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FUNCTIONALITY OF A REDUCED PROBOSCIS: FLUID UPTAKE BY *PHIGALIA STRIGATARIA* (MINOT) (GEOMETRIDAE: ENNOMINAE)

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ABSTRACT. The structure and functionality of the reduced proboscis of males of *P. strigataria* was studied. Scanning electron microscopy revealed a proboscis structurally similar to functional proboscises of other lepidopteran species, including chemo- and mechanosensilla and a tip-region with larger spaces between the dorsal legulae. Drops of red food coloring applied to the dorsal legulae entered the proboscis. Subsequent dissections exposed a complete and functional gut containing food coloring. We suggest that Lepidoptera with reduced proboscises might rely on capillarity as an initial step for fluid to enter the proboscis for subsequent uptake. Field observations are needed to determine if *P. strigataria*, and other Lepidoptera with reduced proboscises, feed in their natural habitats.

Additional key words: feeding habits, fluid uptake, capillarity

Most extant Lepidoptera possess a coilable proboscis that is used for fluid uptake (Scoble 1992; Krenn 2010). The proboscis is composed of two elongated maxillary galeae connected by dorsal and ventral legulae to form a food canal for fluid transport (Eastham & Eassa 1955; Krenn et al. 2001). The lepidopteran proboscis has been assumed to function like a drinking straw (Eberhard & Krenn 2005), only using the sucking pump in the head for fluid uptake (Daniel et al. 1989; Kingsolver & Daniel 1995). According to the drinking-straw model, a proboscis must be a sealed tube to properly function (Borrell & Krenn 2006; Krenn 2010); proboscises that are small and might lack a sealed tube-like arrangement are described as reduced, rudimentary, or vestigial (Rindge 1975; Krenn & Kristensen 2000), implying a lack of functionality without experimental evidence. The lepidopteran proboscis, however, does not have to be completely sealed to be functional (Monaenkova et al. 2012), which suggests that the drinking-straw model is incorrect or incomplete.

Moths in the genus *Phigalia* are medium-sized and have a reduced proboscis; most additional members of the Bistonini have a proboscis described as vestigial or absent (Rindge 1975). Adults of *Phigalia strigataria* (Minot) (Lepidoptera: Geometridae) (Fig. 1) are active from January to March in the southeastern USA (excluding peninsular Florida), and their range continues north into Canada (Rindge 1975). Although adult Lepidoptera use a variety of dietary sources (Adler 1982; Scoble 1992), foods such as floral and extrafloral nectar, fruit, and tree sap, are scarce or unavailable during the winter when adults of *P. strigataria* are active.

The reduced proboscis of *P. strigataria* suggests a lack of functionality; however, studies of *Gluphisia septentrionis* and *Clostera albosigma* (Notodontidae) provide an example of a reduced, but functional proboscis (Adler 1982; Smedley & Eisner 1995). Considering that a reduced proboscis can retain functionality (e.g., *G. septentrionis*), and that functionality is not limited to the drinking-straw model, we hypothesized that the proboscis of *P. strigataria* is functional, even though food sources are scarce when the adults are active.

MATERIALS AND METHODS

Twelve males of *Phigalia strigataria* were captured at lights from 1900 to 2300 h in Central, SC, USA, in February 2011, and placed in glassine envelopes. Two males were deposited in the Clemson University Arthropod Collection. The remaining males were used to study the proboscis with scanning electron microscopy (SEM) or to perform feeding experiments followed by dissections.

Scanning electron microscopy and proboscis measurements. The heads of four males were prepared for SEM using a series of ethanol washes (24

hrs in 80, 95, and 100% each) followed by chemical drying with hexamethyldisilazane. Heads were placed on stubs with carbon-graphite tape and gold sputter-coated for approximately 90 seconds. A Hitachi TM3000 scanning electron microscope was set to full vacuum and 15 kv for imaging. The galea length (= proboscis length), galea width at its widest point, and tip-region length were measured using SEM images and ImageJ software (http://rsbweb.nih.gov/ij/download.html). The tip-region was characterized by larger spaces between the dorsal legulae (Krenn et al. 2001, 2010) and where only a single layer of dorsal legulae existed. The tip-region was measured from the proximal end of the drinking-slit region (sensu Krenn 2010).

Feeding trials and dissections. Four live males of *P. strigataria* were secured with insect pins on dissecting trays, leaving the ventral side exposed. With the aid of a dissecting microscope, a microsyringe (Hamilton Co. Inc., Whittier, CA.) was used to place drops of diluted red-food coloring (Southern Homer Assorted Food Coloring Set, Mauldin, SC) (ca. 5:1 water:food coloring) on the dorsal side of the proboscis. Drops of food coloring (less than 1 µl each) were placed on the proboscis, and larger drops (greater than 1 µl each) were placed near the base of the proboscis, covering the labrum.

After the feeding trials, the dissecting tray was filled with physiological saline, and the abdomen and thorax were dissected to expose the alimentary canal. The crop was removed from one specimen, placed on a slide with 50% acetic acid, and viewed under a light microscope. Two unfed males were stored for three months in a refrigerator and later rehydrated in water and dissected to compare the alimentary canal to males in feeding trials. A Scion Corporation Color Digital Camera (Model CFW 1310C, Scion Corporation, Frederick, MD) was used to acquire images of specimens under the dissecting and light microscope, and ImageJ software was used for measurements.

RESULTS

Proboscis morphology and feeding trials. The average length of the proboscis was less than 0.50 mm (n = 4, Table 1), and appeared fleshy, pliable, and was light tan (Fig. 2a). The proboscis could bend in all directions. SEM imagery revealed dorsal and ventral legulae (Fig. 2b); however, the galeae separated from each other when nudged with an insect pin. Dorsal legulae were flat and lancet-shaped, with a pointed extension at each tip (Fig. 2c). The architecture of the ventral legulae suggested interlinking capability (Fig. 2b), but this could not be verified because the galeae were separated in all SEM images.

The proboscis had at least two distinct structural regions: a tip-region characterized by a single layer of dorsal legulae with larger spaces between them than those more proximal, and a region proximal to the tip-region characterized by a second row of smaller dorsal legulae (Fig. 2d,e); the proximal region comprised most of the proboscis length (76.4%, n = 4, Table 1). The dorsal legulae in the tip-region were shorter (mean = 11.9 µm, n = 4, 2 individuals) than those proximal to this region (30.2 µm). The proximal region of the galeae was covered by microtrichia that declined in number near the transition to the tip-region.

An irregular row of sensilla trichodea lined the dorsal side of the galeae and occurred in two rows in the mid-region of the proboscis, but became a single row in the tip-region; the tip region had an average of 8.3±0.88 sensilla trichodea (12.6±0.92 µm in length, n = 3 individuals, Table 1) (Fig. 2e). Four sensilla basiconica, each with an elongated stylus, occurred on the lateral galeal wall in the tip-region. A sensillum styloconicum with 4 longitudinal ridges and an extended stylus was at the tip of the proboscis (Fig. 2d). The food-canal wall was annulated and had a row of sensilla basiconica (Fig. 2f).

When a drop of red food coloring was applied to the proboscis, it entered the proboscis, leaving no food

TABLE 1. Measurements (µm) of proboscises of males of *P. strigataria* collected in Central, SC, in February 2011.

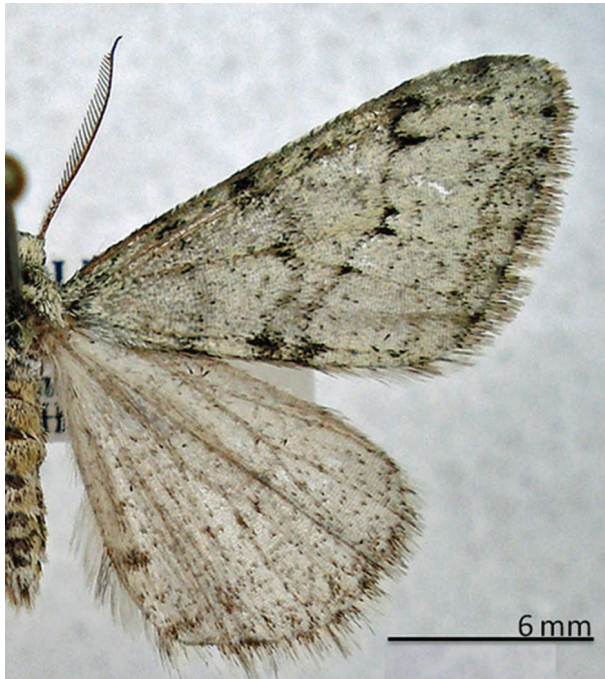| Male ID #	Length of proboscis	Max. width of galea	Length of tip region	% of tip-region	# of sensilla trichodea in tip-region	Mean length of sensilla trichodea in tip-region
1	481.8	75.3	111.3	23.1	8	13.6
2	496.2	63.6	119.8	24.1	-	-
3	488.4	54.8	110.9	22.7	10	13.4
4	495.5	63.0	121.7	24.6	7	10.7
Mean±SE	490.5±3.38	64.2±4.21	115.9±2.81	23.6±0.43	8.3±0.88	12.6±0.92

FIG. 1. Photograph of male of *P. strigataria* captured in February 2011 in Central, SC (Canon Rebel XTI digital camera).

coloring on the external galeal walls; however, the dorsal legulae subsequently had a pink-tinted stain. Fluid entered all tested regions of the proboscis, and anti-parallel movements of the galeae occurred irregularly during the feeding trials. The food canal was stained red after a drop of food coloring was administered. Larger drops placed at the base of the proboscis pulsated and decreased in size until they disappeared.

Gut morphology and functionality. After the feeding trials, red portions of the gut were visible through the cuticle and epidermal layers at the juncture of the thorax and abdomen, and between the abdominal segments. Subsequent dissections revealed a gut with sections of red fluid (Fig. 3). The foregut consisted of a thin tube and the crop, which attached as a separate compartment and was filled with red liquid. Light microscopy revealed spines on the interior crop wall (Fig. 3).

The midgut was wider (323 µm, n = 2) and shorter (986.5 µm, n = 2) than the foregut (52 µm, 3471 µm, respectively) and appeared to be filled with red fluid. Two sets of three Malpighian tubules extended from the juncture of the midgut and hindgut, and each set branched from a single stalk. One Malpighian tubule
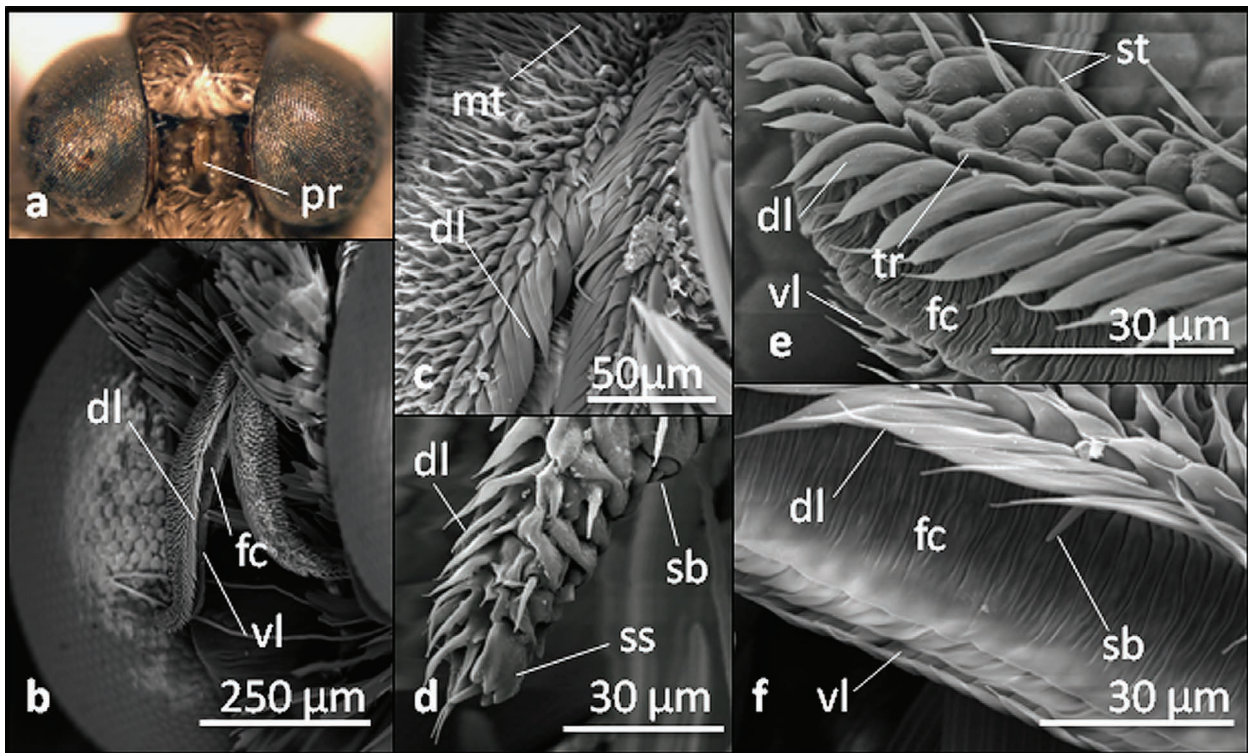

FIG. 2. Reduced proboscises of males of *P. strigataria* (Geometridae). **a.** head of a male of *P. strigataria* displaying the reduced proboscis (pr). b – f SEM photomicrographs of the proboscis of males of *P. strigataria*. **b.** proboscis in its entirety, showing dorsal legulae (dl), ventral legulae (vl), and food canal (fc). **c.** overlapping dorsal legulae and microtrichia (mt) near the base of the proboscis. **d.** tip-region of the proboscis; sensilla basiconica (sb) are at the tip-region and a sensillum styloconicum (ss) is located at tip. **e.** the transition (tr) marking the proximal beginning of the tip-region where two rows of dorsal legulae become a single row, also shown with sensilla trichodea (st). **f.** annulations and sensillum basiconicum in the food canal.
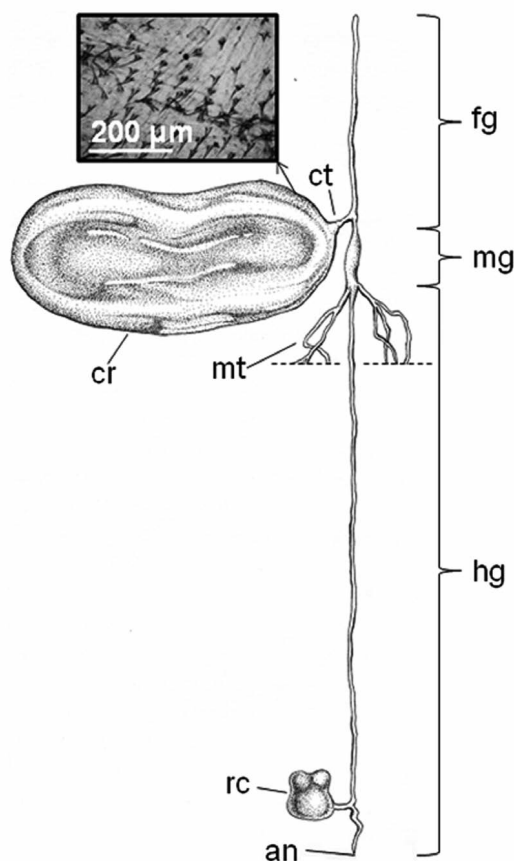
Fig. 3. Gut of a male of *P. strigataria*. The foregut (fg), midgut (md), and hindgut (hg), represent approximately 27%, 9%, and 64% of the entire gut, respectively. The crop (cr) was connected to the foregut by a crop tube (ct). In this illustration, the crop was partially filled with food coloring. The insert from the crop shows a photograph of the cuticular spines on the internal wall of the crop. The Malpighian tubules (mt) (actual length not shown) are at the pyloric juncture of the midgut and hindgut. A rectal caecum arises as an offshoot of the hindgut anterior to the anus (an). of Los Angeles County, science series No. 38.

separated from each base approximately 200 µm from the gut, and the main stalk branched into 2 Malpighian tubules approximately 100 µm distal to the first branch. The longest Malpighian tubule was approximately 3807 µm long (n = 2).

The hindgut was about 8000 µm in length and 50 µm wide (n = 1). Sections of red liquid were interspersed throughout the hindgut, and peristaltic activity moved them toward the rectum. The most noticeable feature of the hindgut was the rectal caecum (Kristensen 2003) (Fig. 3), represented as a red pouch. One male defecated red fluid during dissection.

Discussion

Microtrichia, sensilla trichodea (mechanosensory, Krenn 1998), and sensilla basiconica (chemosensory,

Walters et al. 1998) are plesiomorphic in the glossatan Lepidoptera, and are found in the most primitive clades including the Micropterigidae (Krenn & Kristensen 2000; Krenn 2010); *Phigalia strigataria*, in addition to other Lepidoptera with a proboscis considered vestigial or reduced, also retains these structures. Males of *P. strigataria* also possess derived structures, such as a sensillum styloconicum (chemo-mechanosensory, Altner & Altner 1986; Petr & Stewart 2004), implying that the proboscis can use a combination of sensory equipment for feeding. The occurrence of only a single sensilla styloconicum at the tip of the proboscis might be a trait found in other lepidopteran species with a reduced proboscis, but this requires further study. The presence of a tip-region, considered previously the only place where fluid uptake occurs (Krenn, 2010), and chemo- and mechano-sensilla suggest fluid uptake capability. Males of *P. strigataria*, unlike other Lepidoptera, had smaller dorsal legulae in the tip-region than basally. The galeae, however, were malleable and separated easily, suggesting that if *P. strigataria* feeds in the wild, it might rely on a mechanism of fluid uptake other than the drinking-straw model (Kingsolver & Daniel 1995), which requires a tightly sealed tube (Eberhard & Krenn 2005; Borrell & Krenn 2006).

The feeding trials also indicate a proboscis capable of fluid uptake. Food coloring placed on the surface of the proboscis stained the food canal red. Unlike most reports of fluid uptake being restricted to the drinking-slit region of the proboscis (Krenn et al. 2001; Krenn 2010), fluid also entered the proboscis of *P. strigataria* proximal to the tip-region. The noticeable pulsing of the larger drops placed near the base of the proboscis suggests activity of the sucking pump.

The anti-parallel movements of the galea indicate that these movements are not unique to Lepidoptera with proboscises for specialized feeding, as described for blood-feeding and fruit-piercing moths (Bänziger 1970; Krenn 2010), or as a method for initial proboscis alignment following ecdysis (Krenn 1997). In addition to the movements and fluid uptake by the proboscis, the functional gut provides supporting proof that *P. strigataria* can feed. A functional crop with cuticular spines suggests that these moths are not subjected to selection for a reduced crop in a non-feeding species (Kristensen, 2003).

The morphology, feeding trials, and dissections provide evidence that males of *P. strigataria* are capable of fluid uptake. We suggest that short or reduced proboscises employ capillarity as an important step for fluid uptake into the proboscis, before the sucking pump is used to transport the fluid to the gut. This dual-functioning system has been demonstrated with *Danaus*

plexippus L. (Monaenkova et al. 2012), a butterfly with a relatively long, coilable, and tube-like proboscis. Reduced proboscises that lack a straw-like appearance and are not completely sealed, demonstrated here with *P. strigataria*, probably require capillarity when feeding. The dual-functioning system of capillarity and the sucking pump warrants a reevaluation of proboscises previously labeled as vestigial or reduced.

The lack of available nectar sources during the winter, coupled with the short proboscis, suggests that *P. strigataria* might use sources of nutrition other than floral nectar. Winter temperatures rise above freezing in South Carolina during the flight period of *P. strigataria*, which might provide sources of water for uptake. The fluctuating temperatures might provide adequate humidity for dew droplets to precipitate on the proboscis; however, this method of fluid uptake has not been investigated. Droplets of water available on the surfaces of substrates in the habitat also might be acquired by *P. strigataria*. The uptake of water could be important for longevity of this species, as demonstrated with *Parapediasia teterrella* (Pyralidae) (Marshall 1988). We suggest the need for field observations of *P. strigataria* and other moths with proboscises labeled as reduced or vestigial to determine feeding habits that could provide new insights into the biology of these species.

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