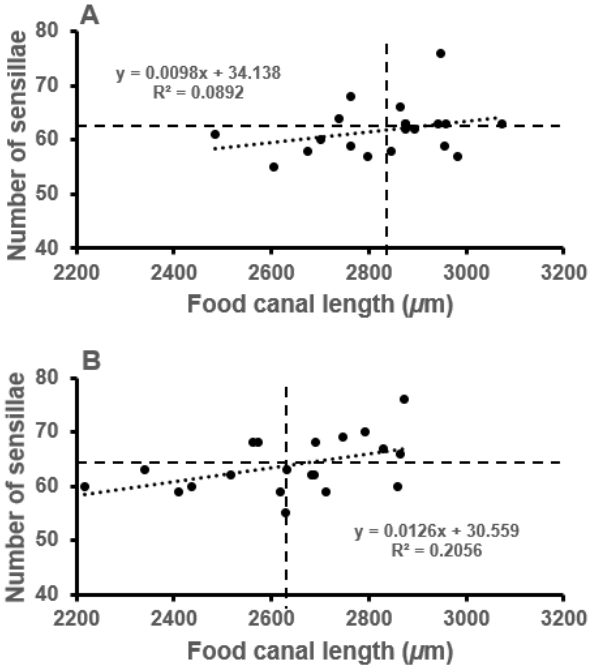

Figure 3. Numbers of setiform sensillae as a function of food canal length in *Glossina m. morsitans* females (A), and males (B). R^2, ANOVA (females): F = 1.763; P = 0.201. R^2, ANOVA (males): F = 4.658; P = 0.045.

A single pair of basiconic (campaniform) sensillae (Fig. 1C) were consistently present in each food canal of all 40 sample individuals (n = 20 females; n = 20 males), but numbers of setiform sensillae in the food canals were variable, ranging from 53 to 74 in both females and males (Table 1). Mean number of setiform sensillae in the food canal of females was less than the mean for males, but the difference was not significant (Table 1; Accept $H_0$3). There was no relationship between numbers of setiform sensillae and food canal length in females (Fig. 3A, reject $H_0$4 for females), but numbers of setiform sensillae were positively, and significantly, correlated with food canal length in males (Fig. 3B, accept $H_0$4 for males).

Setiform sensillae were significantly aggregated in the proximal-most region of the food canal in both females and males, with the proximal canal region contributing 75.0% of the total X^2 value in females and 75.9% in males (Fig. 1A; Table 2; Reject $H_0$5). Moreover, the percent deviation of observed setiform numbers over expected values in the proximal region was far greater than expected at +259.6 and +257.1 for females and males, respectively. The significant lack of setiform sensilla aggregation in the proximal median, distal median, and distal regions of the canal was shown by the relatively small percent X^2 contributions of these regions to the total X^2 value, concomitant with the far lower than anticipated percent deviations from expected values for these regions. Moreover, the X^2 percent contributions to total X^2 value and percent deviations for these canal regions were remarkably consistent for females and males (Table 2). Although the basiconic sensillae were more widely distributed throughout food canal regions, they still exhibited significant aggregation in the proximal and proximal median regions of females, and proximal region of males (Figs. 1A and C; Table 2; Reject $H_0$6).

Cibarial Lengths, Sensilla Numbers and Aggregation

Mean cibarium length of females was significantly greater than that of males (Table 3; Reject $H_0$7). Controlling for body length as a factor, the mean cibarium length of females was still significantly greater (ANCOVA: $F = 7.943$; $P = 0.008$; Reject $H_0$7) than that of males. There was no

Table 2. Chi-square goodness-of-fit test for setiform and basiconic sensilla aggregation in the food canal of *Glossina m. morsitans*, by region (see Fig. 1A). Obs f, observed frequency; Exp f, expected frequency; X^2, Chi-square value; % X^2, percent of Chi-square contributed by region to total X^2 value; % dev, percent deviation from expected frequency.

setiform	Females (n = 20)					Males (n = 20)				
Region	Obs f	Exp f	X^2	% X^2	% dev	Obs f	Exp f	X^2	% X^2	% dev
P	1112	309.25	2083.78	75.0	+259.6	1139	319.0	2107.84	75.9	+257.1
PM	36	309.25	241.44	8.7	-88.4	42	319.0	240.53	8.6	-86.8
DM	45	309.25	225.65	8.1	-85.5	33	319.0	256.41	9.1	-89.7
D	44	309.25	227.51	8.2	-85.5	62	319.0	207.05	7.4	-80.6
Totals	1237	1237		100		1276	1276		100	
	$X^2 = 2778.38$; df = 3; P < 0.0001					$X^2 = 2811.83$; df = 3; P < 0.0001				

basiconic	Females (n = 20)					Males (n = 20)				
Region	Obs f	Exp f	X^2	% X^2	% dev	Obs f	Exp f	X^2	% X^2	% dev
P	18	10	6.40	21.8	+80	25	10	22.50	62.2	+150
PM	19	10	8.10	27.6	+90	11	10	0.10	0.3	+10
DM	3	10	4.90	16.7	-70	4	10	3.60	9.9	-60
D	0	10	10	34.0	-100	0	10	10	27.6	-100
Totals	40	40		100		40	40		100	
	$X^2 = 29.41$; df = 3; P < 0.0001					$X^2 = 36.19$; df = 3; P < 0.0001				

relationship between cibarium length and body length in either sex (Figs. 4A and B).

Table 3. Range and mean cibarium lengths (CL, in μm), and mean numbers of setiform sensilla in the cibarium (CS) of *Glossina m. morsitans* by sex (f = female; m = male). A value of n < 20 indicates some cibaria were damaged in dissection and thus not suitable for measurements.

	sex	n	range	mean	± 1 SD	$t_{.05,35}$	*P*-value
CL	f	18	868 - 1033	947.17	46.61	4.220	0.0002
	m	19	785 - 956	883.68	44.74		
CS	f	18	5 - 12	8.61	1.69	0.560	0.5793
	m	19	7 - 11	8.89	1.37		

No basiconic sensillae were observed within the main body of the cibaria of either females or males, but four minute, peg-like basicones were observed on a small sclerotized plate at the proximal extremity (i.e., posterior wall) of the cibarium (Fig. 1B, insert). Numbers of setiform sensillae in the cibaria ranged from 5 to 12 in females, and 7 to 11 in males, but the mean number of setiform sensillae in females was not significantly different from that of males (Table 3; Accept $H_0$8). There was no relationship between number of sensillae and cibarium length for either gender (Figs. 5A and B; Reject $H_0$9 for both sexes).

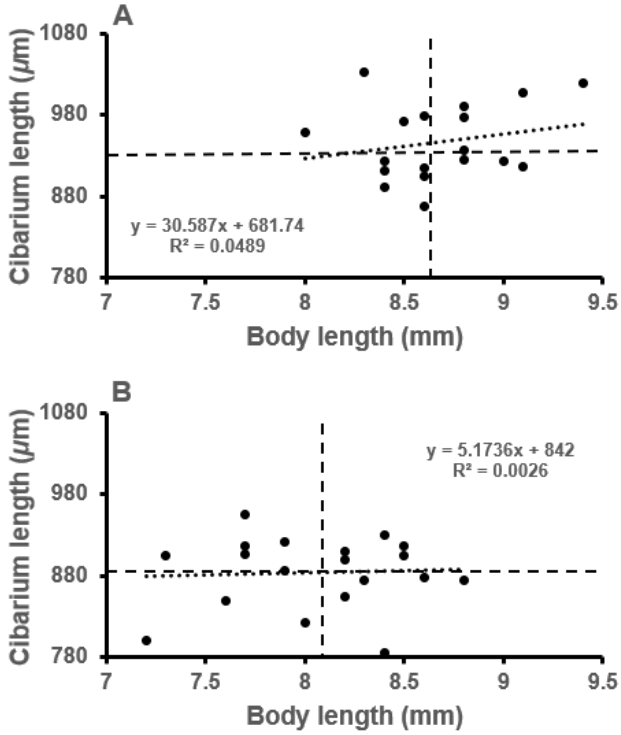

Figure 4. Cibarium length as a function of body length in *Glossina m. morsitans* females (A), and males (B). R^2, ANOVA (females): $F = 0.823$; $P = 0.378$. R^2, ANOVA (males): $F = 0.045$; $P = 0.835$.

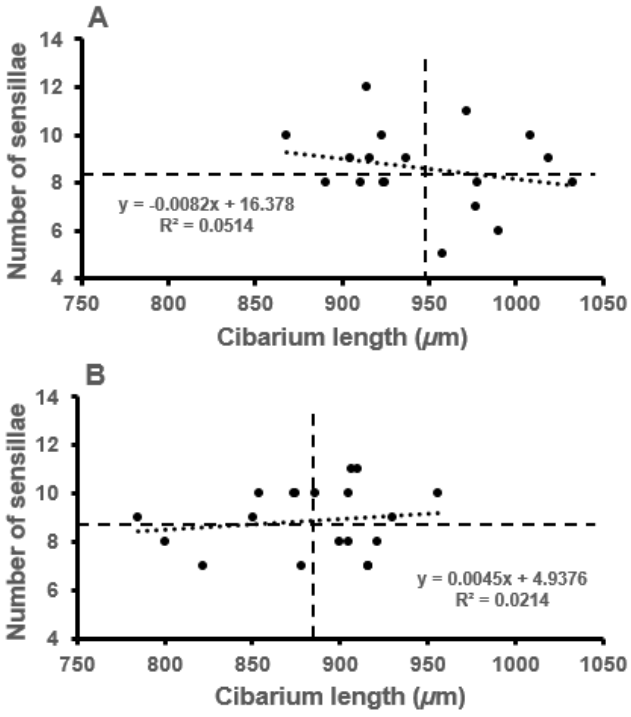

Figure 5. Numbers of setiform sensillae as a function of cibarium length in *Glossina m. morsitans* females (A), and males (B). R^2, ANOVA (females): $F = 0.868$; $P = 0.365$. R^2, ANOVA (males): $F = 0.371$; $P = 0.550$.

Table 4. Chi-square goodness-of-fit test for setiform sensilla aggregation in the cibarium of *Glossina m. morsitans*, by region (see Fig. 1B). Sample size of n < 20 indicates that some cibaria were damaged and thus unsuitable for measurements. Obs f, observed frequency; Exp f, expected frequency; X^2, Chi-square value; % X^2, percent of Chi-square contribution of region to total X^2 value; and % dev, percent deviation from expected frequency.

	Females (n = 18)					Males (n = 19)				
Region	Obs f	Exp f	X^2	% X^2	% dev	Obs f	Exp f	X^2	% X^2	% dev
D	2	38.75	34.85	19.8	-94.8	2	42.25	38.34	19.2	-95.3
DM	53	38.75	5.24	3.0	+36.8	55	42.25	3.85	1.9	+30.2
PM	100	38.75	96.81	55.1	+158.1	112	42.25	115.15	57.7	+165.1
P	0	38.75	38.75	22.1	-100	0	42.25	42.25	21.2	-100
Totals	155	155		100		169	169		100	
	$X^2 = 175.66$; df = 3; P < 0.0001					$X^2 = 199.59$; df = 3; P < 0.0001				

Setiform sensillae were significantly aggregated in the proximal median region of the cibarium in both females and males (Fig. 1B; Table 4). This region accounted for 55.1% of the total X^2 value in females, and 57.7% of the total X^2 value in males. Moreover, the percent deviation of observed setiform numbers over expected numbers in the proximal median region, being far greater than expected, was indicative of significant aggregation. Percent deviations of observed sensilla numbers in the distal and proximal regions, being far below expected numbers, indicated a significant lack of aggregation in those regions for both females and males (Table 4; Reject $H_0$10).

Discussion

The Food Canal

Our finding that the mean food canal length in females was significantly greater than males appears contrary to a previous report that the labra of *G. morsitans* females and males appeared identical (Buerger 1967). Our observations of 53 to 74 setiform sensilla aggregated in the proximal food canal region agrees, in part, with Buerger (1967) who did not provide numbers of sensillae but noted that, "…all sensilla are of the setiform type…", and they are more numerous towards the basal end (i.e., aggregated proximally) of the food canal. Two rows of setiform sensilla situated in the proximal food canal region are described in other previous works (Stephens et al. 1906, Jobling 1933, Rice et al. 1973), but without mention of specific numbers or degree of aggregation. Other investigators did not report the presence of setiform sensilla in the food canal of tsetse flies even though the labrum/food canal was well described (Hansen 1903, Giles 1906). Thus specific documentation of aggregation patterns in the present study, and the remarkable similarity of such patterns between *G. m. morsitans* females and males as shown by Chi-square goodness-of-fit tests (Table 2), is new and suggests that certain regions of the tsetse food canal are more important than others in providing sensory input to the feeding fly. Curiously, while both mean body and food canal lengths were significantly greater for females than males, males possessed more setiform sensillae in the food canal, although the difference was not significant. This finding supports the contention that aggregation patterns of sensillae in the food canal, and the consistency of such patterns in both females and males, are more important in providing sensory information to the feeding fly than total sensilla numbers, which vary considerably from one individual to another.

Variability in numbers of food canal sensilla has also been reported for tabanids. Stoffolano and Yin (1983), working with *Tabanus nigrovittatus*, noted that the number of sensillae not only varied (18-25) from one side of the food canal to the other, but also from one individual to another; adding that the spacing between sensilllae was irregular. Scudder (1953) also reported "…roughly 30…" "…rheometric trichodes…" irregularly spaced along the lateral walls of the food canal of *Tabanus quinquevittatus*. Joy (2017) reported irregularly spaced sensilla in the food canal of *T. atratus* varying in numbers from 54 to 85 in individuals making up the sample population. Joy (2017) also noted that food canal sensillae were significantly aggregated in the distal and distal median regions of the food canal, with relatively few sensillae observed in the proximal canal region; again, supporting the contention that aggregations of sensillae provide essential sensory information rather than total sensilla numbers.

Our observations of a single pair of basiconic (campaniform) sensilla in the food canal of every fly examined (both females and males) also agrees with Buerger (1967) who described a pair of sensilla characterized by a "…round membrane without any projection" in the food canal of *Glossina morsitans*. Buerger (1967) noted that these campaniform sensilla were located at an average distance of 1.7 mm from the distal tip of the labrum, which places them approximately in the proximal median food canal region that we delineate in Fig. 1A; Table 2. Basiconic sensilla of campaniform design in the food canal were not reported in previous studies on *G. pallidipes*, *G. morsitans*, and *G. fusca* (Hansen 1903), *G. palpalis* (Stephens et al. 1906, Giles 1906, Jobling 1933), *G. fusca* and *G. tachinoides* (Stuhlmann 1907), or *G. austeni* (Rice et al. 1973), even though several investigators (Stephens et al. 1906, Jobling 1933, Rice et al. 1973) provided detailed figures to supplement their narratives. Both Scudder (1953) and Stoffolano and Yin (1983) observed occasional basiconic sensilla in the food canal of tabanids, but offered little in terms of their positioning, or function. Romoser and Stoffolano (1998) wrote that sensillae of the campaniform type have always been found to be a mechanoreceptor stimulated by deformation of the cuticle. This agrees with Pringle (1938) who was the first to report that campaniform sensillae were sensitive to compression forces acting down the length of the

palps of cockroaches. Sensitivities to bending forces, or cuticular strains, have also been shown for campaniform sensilla in the tarsi of cockroaches (Zill et al. 2010), and for trochanteral campaniform sensilla in cockroaches (Zill et al. 1999) and stick insects (Hofmann and Bässler 1986, Delcomyn 1991). The putative function of campaniform sensilla in the tsetse food canal remains an open question.

The Cibarium

Like the food canal, aggregation patterns of sensillae in the cibarium have not been reported, although cibarial sensillae of setiform design have been observed by several investigators; most notably Jobling (1933) who reported three or four setiform sensillae lying between the median plate of the pharynx (which serves as the insertion point of pharyngeal, or cibarial, dilator muscles; see Fig. 1B) and lateral pharyngeal (i.e., cibarial) border. Thus this implied total of six to eight such sensillae (i.e., lying on both sides of the median plate) is similar to the range of cibarial setiforms (5 – 12 for females; 7 – 11 for males) found in *G. m. morsitans* individuals of the present study (Table 3). Earlier, such cibarial sensillae had been observed (Kraepelin1883, Frey 1921); the former author believing they served to trap solid particles entering the food canal, with the latter noting they represented true sensory organs. Jobling (1933), however, thought there were too few of these sensilla to function effectively as a sieve mechanism. The question of trapping v. sensory function was put to rest by Rice et al. (1973) who noted that there could be little doubt that these cibarial sensillae were mechanoreceptors stimulated by movement of fluid in the lumen of the cibarium. In fact, two groups of cibarial setiform sensillae were recognized by Rice et al. (1973); one group positioned proximally with setae directed disto-laterally to detect blood flow during ingestion, and the other group situated distally with setae directed proximo-laterally to monitor flow during regurgitation. Our observations on the positioning of cibarial setiforms, and the direction of setae projecting from them (see Fig. 1B), agree with Rice et al. (1973), although we provide more precise information on aggregation patterns of these sensillae. For example, cibarial setiforms in individuals of the present study were significantly aggregated in the proximal median region, as indicated by the high X^2 value contributions (in both females and males) for this region as a percentage of the total X^2 value (Table 4). Additionally, the percent deviation of observed setiform sensillae from the expected number in the proximal median region was far greater than expected, thus supporting rejection of the null hypothesis of an equal distribution of sensillae in regions of the cibarium.

There are no basiconic sensillae in the body proper of the cibarium, but our observations of four minute, peg-like basiconic sensilla on a sclerotized plate consistently located at the posterior margin of the cibarium (see insert, Fig. 1B) corroborated Jobling (1933) who described three or four very short "basio-conical" sensillae positioned on a square-shaped plate (the "sensory plate of the pharynx" in his Fig. VI). Jobling (1933) did not assign a function to these sensillae, but Snodgrass (1935) had noted that basiconic sensillae with thick processes apparently respond to mechanical stimuli and thus may be regarded as tactile in function. Rice et al. (1973), however, believed these peg-like sensillae (their "LC4 receptors"), were the only chemoreceptors within the food canal of the tsetse, and as such they played a decided role in determining whether fluids taken up should be ingested or not. Moreover, adenosine triphosphate (ATP) may be the most important phagostimulants in mosquitoes and tsetse flies (Galun and Margalit 1969, Galun and Rice 1971), and when cibarial receptors detect the presence of blood, and the specific phagostimulants in it, continuous pumping of the cibarium is initiated (Galun 1987).

Cibarium length of females was significantly greater than that of males, but there was no relationship between numbers of setiform sensillae and cibarium length in either sex (Figs. 5A and B), suggesting that relatively few sensillae are necessary for monitoring blood flow regardless of the length of this pumping structure.

Summary

This basic research provides new information on aggregation patterns of sensory sensillae that reside in the food canal and cibarium of tsetse flies and offers a new way of thinking about how these vectors of medical and veterinary importance process sensory information while feeding.

Curiously, aggregation patterns of sensory sensillae in the food canal/cibarium complex of tsetse flies are quite different from those found in blood-feeding tabanids despite both groups of flies being

housed in the Brachycera clade and sharing similar diets. For example, sensory sensillae are aggregated in the distal regions of the food canals of tabanid flies (Buerger 1967, Joy and Stephens 2016, Joy 2017, Ranavaya and Joy, 2017, Setser and Joy 2017), whereas these sensillae are aggregated in the proximal-most canal region of tsetse flies (Stephens et al. 1906, Buerger 1967, Rice, et al. 1973, present study). Why aggregations of sensory sensillae in deer flies and horse flies is so different from that of tsetse flies is uncertain, but a plausible explanation may lie in the structure of the distal labrum/food canal complex (Figs. 6A and B). Both tabanids and tsetse draw blood from a pool under the host's skin which is formed by lacerating blood vessels via the action of serrated mandibles and laciniae in tabanids (Mullens 2009), and the back and forth scraping/penetrating movements of a labellum armed with teeth and rasps (Giles 1906, Margalit et al. 1972, Krinsky 2009) at the labellar tip of tsetse. In tabanids, blood drawn from the pool enters a funnel-shaped vestibule that broadens as it approaches the food canal. As blood passes from the broad vestibule base into a narrower food canal opening, the resulting turbulence would explain the necessity for a distal aggregation of sensory sensillae to monitor this dynamic fluid movement (Fig. 6A). Conversely, blood flows into the tsetse distal labrum unimpeded by any constriction at the food canal opening, and consequently there are few sensillae (Stephens et al. 1906) in this region (Fig. 6B). Presumably, blood flows linearly (i.e., with relatively little turbulence) in the tsetse food canal, as indicated by Margalit et al. (1972), until reaching the proximal canal region where aggregation of setiform sensillae and a pair of basiconic sensillae are positioned to provide critical sensory information to the feeding fly. It may be desirable to construct a fluid dynamics model of the food canal in tabanid v. tsetse to test the plausibility of this blood flow supposition.

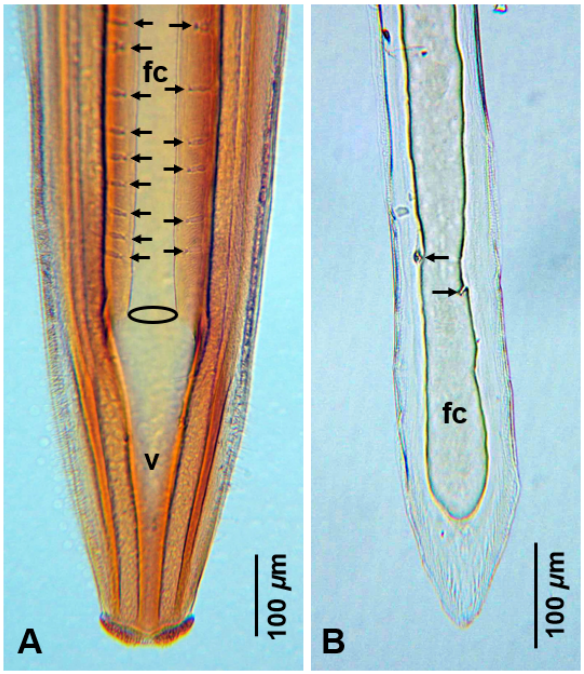

Figure 6. Distal labra (ventral views). A. *Tabanus abdominalis*; B. *Glossina m. morsitans*. Legend: fc, food canal, v, vestibule; oval (in A), vestibule/food canal junction (opening into food canal).

This study focused on aggregation patterns of sensillae rather than structure or function of various sensilla types. Setiform sensillae observed in the present study appear to be mechanoreceptors, corroborating Rice et al. (1973), however, the function of the two basiconic sensillae observed in food canals of specimens in the present study (Fig 1C insert) remains uncertain. They are, however, suitably positioned (see Fig. 1A, box) to monitor stress forces associated with elevation and depression, and protraction and retraction of the haustellum described by Jobling (1933).

Even though the food canal of females is significantly longer than in males, the same number of sensillae are present in the food canals of both sexes, indicating that a set number of sensilla suffices to monitor blood flow in both. Moreover, since aggregation patterns of sensillae are the same in both sexes it would appear such patterns are more important in providing sensory information to the feeding fly than simply total numbers of sensillae.

Acknowledgements

Our appreciation is extended to Jenna Zuzolo for assisting with microdissections, April Fugett for reviewing statistical procedures, and Roman Olynyk for help with color graphics. We also acknowledge the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (R01AI118789 to R.V.M.R.) for support in maintenance of laboratory colonies of *Glossina m. morsitans*.

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