HISTOLOGICAL ATLAS OF FRESHWATER MUSSELS (BIVALVIA, UNIONIDAE):
VILLOSA NEBULOSA (AMBLEMINAE: LAMPSILINII), FUSCONAIA CERINA
(AMBLEMINAE: PLEUROBEMINI) AND STROPHITUS CONNASUGAENSIS
(UNIONINAE: ANODONTINI)

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ABSTRACT

Freshwater mussels (Mollusca: Bivalvia: Unionoida) are a species-rich group of parasitic bivalves comprising approximately 843 nominal species in six families, including 300 species of Unionidae and five of Margaritiferidae in North America. Unionid shells have been studied extensively for the purposes of taxonomy, but less information exists about the cellular anatomy of their “soft tissues” (mantle cavity tissues and visceral tissues). No systematic histological atlas of any unionid has been published in the peer-reviewed literature, and this lack of information hinders basic and applied research topics involving freshwater mussels. Herein, we describe the tissue and cell anatomy of a representative species from each of three lineages (tribes) of Unionidae sensu Graf & Cummings (2006) ranging in North America: Villosa nebulosa (Ambleminae: Lampsilini), Fusconaia cerina (Ambleminae: Pleurobemini) and Strophitus connasaugaensis (Unioninae: Anodontini). Based on necropsy observations and light microscopy of serial histological sections, for each species we describe and compare mantle cavity tissues (i.e., tissue enclosed by mantle: mantle, adductor muscle, pedal protractor, pedal retractor, gill, foot, labial palp) and visceral organs (i.e., internal organs: esophagus, digestive diverticulum, stomach, crystalline style sac, intestine, heart, nephridium, cerebral ganglia, pedal ganglia, visceral ganglia, ovaries and testes). We also present a synoptical review of pertinent literature on histological anatomy of unionids. The present study (i) represents the first histological atlas for freshwater mussels, (ii) comprises a baseline for monitoring mussel health in aquatic ecosystems, and (iii) could assist future workers studying freshwater mussel physiology, life history, toxicology, pathology, systematics and ecology.

Key words: Histology, Unionidae, Villosa nebulosa, Fusconaia cerina, Strophitus connasaugaensis, Mobile River Basin.

INTRODUCTION

Diversity

The order Unionida is a taxonomically and ecologically diverse and geographically widespread group of bivalve mollusks commonly known as “freshwater mussels” including six families (Unionidae, Margaritiferidae, Hyriidae, Etheriidae, Mycetopodidae, Iridinidae), and approximately 161 genera and 843 species, with extant representatives ranging on all continents except Antarctica. Unionidae is the largest family, with approximately 677 species, whereas there are fewer than 100 species in each of the five other mussel families. The greatest concentration of mussel species occurs in the inland waters of North America, with a total of 305 species comprising Unionidae (300 spp.) and Margaritiferidae (five spp.) (Gangloff et al., 2006; Graf & Cummings, 2007; Williams et al., 2009; Jones & Neves, 2010). The southeastern United States is a biodiversity focus for unionids. For example, there are approximately 84–182 unionid species ranging in each of Alabama, Georgia,
Kentucky, Mississippi and Tennessee (Williams & Neves, 1995; Gangloff et al., 2006; Williams et al., 2008, 2009; Jones & Neves, 2010). In addition to their species richness, freshwater mussels are remarkable for their unique life histories. Mussels have a parasitic larval stage (glochidium) that infects fish skin or gill before inhabiting the benthos, and gravid females exhibit parental care by withholding those larvae in their gills for several weeks or months before release. Related to parasitism, some unionids, especially lampsilines, have elaborate modifications of the mantle tissue that mimic specific prey items of the requisite host fish species, essentially acting as a “lure” to hasten transmission of glochidia to the fish (Barnhart et al., 2008). Moreover, freshwater mussels provide an array of well-documented ecosystem services (Vaughn & Hakenkamp, 2001; Atkinson et al., 2011): removing algae and bacteria from stream water, enriching the benthos with organic matter, and comprising a food resource for terrestrial and aquatic animals (Fuller, 1974; Cosgrove et al., 2007).

Conservation Status and Challenges

Many North American unionids are imperiled, specifically an estimated 71% of unionid species in the U.S. are imperiled and 40% of the unionid species in 25 states are imperiled (Williams & Neves, 1995). Furthermore, an estimated 17–37 unionid species already are extinct (Master et al., 2000). Threats to mussel biodiversity and populations include modifications to stream habitat (Hornbach, 2001, and references therein), obliteration of acceptable habitat, toxic contaminants, introduction of exotic bivalve species, and mysterious die-offs (Anderson et al., 1991; Lydeard et al., 2004; McGregor & Garner, 2004; Haag, 2012). Mussel die-offs, short-term high mortality events characterized by multitudes of dead mussels detached from their shells and/or moribund, gaping individuals, are well documented but enigmatic (Pauley, 1968; Ahledt & Jenkins, 1987; Blodgett & Sparks, 1987; Havlik, 1987; Fleming et al., 1995), and most lack a well-established etiological agent or causative mechanism (Pauley, 1968; Neves, 1987). A high diversity of parasites and potential pathogens infect unionids and may impact population levels or the proportion of reproductively viable adults, but the virulence, pathogenicity and epidemiology of these myriad viral, bacterial, fungal and metazoan pathogens (Grizzle & Brunner, 2009) remains understudied relative to related symbionts that infect aquatic vertebrates. Few published descriptions exist of histopathological changes associated with infections or diseases of freshwater mussels (Baker, 1976; Pauley & Becker, 1968; Taskinen et al., 1997; Chittick et al., 2001; Zhong et al., 2011), and although chemical contaminants allegedly cause mussel die-offs, little species-specific information exists on pathological changes to mussel tissues exposed to a toxin (Newton & Cope, 2007).

Need for Histology

The justification for the histological atlas of freshwater mussels presented herein is not fundamentally different from that of an anatomical atlas for any group of organisms: cell and tissue anatomy are “bricks in the foundation” of basic research and contribute to the body of knowledge about a lineage. At present, the literature on freshwater mussels lacks a systematic, comparative atlas of histology with high resolution, color, and labeled micrographs. Herein, we describe the normal appearance, position and overall organization of cells and tissues in three freshwater mussel lineages: Lampsilini, Pleurobemini and Anodontini. Given the conservation status of freshwater mussels and the notion that they are useful as ecosystem indicators, a systematic description of the appearance of normal (“healthy”) cells and tissues comprises baseline information for future studies involving not only physiology but also toxicology and histopathology. As such, we think the present study is timely and relevant to conservation biologists and wildlife managers. Beyond them, taxonomists also may find these results useful if comparable data from related species/lineages are published.

ANATOMY OF FRESHWATER MUSSELS

A complete synopsis of the available anatomical information on freshwater mussels is beyond the scope of the present work, but we provide some helpful “first stop” references below, as well as present specific details of their results in order of organ system in the subsequent sections.

Marquee synopses of the biology of freshwater mussels are provided by, among others, Burch (1973), Fuller (1974), Parmalee & Bogin (1998), Bauer & Wächter (2001), Strayer
HISTOLOGICAL ATLAS OF FRESHWATER MUSSELS

(2008), Williams et al. (2008), Watters et al. (2009), Cummings & Graf (2010) and Haag (2012). Anatomical information on freshwater mussels is largely based on shell morphology of adults and glochidia, while details concerning soft tissue structure mainly pertain to the reproductive system including the gill. Most of the earlier published works (late 19th century-early 20th century) consisted of invertebrate textbooks, handbooks or manuals that included a unionid as an exemplar bivalve, including illustrations of the mussel and selected organ systems or cells. Work published since the early 1900’s contains more detailed, species-specific anatomical treatments that include gross micrographs, histological sections and illustrations. In general, we found that high quality histological micrographs of mussel tissues were sparsely represented in the literature. In fact, with exception to a few works (some of which are detailed below), most either excluded histology or included confirmatory, low resolution histological micrographs that accompanied gross or functional anatomical treatments of the tissue, organ or system being described. The lack of that fundamental information made it difficult for us to sit at the microscope with sections of mussel tissue and reconcile specific cell types, which was an impetus for embarking on this monograph.

Cooke (1895), a general mollusk textbook, generally described gill, foot, mantle, crystalline style, heart, nephridium and gonad of bivalves and compared members of Anodonta and Mytilus spp. Brooks (1882), Howes (1885) and Girod (1889), also textbooks or dissection manuals, described the general habitus of organs of Anodonta anatina, and Anodonta cygnea, including dorsal tissues (nephridia, intestine, and adductor muscles), paired nephridia, pericardial cavity, position of intestinal limbs and gill-mantle junction. Simpson (1884) illustrated myofibers of pedal protractors and retractors fanning into the foot of Pyganodon cataracta (as Anodontia fluviatilis), and provided a helpful nervous system schematic showing ganglia forming a neural circuit and nerve fibers radiating from each ganglion. Raßbach (1912) described mantle morphology, cellular constituents of the epithelium and connective tissue, periostracum secretion and shell structure, including external and internal morphology, mineral layers, regeneration following mechanical damage, and morphology of mineral crystals. Siebert (1913) described incumbent and excurrent apertures, foot, mantle edge, labial palp, ciliated and non-ciliated columnar cells, mucus cells, granular secretory cells and sensilla. Similarly, Guthiel (1912), Splittstößer (1913), Motley (1932), Beedham (1958), Purcheon (1958) and Smith (1988) characterized specific organ systems and their associated tissues; whereas, others have described embryos (Lillie, 1895, 1897, 1898), larvae (Lefevre & Curtis, 1912; Fryer, 1961; Schwartz & Dimock, Jr., 2001), and juveniles (Lefevre & Curtis, 1912; Lasee, 1991). By phylogenetic inference, also helpful to those interested in understanding tissues of freshwater mussels are several synoptical references regarding anatomy and physiology of marine bivalves (Galtsoff, 1964; Norton & Jones, 1992; Eble, 2001; Grizel, 2003).

The unpublished dissertation by Yokley (1968) detailed the shell, mantle tissues and viscera of Pleurobema cordatum. Gross photographs accompanied low magnification histological sections of select tissues, and some helpful drawings of select cells highlighted in those sections were provided. Yokley’s work comprises the most extensive treatment of cellular anatomy of a freshwater mussel, but we view the present contribution as a much-needed expansion of that work and including three additional unionid species. Another unpublished dissertation, by Lasee (1991), included scanning electron microscopy and some accompanying histology of larval and post-larval development of Lampsilis ventricosa (as Lampsilis cardium). It also included a study documenting the effects of cadmium on those life history stages, showing marked pathological changes to mantle, ganglia, and digestive gland.

Shell morphology of glochidia and adults is useful towards species-level or higher-level taxonomy (Graf & Cummings, 2006), but some taxonomy is based on viscera morphology (Ridewood, 1903; Ortmann, 1910a, 1911a; b; Purcheon, 1958; Pain & Woodward, 1968; Fuller, 1973; Heard & Dougherty, 1980; Smith, 1988; Mansur & Da Silva, 1990; Graf & Cummings, 2006; Avelar & Cunha, 2009). The visceral mass, gills, and mantle are typically portrayed in a lateral view to show foot, inner and outer gills, labial palps, siphon and adductors (e.g., Pain & Woodward, 1968; Fuller, 1974; Heard & Dougherty, 1980; Smith, 1988; Mansur & Da Silva, 1990). Three contributions by Ortmann included morphological details of representative species of Anodontini (Anodontinae), Pleurobemini (Unioninae) and Lampsiliini.
focusing on coloration, geometry and sculpture of shell but also position, coloration and shape of the labial palps, gill and siphon papillae. Herein we describe twenty-five major tissues of *Villosa nebulosa* (Conrad, 1834), *Fusconaia cerina* (Conrad, 1838) and *Strophitus connasaugaensis* (Lea, 1858) (Table 1). *Villosa nebulosa* was originally described as *Unio nebulosus* by Conrad (1834). *Fusconaia cerina* (Conrad, 1838) was originally described as *Unio cerinus* by Conrad (1838). *Strophitus connasaugaensis* (Lea, 1858) was originally described as *Margaritana connasaugaensis* by Lea (1858).

### MATERIALS AND METHODS

Mussels were collected by hand while snorkeling, transported to Auburn University in an aerated cooler filled with stream water from the collection site. Specimens of *Villosa nebulosa* were collected from the South Fork of Terrapin Creek near the Cleburne County Road 55 crossing (33°51’36.56"N, 85°31’28.15"W) in May 2010 (n = 36), August 2010 (n = 39) and August 2011 (n = 5), plus Shoal Creek near the Talladega National Forest Road 500 crossing (33°43’30.46"N, 85°36’05.05"W) (Table 2). *Fusconaia cerina* were collected from the Cahaba River, in shoals, upstream and downstream of a canoe launch (33°10’10.04"N, 87°01’12.73"W) located on Slab Road, Shelby County. Specimens of *Fusconaia cerina* were collected in May 2011 (n = 10), August 2011 (n = 28) and June 2012 (n = 7) (Table 3). Specimens of *Strophitus connasaugaensis* were collected from Shoal Creek near the Talladega National Forest Road 500 crossing, in May 2011 (n = 5), January 2012 (n = 3), and from Shoal Creek near the Cleburne County Road 61 crossing (33°46’14.57"N, 85°33’20.59"W), as well as from the South Fork of Terrapin Creek near the Cleburne County Road 55 crossing in August 2011 (Table 3). A separate sample of *V. nebulosa* was collected from pond and recirculating culture systems of the Alabama Aquatic Biodiversity Center (AABC; Marion, Alabama) in July 2010 (n = 20), March 2011 (n = 10) and October 2011 (n = 5) (Table 2). Hatchery-reared specimens of *Strophitus connasaugaensis* were also collected from a pond culture system at the AABC in March 2011 (n = 10) (Table 4).

In the laboratory, each mussel was measured, photographed, and, to minimize the possibility of damaging the microtome blade and simultaneously causing chatter or tears in the paraffin sections, allowed to purge sand and particulate matter for 24 h before fixation. Prior to fixation, the shell of each living mussel was propped open with a wooden dowel to enable adequate infiltration of fixative into the visceral tissues. Mussels were then immersed in 10% neutral buffered formalin for 48 h, rinsed in tap water to remove buffer salts from tissue, and dehydrated in a graded series of ethanol using a Tissue-Tek VIP E300 tissue processor (Sakura Finetek, Inc., Tokyo, Japan). Formalin-fixed mussels with shell then were de-shelled by excising mantle and musculature from the nacre with aid of a scalpel and divided into

<table>
<thead>
<tr>
<th>Tissue types</th>
<th>Mantle edge</th>
<th>Middle mantle</th>
<th>Mantle isthmus</th>
<th>Non-marsupial gill</th>
<th>Marsupial gill</th>
<th>Foot</th>
<th>Pedal protractor/retractor</th>
<th>Adductor</th>
<th>Labial palp</th>
<th>Esophagus</th>
<th>Digestive diverticulum</th>
<th>Stomach</th>
<th>Crystalline style sac</th>
<th>Intestine</th>
<th>Cerebral ganglion</th>
<th>Pedal ganglion</th>
<th>Visceral ganglion</th>
<th>Statocyst</th>
<th>Nerve</th>
<th>Heart</th>
<th>Hemolymph vessel</th>
<th>Pericardial gland</th>
<th>Nephridium</th>
<th>Ovarian acinus</th>
<th>Testicular acinus</th>
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</table>
TABLE 2. Size, site, and collection dates of Alabama rainbow (Villosa nebulosa) specimens used for histology. *Dorsum sectioned in a coronal plane, gonad not sectioned; †Embryos and/or glochidia present in marsupia.

<table>
<thead>
<tr>
<th>Specimen code</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Height (mm)</th>
<th>Sex</th>
<th>Collection site</th>
<th>Collection date</th>
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</table>

pieces by cutting through the visceral mass with aid of a grossing knife. The mussels sampled for histology consisted of 19 wild and four cultured V. nebulosa (Table 1), 29 F. cerina (Table 2) and 12 wild and 2 cultured S. connasaugaensis (Table 3): yielding 57 blocks and 416 slides from V. nebulosa, 111 blocks and 397 slides from F. cerina, and 49 blocks and 376 slides from S. connasaugaensis. Typically, a mussel having a shell length of 40–50 mm would render 4–6 pieces of viscera from anterior to posterior. Each sample was placed into a labeled mega tissue cassette and processed for routine paraffin embedding using the aforementioned automated tissue processor and a Tissue-Tek Thermal Console 4585/7 (Sakura Finetech, Tokyo, Japan). Following tissue processing, pieces of visceral mass were embedded in a mega base mold to obtain a whole dorsal to ventral profile of the body.

Before sectioning, paraffin blocks (especially needed for the visceral mass) were immersed for 1–2 min in cold water (5°C) immediately before sectioning. Paraffin blocks were serially sectioned at 4 μm thickness using a Reichert-Jung Biocut 2030 microtome (Wetzlar, Germany), immediately thereafter moved to a Boekel Scientific 145701 lighted tissue floatation bath water (Feasterville, Pennsylvania) at
TABLE 3. Size, site, and collection dates of Gulf pigtoe (*Fusconaia cerina*) specimens used for histology. *Only a small sample of gonad sectioned; †Embryos and/or glochidia present in marsupial; ‡Acini contained a mixture of small, immature ova, and larger degenerative ova.

<table>
<thead>
<tr>
<th>Specimen code</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Height (mm)</th>
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43°C and pre-mixed with histology adhesive, and lifted with glass slides. Slides with paraffin sections were heated to 63°C for 45 min to remove excess paraffin, stained in an Sakura Finetek automated slide stainer with fume hood (Tiyoda MFG, Torrance, California, U.S.A.) using Harris’s hematoxylin and eosin as per Luna (1968). Stained slides were photographed using a digital single lens reflex camera mounted on a Leica DM 2500 compound microscope (Wetzlar, Germany).

Each mussel was identified to species by the anatomical diagnostic features provided by Burch (1973) and Williams et al. (2008). Nomenclature and higher level systematics of Unionoida follows Graf & Cummings (2007), of *Galba truncatula* follows Bank (2004), and of marine representatives of Bivalvia follows Appeltans et al. (2012). Morphological terms for Unionidae follows Giribet & Wheeler (2002), Graf & Cummings (2006) and Cummings & Graf (2010). Histologi-
cal terminology for Unionidae follows Ridewood (1903, gill), Gutheil (1912, digestive system), Nelson (1918, digestive system), Motley (1932, cardiovascular system), Atkins (1937, gill), Beedham (1958, mantle), Galtsoff (1964, renal system), Sumner (1966a, digestive system), Yokley (1968, foot, nervous system), Brand (1972, cardiovascular system), Kraemer (1978, nervous system), Woody & Holland-Bartalls (1993, stages of gametogenesis) and Henley (2007, reproductive system). General histological terminology follows Dorland (2007). Shell vouchers for each individual mussel sectioned are retained in the collection of the Aquatic Parasitology Laboratory, Auburn University.

**ANATOMICAL PART**

**Villosa nebulosa**

Shell Morphology

Valves of *Villosa nebulosa* are elliptical, moderately thin and slightly compressed. The epidermis is yellow to light brown with thick, green to black interrupted rays. The anterior margin is narrow, rounded and the posterior margin is bluntly pointed to trapezoidal. The dorsal margin is somewhat convex and the ventral shell margin is straight to slightly convex. The umbo is low directed slightly anteriorly. The posterior ridge is low, not deeply furrowed and the posterior slope is flat to concave (Figs. 1, 2) (Williams et al., 2008). The nacre is silver to white with an elliptical pallial line. The anterior adductor scar is deeply inset, perpendicular to the long axis of the shell. The posterior adductor scar is flat and obliquely oriented to the long axis of the shell. The pseudocardinal teeth are triangular, with two slightly divergent teeth on the left valve and one tooth on the right valve (Figs. 3, 4) (Williams et al., 2008).

Gross Anatomical Features of the Mantle Cavity

Unionids can form a reduced and incomplete siphon comprising a pair of incurrent and excurrent apertures. Apertures are formed when the sinistral and dextral mantle edges are cupped and slightly overlap. The incurrent aperture of *V. nebulosa* is papillose, with uniramous, fine

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**TABLE 4. Size, site, and collection dates of Alabama creekmussel (*Strophitus connasauagaensis*) specimens used for histology. *Glochidia present in marsupial.**

<table>
<thead>
<tr>
<th>Specimen code</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Height (mm)</th>
<th>Sex</th>
<th>Collection site</th>
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"Glochidia present in marsupial."
papillae and with shorter papillae along the excurrent aperture. Species of *Villosa* have a mottled mantle edge with brown and black transverse bars and the mottling is most pronounced along the siphonal apertures (Williams et al., 2008). Incurrent and excurrent apertures are partitioned by when the mantle edge is slightly curled and forms trapezoidal flaps (Fig. 5).

The mantle edge is pale, orange and mottled with black transverse bars that become conspicuous posteriorly along the siphonal apertures. The middle mantle extends from the pallial line to the umbo cavity and it is pallid, translucent, skin-like tissue. The mantle isthmus is translucent and trapezoidal, extending dorsally into a cleft between the left, right valves. The gills are
translucent, elongated sheets extending into the mantle cavity. The gills are comprised of two pairs of demibranchs located between the mantle and visceral mass. Each demibranch consists of a series of transversely oriented, ciliated filaments (Figs. 6, 7). Outer gills and inner gills resemble each other in males, but females have an enlarged group of water tubes located along the posterior third of the outer gill. Marsupia have a blackened ventral margin, and the marsupium becomes distended when filled with glochidia (Fig. 7). The apertures are sexually dimorphic; the apertures are flattened in males except for a series of fine branchial papillae. Females however, have enlarged, brown papillae (Fig. 7). The foot of *V. nebulosa* is orange, and there is a black spot located at the posterior margin. The visceral mass is white and extends dorsally to the base of the gills. Labial palps are translucent, triangular lips that extend laterally from the mouth between the foot and visceral mass. The anterior adductor is pale, white, oval in outline, and oriented vertically between the anterior shell margin and foot. The posterior adductor is grossly identical to the anterior adductor but is parallel with the posterior shell margin (Fig. 8).

**Anterior Mantle Edge**

The free distal margin of the mantle edge is lobular, with three epithelial folds, or mantle lobes, extending medially into the mantle cavity (Fig. 9). From ventral to dorsal, there is an outer lobe, a middle lobe and an inner lobe. Mantle lobe morphology varies throughout the length of the mantle edge, but for simplicity only a representative area from the anterior and posterior end will be described. A conspicuous component of the mantle edge is a ribbon of periostracum secreted by a group of cells called the basal bulb located at the base of the outer lobe (Figs. 9–12). Periostracum bends around a semicircular groove called the periostracal groove and extends medially towards the mantle cavity (Figs. 11, 12). Outer lobe is characterized by a flattened ventral margin of columnar cells (Figs. 9, 15), and the epithelium becomes pleated along the dorsal surface for approximately half the length of the outer lobe (Figs. 9, 11, 12). The epithelium becomes pleated again from the base of the middle lobe and plicae extend along the ventral surface of the mantle edge to the junction of the middle mantle (Figs. 9, 13, 14). Columnar epithelial cells of the outer lobe are characterized by a basophilic cytoplasm, and an oval, monochromatic nucleus (Fig. 11). The simple columnar epithelium terminates at the basal bulb, where it abruptly transitions to a simple, squamous epithelium (Fig. 10). The middle lobe is ventrally flattened, with a simple squamous epithelium, transitioning to columnar epithelium from the distal margin where the slope begins to decline dorsally. The outer lobe epithelium, cells lining the middle lobe have monochromatic nuclei, but the cytoplasm is eosinophilic (Fig. 12). The base of the middle lobe is plicate, with several short, broadly rounded epithelial folds. Plicae span the length of the dorsal mantle.
edge surface from the inner lobe to the pallial line, where the dorsal surface of the mantle edge meets the nacre. However, plicae are irregularly spaced and the shape and size of each fold is variable (Fig. 13). Columnar cells continue throughout the length of the mantle edge to the pallial line. In contrast to the columnar epithelium of the outer lobe, cells representing the inner lobe have brown granular inclusions (Fig. 14).

Muscle tissue comprises the main histological constituent of mantle edge with a central region of adipose tissue (Figs. 14, 15). Fibers are organized into dense bands in the straightened, proximal portion of the mantle edge (Fig. 9) and become less regular between each lobe. Small groups of fascicles are scattered throughout a region of adipose tissue in the proximal portion of the mantle edge. Muscle fibers in the outer and middle mantle lobes are wavy but generally regular (Figs. 11, 12). Myofibers in the inner lobe become branched and overlap each other (Fig. 13). Additionally, there are spherical cells, with a basophilic, granular cytoplasm located directly beneath the epithelium of the middle and inner lobes (Fig. 13).

**Posterior Mantle Edge, Middle Mantle and Mantle Isthmus:** The mantle edge becomes blackened near the posterior terminus of the visceral mass (Figs. 6–8). Epithelial cells in this region contain brown or black granules, which may be melanin. The concentration of granules in a cell may be so great that the nucleus and cytoplasm may be occluded (Figs. 16–18). Mantle lobe morphology near the posterior adductor muscle is considerably different from the morphological features characterizing the lobes near the labial palps.

In the posterior region of the mantle edge, the outer and middle lobes are a forked extension of mantle tissue with each fork appearing equally long. The inner lobe is bulbous and enlarged, with conspicuous plications that have deeper crypts between each peak. Furthermore, epithelial folds around the inner lobe range from single lamellae to forked or branched papillae, giving the inner lobe a high surface area (Fig. 16). Branchial papillae are thin, conical extensions of the mantle located near the narrow, posterior shell margin. Papillae consist of an irregular matrix of muscle and connective tissue fibers surrounding an irregularly shaped hemolymph sinus. Papillae have a highly pleated surface with obliquely oriented epithelial folds directed towards the mantle cavity (Figs. 17, 18).

As the mantle tissue separates from the visceral mass, it is composed of simple, cuboidal epithelium along the shell side, and simple, squamous epithelium along the visceral side (Fig. 19). Middle mantle subepithelium consists of loose connective tissue and hemolymph. The isthmus is a tear drop-shaped extension of the mantle located dorsally in relation to the visceral mass. It originates near the esophageal-digestive gland junction and terminates posterior to the posterior adductor. The isthmus has an irregular surface with simple columnar epithelium and a fibrous subepithelium consisting of muscle and connective tissue. Columnar cells of the isthmus are basophilic with a monochromatic, elliptical nucleus (Figs. 20, 21).

**Cellular Structure of Gill**

**Non-Marsupial Demibranch:** Gills, ctenidia or demibranches of freshwater mussels

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FIGS. 9–15. Anterior mantle edge of *Villosa nebulosa*. FIG. 9: Transverse section of the anterior portion of the mantle edge showing the position of the outer lobe (OL), periostracal ribbon (PE), middle lobe (ML), and inner lobe (IL); FIG. 10: Transverse section of the mantle edge emphasizing columnar epithelium (CE) of the outer lobe, basal bulb (BB), periostracal groove (PG), squamous epithelium (SE) of the middle lobe, and periostracum (PE); FIG. 11: Transverse section of the distal end of outer and middle mantle lobes where obliquely oriented plicae (PL) line the outer lobe and a periostracal ribbon (PE) leaves the interlobular space; FIG. 12: Transverse section of the middle lobe showing plicae (PL), periostracum (PE), squamous epithelium (SE), violet granulocyte (VC), columnar epithelium of the dorsal surface (CE), and brown intracellular granules (BG); FIG. 13: Transverse section of inner lobe featuring plicae (PL), violet cells (VC), and myofibers (MF); FIG. 14: Transverse section of the dorsal part of the inner mantle edge emphasizing plicae (PL), ciliated columnar cells (CC), goblet cells (GC), and adipocytes (AC); FIG. 15: Transverse section of ventral epithelium of the base of the mantle edge showing columnar epithelium (CE), myocytes (MC), and adipocytes (AC).
HISTOLOGICAL ATLAS OF FRESHWATER MUSSELS 111

(Unionoida) are paired structures extending into the mantle cavity between the mantle and visceral mass. Outer demibranchs are located medially to the mantle while inner demibranchs are lateral to the visceral mass. Each demibranch consists of an ascending and descending lamella bearing a series of cylindrical filaments running vertically along the lamellar surface. The lateral lamella of an outer demibranch is referred to as ascending whereas the medial lamella of an outer demibranch is referred to as descending. Concerning the inner demibranch, the lateral lamella is descending and the medial lamella is ascending. Ascending and descending lamellae are united by a series of interlamellar septa. When viewed in a transverse orientation, demibranchs are triangular such that one set of outer and inner demibranchs resembles a “W” (Ridewood, 1903; Brusca & Brusca, 2002; Allaby, 2003).

Demibranchs of *V. nebulosa* are non-plicate, with the main functional aspect of a ctenidium consisting of horizontally oriented, cylindrical filaments. Filaments are occasionally separated by pores called ostia (Figs. 22, 23). The inner and outer lamellae of a demibranch are generally separated from each other along the anterior-posterior axis and only joined by a small number of interlamellar septa (Fig. 22). The space between each lamella accommodates water entering through ostia and is referred to as a water tube (Fig. 23). Water travels dorsally and enters the suprabranchial cavity at the dorsal terminus of each demibranch. The superbranchial cavity is a conduit for vertical water currents created by the lateral expansion of the inner and outer lamellae. Each gill is supported with loose connective tissue and large fascicles of muscle located dorsal and lateral to each suprabranchial cavity (Figs. 26–28).

Branchial filaments are cylindrical, bearing cilia organized into three groups (lateral, lateral-frontal, and frontal) along the lateral and distal surfaces. A single layer of squamous cells extends along the lateral and medial portions of each filament. Each filament is supported by a set of basophilic skeletal rods located directly under the squamous epithelium, and a pale eosinophilic matrix may be observed throughout the long axis of each filament. The medial portion of each filament contains a hemolymph sinus, or lacuna, where a preponderance of hemocytes is typically observed (Fig. 23). The inner and outer demibranchs appear to be functionally identical (except for marsupial demibranchs, see below) until the free distal tip. Outer demibranchs have a rounded ventral margin (Fig. 24) while the inner demibranchs have a furrow along the ventral surface (Fig. 25).

Ascending lamellae of inner demibranchs are united with the visceral mass anteriorly and become disconnected posteriorly at the gonopores. Ascending lamellae of inner demibranchs are fused posteriorly between the posterior margin of the visceral mass and posterior adductor (Fig. 26). Descending lamellae of inner demibranchs and descending lamellae of outer demibranchs are united with the abdomen. Furthermore, the gill tissue in this region is supported by fascicles spanning the length of the body (Fig. 27). Ascending lamellae of outer demibranchs are united with the abdomen in a medial position to the middle mantle (Fig. 28).

*Marsupial Demibranch*: Female *V. nebulosa* may be distinguished from males in having

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FIGS. 16–21. Posterior mantle edge, middle mantle, and mantle isthmus of *Villosa nebulosa*. FIG. 16: Sagittal section of posterior mantle edge showing a thin outer lobe (OL) and middle lobe (ML) and a bulbous inner lobe (IL) featuring conspicuous plications (PL); FIG. 17: Sagittal section of posterior mantle edge characterized by conical papillae (PA) with deep, obliquely oriented plications (PL) along the surface; FIG. 18: Sagittal section of a papilla emphasizing a columnar epithelium containing brown intracellular granules (BG) as well as an irregular composition of connective tissue fibers (CT) and hemolymph sinuses (HS) within the subepithelium; FIG. 19: Transverse section of middle mantle displaying a outer epithelium (OE) of columnar cells, inner epithelium (IE) of squamous cells, cilia (CI), subepithelial connective tissue (CT), and hemocytes (HC); FIG. 20: Transverse section of mantle isthmus (MI) portraying a thin dorsal extension of tissue from the visceral mass (VM) and a terminal, dorsally located bulb (BU); FIG. 21: Transverse section of mantle isthmus bulb is emphasizing a basophilic columnar epithelium (BE) with occasional goblet cells (GC) and a subepithelium consisting of irregular muscle fibers (MU).
a localized enlargement of the outer demibranch known as a marsupium (Figs. 7, 29). When gravid, the posterior quarter of the ctenidia becomes distended and comprises the distal half of its vertical length. A marsupial demibranch structurally differs from both the anteriorly located non-marsupial outer demibranch, and inner demibranch in many respects. The most obvious characteristic of marsupial gill are the greatly expanded water tubes, which become progressively larger dorsal to ventral. In one individual collected in May 2010, gills were asymmetrically filled allowing a comparison between filled and empty marsupia. The filled marsupium was appeared turgid and symmetrical, while the empty marsupium was flaccid from partially collapsed water tubes (Fig. 29). Glochidia within a filled marsupium appear to be held together by strands of fibrous tissue, possibly representing a binding substance of a conglutinate of embryos (Fig. 30). Walls of marsupial water tubes may be pleated with ciliated plicae. Ciliated plicae also occur along the septa at the base of the marsupium (Fig. 31). The interlamellar septa of the ventral marsupial chambers have a fibrous composition with a lattice of connective tissue (Fig. 32). The epithelium representing the lateral surface of a marsupium appears to be less porous than non-marsupial gill tissue. In contrast to non-marsupial demibranchs, the subepithelium along the vertical surface of the water tubes as well as the septa of marsupia is thickened with connective tissue fibers and a pale staining, ground substance (Figs. 30, 32, 33). The luminal surface of the water tubes is lined with teardrop-shaped, ciliated columnar cells. Ciliated cells lining the lumen of marsupial water tubes contain brown intracellular granules, which may explain why the ventral margin of marsupium is blackened (Figs. 7, 30, 32).

Marsupia may contain a mixture of glochidia and spherical to ovular masses of cells representing embryos in various stages of development. Early stage embryos consisted of clusters of eosinophilic, spherical cells within a shapeless, eosinophilic mass. Each cell contains a spherical nucleated mass, surrounded by a heterochromatic, eosinophilic matrix (Fig. 34). Later stage embryos were more differentiated than the largely eosinophilic cellular masses just described. Later stage embryos were ovular, possessing a spherical mass of myocytes, and distinct dorsal and ventral end characterized by basophilic cells. Glochidia were semi-circular with a centrally located adductor muscle flanked by falcate lines of eosinophilic to basophilic tissue presumably representing the mantle (Fig. 35).

Cellular Structure of Foot and Associated Tissues

Pedal Musculature and Byssal Gland: The ventral margin of the foot consists of irregular, overlapping muscle fibers, subepithelial granulocytes, and a highly plicated integument (Fig. 36). Violet staining granulocytes are located directly beneath the pedal epithelium, while pale, blue-staining granulocytes are medial in respect to violet cells (Fig. 37). Foot muscle is composed of regions of irregular and regular muscle fibers. Musculature in the ventral region of the foot has a woven appearance with bundles of fibers arranged

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FIGS. 22−28. Outer demibranch, and inner demibranch of *Villosa nebulosa*. Fig. 22: Transverse section of inner demibranch displaying transversely oriented filaments (FI), a septum (SE) linking the inner and outer lamina, and a water tube (WT); Fig. 23: Transverse section of the inner lamina of inner ctenidia showing the main tissue components including filament (FI), lateral cilia (LC), frontal lateral cilia (FL), frontal cilia (FC), skeletal rods (SR), and hemolymph sinuses (HS), in addition to ostium pores (OS), and corresponding water tube (WT); Fig. 24: Transverse section of the distal tip of outer gill characterized by a rounded distal margin (DM), resembling the merger of two or more filaments (FI); Fig. 25: Transverse section of the free distal inner gill tip characterized by a bending layer of filaments (FI) around the distal margin (DM) creating a pleated surface; Fig. 26: Transverse section of the inner lamina (IL) and outer lamina (OL) of the inner ctenidia joined separately at the base of the visceral mass (VM); Fig. 27: Transverse section of ctenidia where muscle fascicles (MU) are aligned anterior to posterior where the inner lamina (IL) of inner gill and outer lamina (OL) of outer gill unite with the abdomen (AB); Fig. 28: Transverse section of gill revealing musculature (MU), outer lamina (OL) of outer gill, and mantle (MA).
into sagittal and transverse planes, giving the tissue a woven appearance (Fig. 38).

In contrast, musculature of the base of the foot is more organized with distinct layers of transverse and sagittal fibers (Fig. 39).

The byssal gland is triangular, located medi ally at the ventral margin of the coelom, just posterior to the pedal ganglion. The byssal gland has a wide, triangular dorsal chamber and a smaller, ovoid ventral chamber (Fig. 40). The luminal surface of the byssal gland features a simple, ciliated columnar epithelium, and these cells have an eosinophilic cytoplasm and a monochromatic, ovolar nucleus. The lumen of the dorsal chamber contains a heterochromatic, eosinophilic mass, possibly representing the remnants of the byssal thread (Fig. 41). The byssal gland of adult *V. nebulosa* is blind-ended, not connected to a dorso-ventral, byssal canal. The byssal canal consists of a series of disconnected, ciliated ducts located medially throughout the pedal musculature. Remnants of the byssal canal have a ciliated columnar epithelium and these cells are surrounded by a pale, eosinophilic matrix of fibrous connective tissue. These ciliated remnants of the byssal canal have an irregular shape and a narrow lumen (Fig. 42).

**Pedal Integument and Mesentery:** The integument of the foot and visceral mass is pleated, and there are five different epithelial regions ventral to dorsal. In the first two regions, the epithelium appears to be folded and each fold is rounded with some resembling a “Y”. The first region possesses ciliated, columnar cells with a subepithelium consisting of irregular muscle fibers and violet granulocytes (Fig. 43). Ciliated cells have an eosinophilic cytoplasm, similar to the coloration of the underlying muscle tissue, and cilia are short and nearly straight. Epithelial cells in the second region have similar cytological characteristics as region one, however ciliated cells are sparsely distributed. Additionally, muscle tissue beneath the epithelium is generally more regular than in region one, and with fewer violet chromatocytes (Fig. 44). Region three possesses shorter epithelial folds, sparsely distributed ciliated cells, and mucus cells. Unlike epithelia of the first two regions, the basal portion of region three epithelial cells is parallel to the underlying musculature. Muscle underlying type-three epithelium consists of an outer, thin, transverse layer and a deeper, thicker, sagitally oriented layer (Fig. 45). The fourth and fifth regions are dorsally located along the oblate portion of the visceral mass. The fourth type of integument surrounding the foot is similar to the third region, but is more irregular and a dark hemolymph sinus is located between the epithelium and muscle (Fig. 46). Region five is located near the junction between visceral mass and inner gill, and has the shortest epithelium of all five regions. Cells representing region five vary in height from a flattened, squamous type to slightly taller, domed to tear drop shaped. Mucus secreting cells also vary in morphology from dome-shaped to tear drop cells (Fig. 47). In a longitudinal orientation the pedal integument has numerous irregular cavities. Additionally, the columnar epithelium at the location of the black spot features brown intracellular granules (Figs. 8, 48).

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**FIGS. 29−35.** Marsupium, and embryonic development of glochidia of *Villosa nebulosa.*** FIG. 29: Transverse section of an empty marsupium revealing distended water tubes (WT), thickened septa (SE), and reduced filaments (FI) along the distal margin; **FIG. 30:** Transverse section of a marsupium portraying a wall of concentric musculature (MU), and brown-pigmented columnar cells (BC), and a central mass of fragmented to intact glochidia (GL) enclosed by gray, wispy filaments (FL), possibly representing a substance that binds glochidia together within the conglutinate; **FIG. 31:** Transverse section of the marsupium at its dorsum, characterized by a thickened base of fibrous connective tissue (CT) and a series of ciliated papillae (CP) extending into the lumen; **FIG. 32:** Transverse section of a marsupial water tube septum emphasizing a lattice of connective tissue fibers (CT), and brown-pigmented, ciliated cells (BC); **FIG. 33:** Transverse section of a marsupium showing ctenidial filaments (FI) supported by a continuous subepithelium of connective tissue (CT); **FIG. 34:** Transverse section of a marsupium containing a mixture of early stage embryos (EE) resembling a mass of spherical cells, and late stage embryos (LE) bearing a resemblance to glochidia; **FIG. 35:** Transverse section of a marsupium portraying an intact glochidium with well-defined, semicircular valves (VA) and a median adductor muscle (AM).
The left and right halves of the visceral mass are united by bundles of mesentery and mesentery fascicles are distributed throughout the hemocoel (Fig. 49). Each fascicle has a heterogeneous composition in terms of the staining characteristics and geometry of its fibrils. Fibrils are eosinophilic, but staining intensity varies from a pale, almost white, to dark red. Each filament is quadrangular, but varies in shape from square, to a triangular. Furthermore, there does not appear to be an obvious pattern of fibril arrangement within a fascicle (Fig. 50).

**Labial Palps:** Labial palps of *V. nebulosa* have an inner palp surface lined with a series of ciliated, rectangular plicae, whereas the outer surface is generally smooth (Fig. 51). Plicae have a flattened posterior margin and a pleated anterior surface (Fig. 52). Ciliated columnar cells represent the main constituent of the inner palp epithelium and extend along the palp interior until the distal margin of each lip. Ciliated cells of the inner palp surface are characterized by a pale, basophilic cytoplasm and an ovular, monochromatic nucleus. The surface of the free distal palp tip transitions from a ciliated epithelium to a non-ciliated mucosa characterized by basophilic, granular mucus cells (Fig. 53). Although the outer surface of each palp is generally flattened, the integument is composed of teardrop-shaped columnar cells. Teardrop cells of the outer palp surface possess an ovular monochromatic nucleus, an eosinophilic cytoplasm, and a transparent, apical vesicle (Fig. 54). Subepithelial tissue of the palps consists of a somewhat dense matrix of pale, eosinophilic fibers within a membranous ground substance, especially the supporting tissue directly beneath the epithelium. However, the medial layers of each palp have a more loose consistency. Additionally, there are a series of well-defined hemolymph sinuses, and wandering hemocytes are widely distributed throughout the subepithelium.

**Anterior Adductor and Anterior Pedal Retractors:** The anterior pedal retractors are located medial to the labial palps and may be observed in histological sections of palp. Retractor muscle is dark staining and eosinophilic, with distinct fascicles and fasciculi. Furthermore, fibrils are quadrangular and separated by endomysium, however perimysium appears to be more delicate since there are pale, eosinophilic wisps adjacent to fascicles and fasciculi (Fig. 55). Adductor muscle is organized into large fascicles containing dark, red eosinophilic fibers. Adductor myocytes are filamentous with a pale, granular, basophilic nucleus (Fig. 56).

**Oral Groove and Esophagus:** In transverse sections of the anterior visceral mass, the oral groove resembles a U-shaped canal, traveling dorsally from the labial palps. Oral groove epithelium consists of transversely pleated, ciliated tracts. In a sagittal plane, oral groove appears multinucleated since the walls of the oral groove are curved. Oral groove epithelium is mucociliary with a preponderance of transparent vesicles, especially along the folds (Fig. 57). Additionally, there are wandering hemocytes nestled between columnar cells of the ventral oral groove wall (Fig. 58). Subepithelial tissue of the oral groove consists of a loose meshwork of thin connective tissue fibers and hemocytes. A single layer of cuboidal epithelial cells appears ventrally.
in sections of oral groove, and represents a short stretch of integument between the oral groove and foot (Fig. 59).

The esophagus is essentially a posterior continuation of the oral groove, a short tube connecting the oral groove with the stomach and digestive gland. The esophagus is located at the dorsal end of the visceral mass and is generally between digestive gland tubules and the mantle isthmus (Fig. 60). Ciliated, rectangular folds extend from the dorsal and ventral esophageal walls. Epithelial cells are pseudostratified and ciliated, with an eosinophilic cytoplasm and an elliptical, monochromatic nucleus. Cilia along the apical surface of esophageal cells are short and generally straight. The lumen of the esophagus may contain a shapeless, eosinophilic mass, which may represent mucus and ingested substances. Additionally, the esophageal epithelium is supported by concentric layers of pink and red eosinophilic fibers, creating a heterogeneous lamina propria (Fig. 61).

**Cellular Structure of the Alimentary Canal**

**Digestive Diverticulum:** The esophagus expands laterally into a vestibular chamber at the visceral mass dorsum. Particulate matter is transported, by means of ciliary action, through small tubules located on the left and right sides of the body (Fig. 62). There are three types of tubules distinguished by variations in the diverticulum lining. Ciliated primary tubules have folds of epithelial tissue that give tubule walls a wavy or serrated appearance (Figs. 62–64). Primary tubules consist of eosinophilic, columnar cells with cilia. Additionally, primary tubules have low, broadly rounded plicae (Fig. 64). Ciliated epithelium transitions into a microvillar lining in the secondary tubules. Secondary tubules connect to tertiary tubules, which are the most widely distributed diverticulum tubules. Microvillar cells of secondary tubules are characterized by a red, vesiculated cytoplasm, and a pale, basophilic nucleus with a distinct nucleolus (Fig. 65). Tertiary tubules have two types of cells. The most abundant cell type is the vesiculated cell. These cells have a pale, eosinophilic cytoplasm and numerous intracellular vesicles. The second cell type is triangular with a dark staining, basophilic cytoplasm, and a dark staining nucleus containing a distinct nucleolus (Fig. 66).

**Stomach:** The stomach is a large bag-like chamber located between the digestive diverticulum and style sac. The stomach is somewhat dorsoventrally flattened, with plicated walls and a gelatinous, eosinophilic matrix within the lumen (Fig. 67). The ventral stomach wall features a columnar epithelium with an eosinophilic cuticle (Fig. 68). The lumen may contain a mass of chyme. The composition of chyme may be an irregular eosinophilic mass that has a slightly lighter staining characteristic as compared to the cuticle. Chyme may also consist of darker, eosinophilic granules or spherical objects (Fig. 68). The columnar epithelium has a dense cuticle, embedded in cilia, that is rela-
tively uniformly distributed across the apical surface of the epithelium. The cuticle represents the gastric shield and it lines dorsal and ventral surfaces at the posterior end of the stomach. Columnar cells are eosinophilic with an elliptical nucleus and a distinct nucleolus (Fig. 69). The medial aspect of the ventral wall consists of a series of irregular plicae (Fig. 70). The cuticle disappears dextrally where the epithelium becomes pleated (Fig. 71). Plicae are also located along the dorsal aspect of the stomach. Dorsal plicae are tall and narrow in comparison to plicae along the ventral wall (Fig. 72). Additionally, cells lining the dorsal gastric wall may appear vesiculated and may be secretory in nature. The sinistral wall features a rectangular typhlosole extending into the lumen. The typhlosole is slightly forked and possesses a gelatinous cuticle (Fig. 73).

**Crystalline Style Sac:** The crystalline style is generally a cylindrical rod spanning the visceral mass from the stomach to the posterior margin of the visceral mass. The diagnostic character of the style sac is a pale, eosinophilic rod, the style, within a circular chamber on the sinistral side. The style may be closely associated or attached to ciliated cells along the ventral sac wall, and there are three different types of epithelia throughout the style sac. The crystalline style sac has a lamina propria, and it varies from a thickened connective tissue support underneath the epithelial folds of the style sac, to a thin line of connective tissue surrounding the style sac (Fig. 74).

Type one epithelium is located within the circular portion of the sac and consists of ciliated columnar cells distinguished by a red cytoplasm and a heterochromatic, elliptical nucleus with a distinct nucleolus. The cell membrane along the apical surface of these cells is a thick line and cilia of type one cells are straight and thickened in comparison to cilia of type two epithelia (Fig. 75). Type-two epithelium originates along the left side of the median ventral fold and terminates near the fork. Ciliated cells characterizing this region have a basophilic cytoplasm, an elliptical, heterochromatic nucleus with a distinct nucleolus. The apical portion of the cell membrane is noticeably thinner than the apical membrane of type one cells, and cilia appear to be more flexible than type-one cilia. The ventral fork of the style sac also has a series of ellipsoid, basophilic mucocytes interspersed among ciliated epithelial cells (Fig. 76). The dorsal wall of the style sac, dextral to the median fold, is characterized by a basophilic columnar epithelium. Cells in type-three epithelium appear to be densely packed, supporting thin, flexible cilia similar to cilia featured in type-two epithelium (Fig. 77). The style is eosinophilic, and homogenous except for a series of fine circumferential rings, which may contain fine, black particulate matter (Fig. 78).

**Intestine:** The crystalline style represents the first limb of the intestine as it curves dorsally along the posterior margin of the visceral mass. The epithelium of the first intestinal limb maintains the same cell types characterizing the horizontal portion of the style sac (Fig. 79). However, the epithelium has large plicae extending into the lumen and the style sac epithelium is convoluted such that each cell type of the style sac may be located on the anterior or posterior wall. The lumen of the descending intestine contains an eosinophilic material likely representing chyme. Within crypts of adjacent folds, and around the distal tips of plicae, chyme appears broken up or shaped according to the contours of the tissue (Fig. 80).

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**FIGS. 51–56. Labial palps, anterior pedal retractor, and anterior adductor of *Villosa nebulosa.* FIG. 51: Sagittal section of labial palps revealing inner plicated surface (IS), distal palp margin (DM), outer palp surface (OS), anterior pedal retractor (PR); FIG. 52: Sagittal section of the inner palp surface featuring plicae (PL), cilia (CI), and hemocytes (HC) in the subepithelium; FIG. 53: Sagittal section of distal labial palp margin featuring cilia (CI), goblet cells (GC), and connective tissue (CT), hemolymph (HL), and hemocytes in the subepithelium; FIG. 54: Sagittal section of outer palp surface highlighting tear drop-shaped columnar cells (TC), and subepithelial connective tissue (CT), and hemolymph sinuses (HS); FIG. 55: Transverse section of anterior pedal retractor showing a fascicle (FA), with a thin perimysium (PM), and dark-staining myocyte nuclei (MN); FIG. 56: Sagittal section of anterior adductor showing the thin myofibers (MF), and oval myocyte nuclei (MN).**
The second and third intestinal limbs, as seen in transverse sections, are located ventrally in relation to the style sac. The second and third intestinal limbs are characterized by the presence of small to large plicae and both intestinal limbs are histologically identical (Fig. 81). In a longitudinal orientation, the intestine arches along the posterior margin of the visceral mass. The epithelium is lined with a regular series of plicae that appear to
be somewhat “Y”-shaped (Fig. 82). Further down the length of the initial limb, villi are replaced by shorter epithelial extensions that are generally plateau-shaped. The lower portion of the first intestinal limb appears to be an area of active secretion and mixing. Plateaus along the anterior and posterior walls are vesiculated and releasing transparent bubbles into the lumen. The center of the lumen has a large mass of fused bubbles surrounded by series of crescent-shaped bubbles (Fig. 83). However, the main constituent of the second and third intestinal limb is the ciliated columnar epithelium. Columnar cells have a

FIGS. 62–66. Digestive diverticulum, and stomach of *Villosa nebulosa*. FIG. 62: Transverse section of the junction between the esophagus (ES) and digestive diverticula highlighting ciliated tubules (CT) leading toward digestive tubules (DT); FIG. 63: Transverse section of digestive diverticulum emphasizing a transition from ciliated primary tubules (PT), to vesiculated secondary tubules (ST); FIG. 64: Transverse section of digestive diverticulum portraying fine structure of primary tubules including cilia (Cl); FIG. 65: Transverse section of the digestive diverticulum showing microvilli (MV) of secondary tubules; FIG. 66: Transverse section of digestive diverticulum showing the basophilic basophil cells (BC), and vesiculated digestive cells (DC).
pale, eosinophilic cytoplasm, and an ovular nucleus with a distinct nucleolus (Fig. 84).

In sagittal sections of the viscera, the second intestinal limb is posterior to the first limb. The lining of the second intestinal limb consists of short plicae that are generally Y-shaped. Between each Y-shaped structure, there may be a triangular cusp creating a set of oblique crypts between each Y-shaped unit (Fig. 84). Ciliated columnar cells are narrow, eosinophilic with a distinct nucleus within each nucleus. Additionally, wandering hemocytes are present within the epithelium of the second intestinal limb and typically resemble a spherical mass of eosinophilic vesicles.

The most distinctive feature of the fourth intestinal limb is the centrally located, ventrally directed major typhlosole (Fig. 85). Surrounding the typhlosole, the ventral lining of the intestine is pleated with a series of flattened epithelial folds. The height of each fold, and the length and width of the crypts increases from the base of the typhlosole to the ventrolateral region. Epithelial tissue surrounding the lumen consists of ciliated columnar cells. In general, the cells lining the ventral and dorsal surfaces have similar characteristics. However, the ventral epithelium has a preponderance of red, elliptical granulocytes. The granular component of the columnar cell is tear drop-shaped to elliptical, and apically positioned within the cell (Fig. 86). Granulocytes do not appear to be wandering hemocytes because the granular structure seems to be continuous with the cytoplasm, and there is usually a slight gap surrounding a hemocyte. Furthermore, hemocytes are typically circular, with a distinct, circular nucleus and the cytoplasm may contain prominent vesicles. Cells along the circumference of the typhlosole are well organized, with short bristle-like cilia along the apical surface (Fig. 87). Connective tissue of the fourth intestinal region is represented by a well-defined lamina propria supporting the typhlosole, and a thin wrapping around the lateral and ventral walls. Fibers within the major typhlosole are loosely aggregated, and the tissue has a pale, eosinophilic quality in comparison to the connective tissue surrounding the lateral and ventral walls (Figs. 85–87). The lamina propria is supplied with hemolymph from a series of small sinuses throughout the tissue that are just large enough for a small number of hemocytes to fit within the lumen.

The fifth limb of the intestine is a reflection of intestinal region four in terms of morphological and cytological features (Fig. 88). Morphologically, the intestine is circular along the hinge line, but becomes flattened as it nears the posterior adductor. The lateral and dorsal portions of the intestinal wall are pleated, but the dorsal wall has more plications than the fourth intestinal limb (Fig. 89). The ventral epithelium is a continuation of the typhlosole of the fourth intestinal limb (Fig. 90). Nuclei of the ciliated columnar cells, along the dorsal and ventral walls, are more regular, clearly revealing the presence of a single layer of cells within the ventral and dorsal walls. Unlike region four, granulocytes are absent from the outer wall of the fifth intestinal region. The connective tissue lamina of the typhlosole consists of loose connective tissue and fibroblasts as in region four, but the connective tissue wrapping around the outer wall supports taller epithelial folds than in region four.
FIGS. 74−78. Crystalline style sac of *Villosa nebulosa*. FIG. 74: Transverse section through the crystalline style sac showing the style sac (SS), crystalline style (ST), midgut (MG), epithelium type 1 (E1), epithelium type 2 (E2), epithelium type 3 (E3), lamina propria (LP), and adipose tissue (AT); FIG. 75: Transverse section of the crystalline style sac showing bristle-like cilia (CI) of type-one epithelium (E1), whispy cilia (CI) of type-two epithelium (E2), and the underlying lamina propria (LP); FIG. 76: Transverse section of the crystalline style sac showing wispy cilia (CI) and goblet cells (GC) of type-two epithelium, and the underlying lamina propria (LP); FIG. 77: Transverse section of the crystalline style sac showing the transition from bristle-like cilia (CI) of type-one epithelium (E1), and whispy cilia (CI) of type-three epithelium (E3); FIG. 78: Transverse section of the crystalline style sac showing the gelatinous style (ST) with ring of particulate matter (PM).
Cellular Structure of Cardiovascular System Tissues

Heart: The heart and nephridium are located dorsally in relation to the visceral mass, and cardiac muscle encircles the fifth limb of the intestine from the posterior margin of the visceral mass to the visceral mass midpoint. Bivalve have a three-chambered heart consisting of a laterally positioned pair of auricles and a large medial ventricle (Fig. 91). The ventricle of *V. nebulosa* is conical and contains a distinct epicardium and myocardium (Fig. 92). The epicardium is a sinuous layer of squamous cells, and the myocardium consists of two layers of cardiac muscle. There is a thickened outer region, located directly underneath the epicardium and characterized by a compact network of branched muscle fibers, while the medial portion of the ventricle contains widely separated cardiac myofibers. The heart is situated within a pericardial cavity with a delicate pericardial sac lined with squamous cells. Ventricles contain branched cardiac myocytes having a fibrous consistency and a pale, basophilic nucleus with a distinct nucleolus (Fig. 93). Auricles are generally delicate cylindrical tubes of cardiac muscle and squamous epithelium linking the ventricle to the pericardial sac (Fig. 94). Hemolymph flow between the auricles and ventricle appear to be regulated by auriculoventricular valves. Valves are long, flaps extending into the lumen of the ventricle consisting of a compact layer of irregular muscle fibers surrounded by endothelium (Figs. 91, 95).

Arteries, Veins, Capillaries and Pericardial Gland: Bivalves have an open circulatory system and hemolymph is distributed throughout the body among interstitial spaces and hemolymph sinuses. However, we observed distinct vessels resembling arteries, veins and capillaries. Hemolymph has a distinct cellular and non-cellular component consisting of spherical, eosinophilic hemocytes (e.g., Fig. 93) and black, ink-like granules of hemolymph (e.g., Fig. 96). Hemolymph sinuses are distinct cavities within tissue that are not lined by endothelial cells (e.g., Figs. 59, 86). Hemolymph vessels resemble veins, arteries or capillaries and are lined with squamous cells. Arteries and veins are difficult to distinguish because they each may be circular to oval or somewhat irregular in shape. Potential arteries have layers of musculature surrounding the lumen (Fig. 96). Veins, however, have thinner walls of fibrous tissue (Fig. 97). Capillaries are simple, circular structures lined with squamous cells and are typically seen in the mantle edge and adductors (Fig. 98).

The pericardial gland is a large network of connective tissue fibers in the dorsal region of the body bordered by gill, mantle isthmus, heart and nephridia. Fibrous connective tissue is located in the dorsal region of the body, anterior to posterior, and is similar to the composition of the middle mantle. The most distinctive feature of this tissue is the preponderance of discolored hemocytes. Eosinophilic hemocytes are present in the sinuses between connective tissue fibers, but many of the hemocytes have a brown or yellow cytoplasm. Discolored hemocytes seemingly have a cytoplasm with a low affinity for eosin given their subtle red coloration, and, additionally, some discolored hemocytes seemingly lack a nucleus or the nucleus appears to be emarginated or slightly separated from the cytoplasm (Fig. 99).

Renal System

Anterior Nephridium: The nephridium is a large tubular organ located dorsally to the left and right of the visceral mass. Nephridial tissue spans the length of the body from the anterior-medial point of the visceral mass to the posterior adductor. Additionally, there are distinct morphological features characterizing the anterior and posterior regions of the nephridia. Anterior nephridia have distinct ventral and dorsal limbs, while the posterior nephridia lacks distinct dorsal and ventral regions (Fig. 100). The ventral nephridial limb is characterized by large plications of the dorsal and ventral walls, while the walls of the dorsal nephridia have fewer plications. Epithelial folds of the ventral limb are composed of columnar cells with an eosinophilic, granular cytoplasm, and a dark-staining, monochromatic nucleus (Fig. 101). The nucleus of a columnar cell is located in the basal portion of the cell, and the apical region may contain a large vesicle. Some columnar cells have a transparent or a pale eosinophilic vesicle, while other cells in the ventral nephridial limb contain a brown vacuole. Subepithelium of the ventral limb consists of connective tissue supporting the epithelium and a hemolymph
sinus. The lining of the dorsal nephridium is generally flattened, but there are localized regions within the dorsal nephridium that contain plicated or features teardrop-shaped cells. For example, the ventral wall of the dorsal nephridium has a small number of plicae and the surface of these structures is irregular (Fig. 102). Plicae of the dorsal nephridium are sparsely distributed anterior to posterior. Secondly, the dorsal nephridium contains patches of teardrop-shaped cells with an apical vesicle (Fig. 103). Teardrop cells are typically located at the dorsolateral corners of the dorsal nephridium.

While the above-mentioned features of the walls of the ventral and dorsal limbs of the nephridium are representative of the anterior nephridial region, there are two structures that are unique to the anterior region. The urethra is a communication between the dorsal nephridium and suprabranchial cavity, and located in the anterior end of the nephridium. Additionally, there is a ciliated tubule, the renopericardial canal, located in the lateral portion of the ventral nephridial limb, adjacent to the urethra (Fig. 104). Squamous epithelial cells lining the dorsal nephridial limb change to a ciliated, columnar epithelium at the ventral end of the urethra (Fig. 105). The renopericardial canal is the ciliated tubule at the lateral end of the ventral nephridial limb and transfers fluid from the pericardial cavity to the lumen of the ventral nephridium. The renopericardial canal is composed of conical epithelial folds that extend far into the lumen and these epithelial folds feature ciliated columnar cells, and conspicuous goblet cells. Surrounding the canal is a well-defined lamina propria consisting of concentric layers of fibrous tissue (Fig. 106).

Posterior Nephridium: The nephridium is enlarged between the posterior margin of the visceral mass and posterior adductor. Nephridial epithelium is convoluted, consisting of rounded, and irregular epithelial folds (Fig. 107). Additionally, epithelial folds of the posterior nephridium are typically spaced farther apart from each other than in the anterior nephridium. Regarding the fine structure of posterior nephridial epithelium, cells are columnar, with an eosinophilic, vesicular cytoplasm and a pale, basophilic nucleus typically lacking a well-defined nucleolus (Fig. 108). Additionally, the lumen between the branches has expanded and is significantly larger than the lumen in the anterior region. Nephridial branches have a noticeably different composition from branches within the ventral limb of the anterior nephridium. Epithelial cells characterizing the posterior nephridial branches are columnar and vesiculated and appear to be bubbling at the apical surface. Additionally, the subepithelium of posterior nephridial branches consists of a simple squamous epithelium and hemolymph (Fig. 108). Posterior nephridium has sinistral and dextral limbs divided by a septum of loose connective tissue fibers and a pair of nerves. The posterior nephridial septum also contains a large artery located ventrally in relation to the pedal retractors. The artery is composed of wavy muscle fibers and runs along the hinge line towards the posterior end of the mantle.

Posterior Adductor and Posterior Pedal Retractors: Posterior pedal retractors are located within the nephridial septum, between the posterior adductor and visceral mass. Posterior nephridial branches encircle the posterior pedal retractors (Fig. 109). In transverse sections of the viscera, posterior retractors represent large fascicles. The musculature has a distinct perimysium and endomysium and myofibers are polygonal in outline (Fig. 110). The posterior adductor is located posterior to

FIGS. 79−84. First intestinal limb, and second intestinal limb of *Villosa nebulosa*. FIG. 79: Sagittal section through the first intestinal limb portraying tall plicae (PL) and chyme (CH) in the intestinal lumen; FIG. 80: Sagittal section of posterior wall of the first intestinal limb emphasizing the tall plicae (PL), with densely ciliated surfaces (CI); FIG. 81: Transverse section through the visceral mass showing the second limb of the intestine (IN), the surrounding lamina propria (LP), and testicular acini (TA); FIG. 82: Sagittal section through the initial portion of the second intestinal limb representing a region of short plicae (PL), and chyme (CH); FIG. 83: Sagittal section of second intestinal limb portraying cilia (CI), mucus secretions (MS), and chyme (CH); FIG. 84: Sagittal section of the second intestinal limb showing cilia (CI) and wandering hemocytes (HC).
the retractors and oriented perpendicularly in relation to the retractor muscles. Adductor myofibers are organized into large fascicles, with a simple, squamous epimysium and a delicate, fibrous perimysium. Myofibers of the posterior adductor are long, eosinophilic filaments with elliptical, heterochromatic nuclei and a distinct nucleolus (Fig. 111).

Nervous System

Pedal Ganglion, Cerebral Ganglion and Visceral Ganglion: The nervous system of *V. nebulosa* consists of four ganglia, each with a series of nerve fibers that extend throughout the body. There is a pair of ganglia at the anterior end of the visceral mass, each located in the lateral portion of the body between the anterior adductor and labial palp. The third ganglion is the pedal ganglion located in the ventral portion of the hemocoel between the digestive diverticulum and gonad, dorsal to the base of the foot. Finally, the posterior ganglion is situated along the ventral surface of the posterior adductor. Each ganglion has a well-defined outer neural cortex of neuron cell bodies, and a fibrous inner medulla. Furthermore, there is not an obvious difference between the cellular characteristics of all ganglia and therefore the foregoing description of the neural cortex and medulla of the pedal ganglion is representative of the anterior and posterior ganglia.

Pedal ganglion is a bilobed group of neurons at the base of the foot between the gonad and digestive diverticula. Each hemisphere is surrounded by well-defined epineuria, and separated by a median fissure. A series of central commissures communicate horizontally with each hemisphere at the vertical midpoint (Fig. 112). The cortex of the pedal ganglion consists of conical to polygonal cell bodies of unipolar neurons. Neuron cell bodies have a pale, basophilic cytoplasm and a spherical heterochromatic nucleus and a distinct nucleolus. Additionally, neurons have small, brown, granular inclusions located around the nucleus (Fig. 113). Fissures are composed of fibrous connective tissue with a thickened, homogenous appearance. The medulla is distinguished by its web-like appearance formed by overlapping bundles of axons. Gial cells of the medulla have a spherical to spindle shaped nucleus with only a small amount of cytoplasm surrounding the nucleus (Fig. 114). Commisures represent a continuation of the medulla serving to link each half of the ganglion by means of horizontal tracts of axons (Fig. 115). Axons leave the hemispheres via fibrous, lateral extensions of the medulla at the lateral, dorsal and ventral margins of the tissue. Roots along the lateral margins of the pedal ganglion are circular to sinuous bundles of axons and supporting cells (Fig. 116). Histological sections of the visceral mass have revealed ventral pedal nerves threaded through the foot musculature and dorsal pedal nerves extending along the lateral portions of the visceral mass.

The anterior ganglia may be observed in sections of the anterior muscle group and palp. Anterior ganglia appear to control the adductor and retractor muscles since there are nerves inserted into the fascicles of each muscle (Fig. 117). Additional nerves from the anterior ganglia travel through the foot, and mantle edge. Sinistral and dextral nerve centers are also linked to one another by means of a nerve spanning the length of the anterior adductor.

The posterior ganglion is a cylindrical structure along the anteroventral side of the posterior adductor (Fig. 118). Nervous tissue also extends up into the base of the

FIGS. 85−90. Fourth intestinal limb, and fifth intestinal limb of *Villosa nebulosa*. FIG. 85: Transverse section through the fourth intestinal limb showing a major typhlosole (TY) along the dorsal wall, and surrounding lamina propria (LP), and adipose tissue (AT); FIG. 86: Transverse section of the fourth intestinal limb showing cilia (CI), eosinophilic granulocytes (EC), lamina propria (LP), and adipocytes (AC); FIG. 87: Transverse section through the fourth intestinal limb showing the cilia (CI), lamina propria (LP), and hemolymph sinuses (HS) of the major typhlosole; FIG. 88: Transverse section of the fifth intestinal limb showing the major typhlosole (TY), lamina propria (LP), and cardiac muscle (CM); FIG. 89: Transverse section of the fifth intestinal limb showing cilia (CI) along the surface of plicae; FIG. 90: Transverse section of the fifth intestinal limb emphasizing ciliated columnar epithelium (CI) of the typhlosole.
gill where the outer face of the inner gill and inner face of outer gill unite (Fig. 119). A thin, but dense stratum of fibrous connective tissue surrounds the posterior ganglion, and on the ventral surface there is a simple epithelium of brown, granular cuboidal cells and goblet cells (Fig. 120). Regarding nerves of the posterior ganglion, the pair of nerves seen in histological sections of the nephridia originates at the anterior end of the ganglion, and the sinistral and dextral posterior pallial nerves are rooted in the posterior ganglion.
Nerves and Statocysts: Nerves are located throughout the body and can be traced from their origin by serial sectioning the body. In a longitudinal orientation, nerves appear as ribbons of fine, eosinophilic filaments that have a slightly wavy appearance. Nerves may be distinguished from connective tissue by observing spindle-shaped gaps between fibers. Additionally, there are small, spindle-shaped nuclei throughout the ribbon of axons, and nuclei have short, brown granular extensions, suggesting these cells are bipolar neurons (Fig. 121). Cross sections of nerves, such as the nephridial nerves, reveal a thick, homogenous, eosinophilic band surrounding the tracts. It appears that this connective tissue wrapping may be myelin or a similar substance and emarginated nuclei are present within this wrapping (Fig. 122).

Statocysts are small sacs located near the lateral margin of the hemocoel and linked to the pedal ganglion via a horizontal band of eosinophilic tissue (Fig. 123). Each statocyst is an ovular chamber formed by a ciliated, columnar epithelium. Epithelial cells lining the sac have a pale, basophilic cytoplasm, and a dark-staining, ovular nucleus. Statocyst epithelium has a stratified appearance, and there are dark-staining, spherical nuclei located in the basal layer of the epithelium. The lumen of the statocyst contains the statolith, a basophilic ring of fluid with a smooth inner surface and a rough outer surface, possibly from ciliary action (Fig. 123).

Reproductive System

Gonadal tissue of *V. nebulosa* is organized into a series of spherical to ovular acini and ciliated gonadal ducts (Fig. 124). Gametogenesis occurred in males and females collected in May 2010, but production was limited in terms of the

FIGS. 96–99. Hemolymph vessels of *Villosa nebulosa*. FIG. 96: Transverse section of visceral mass emphasizing the squamous epithelium (SE) and musculature (MU) of a potential artery, and surrounding adipocytes (AC); FIG. 97: Transverse section of visceral mass showing squamous epithelium (SE) of a potential vein and surrounding hemolymph (HL); FIG. 98: Sagittal section of posterior adductor (PA), emphasizing a capillary composed of squamous epithelium (SE); FIG. 99: Transverse section of the pericardial gland characterized by a network of loose connective tissue fibers (CT), hemocytes (HC), and senescent hemocytes (SH).
number of oocytes and spermatozoa produced as compared to stage 3 individuals collected in August 2010. In females collected in May, ova appeared to develop and arrest at an early stage. Oocytes of stage 1 ovarian acini are basophilic with a distinct, spherical nucleus, and a series of transparent cytoplasmic vesicles. Furthermore, oocytes were typically attached to the inner wall of the acinus and the lumen contained an abundance of spherical, eosinophilic granules. Upon closer inspection, the eosinophilic granules appeared to be apoptotic oocytes given that they were usually enclosed within a membrane, with a small, polygonal nucleus (Fig. 125). Stage 3 females sampled in August possessed a mixture of early stage and late stage oocytes. Oocytes begin as a small basophilic cell with a distinct nucleus and nucleolus. As oocytes mature, they become larger and eosinophilic cytoplasm becomes more prominent. Mature oocytes however, occupied the majority of the acinus volume as they were significantly enlarged. Mature oocytes are enclosed within a membrane that is indistinct and separated from the main part of the oocyte by an expansive fluidic mass. Specifically, the cytoplasm of a mature oocyte is eosinophilic, granular, and contains a pale basophilic nucleus. The nucleus of some oocytes seemingly contained a loose mass of basophilic chromatin, while other oocytes possessed a distinct nucleolus (Fig. 126).

The most distinctive feature of testicular acini from individuals sampled in May 2010 was the preponderance of sperm morula (Fig. 127). Furthermore, these animals exhibited acini in stages 1 and 2. Spermatozoa develop from single-celled spermatocytes and then proceed to divide multiple times and produce a cluster of spherical cells resembling a morula stage embryo. Spermatocytes of sperm morula each differentiate into a pair of oval spermatoid. Spermatocytes of a sperm morula are housed within a thin, eosinophilic cytoplasm that is nearly obscured by the strongly basophilic heterochromatin. Furthermore, spermatocytes and sperm morula have such a strong staining quality that individual chromosomes and stages of meiosis are not visible with hematoxylin and eosin preparations. Like stage 1 ovarian acini, testicular acini in males collected in May contained numerous eosinophilic granules, possibly representing apoptotic spermatocytes (Fig. 128). Acini of males collected in August were significantly enlarged and contained a large, central mass of spermatozoa. Additionally, spermatogenesis appeared to be different than in stage 1 individuals. Spermatocytes in stage 3 individuals possessed a pale, basophilic nucleus containing a blend of euchromatin and heterochromatin. Spermatozoa in the lumen of the acini are small, basophilic, and cylindrical, with an eosinophilic flagellum (Fig. 129).

Female and male gonadal tissue contained circular to oval, ciliated ducts. Gonadal ducts consist of pale, eosinophilic columnar cells with a densely ciliated apical surface (Figs. 124, 128). Ciliated gonadal ducts of stage 3 male V. nebulosa contained a condensed mass of spermatozoa traveling towards the dorsal gonadal pore (Fig. 130). The sinistral and dextral gonadal pores are ciliated tubules located at the dorso-lateral margin of the visceral mass between nephridium and inner lamina of the inner gill (Fig. 131).
**Fusconaia cerina**

**Shell Morphology**

The valves of *Fusconaia cerina* from Cahaba River are circular to rhomboidal, and a well-defined posterior ridge may be present, represented by an obliquely oriented convex line. Shells are typically thick or heavy with a dark brown epidermis. The anterior and posterior margins are broad, but truncated in length. The umbo is broadly conical, high, extending...
FIGS. 112–116. Pedal ganglion of *Villosa nebulosa*. FIG. 112: Transverse section of pedal ganglion portraying neural cortex (CO), neural medulla (ME), ventral fissure (VF), dorsal fissure (DF), central commissures (CC), and roots (RO); FIG. 113: Transverse section of the dorsal margin of the pedal ganglion revealing the median dorsal fissure (DF), hemocytes (HC), central commissures (CC), and neuron cell bodies (CB); FIG. 114: Transverse section of pedal ganglion medulla displaying cell bodies (CB), and axons (AX); FIG. 115: Transverse section of the pedal ganglion median where central commissures (CC) of axons communicating with the sinistral and dextral hemispheres; FIG. 116: Transverse section of the ventro–lateral aspect of the pedal ganglion revealing the medulla (ME), neural cortex (CO), perineurium (PN), and a ventral root (VR).
FIGS. 117−120. Cerebral ganglion, and visceral ganglion of Villosa nebulosa. FIG. 117: Sagittal section of anterior visceral mass showing the anterior adductor (AD), anterior pedal retractor (PR), and anterior ganglion (AG) with distinct neural cortex (CO), neural medulla (ME), and nerves (NE); FIG. 118: Sagittal section of posterior adductor (AD) emphasizing the posterior ganglia (PG) with distinct neural cortex (CO) and neural medulla (ME) located along the ventral surface of the posterior adductor (AD); FIG. 119: Transverse section of the free dorsal margin of the ctenidia anterior to the posterior adductor, emphasizing nervous tissue (NE) enclosed by musculature (MU) and teardrop cells (TC); FIG. 120: Sagittal section of the ventral margin of the posterior ganglion highlighting neuron cell bodies (CB), axons (AX), connective tissue (CT), goblet cells (GC) and cuboidal epithelium (CE) of the overlying the ganglion.
beyond the hinge line. Furthermore, the shell is typically inflated with an umbo that arches or curls dorsally to meet the hinge line (Figs. 132, 133) (Williams et al., 2008). The nacre is white, with a strongly developed and somewhat cuboidal pallial line. Anterior and posterior adductor scars are conspicuous, with a somewhat irregular surface. Anterior adductor scar is deeply inset and parallel with the anterior margin, while the posterior adductor scar is shallow, parallel with the posterior margin. Pseudocardinal teeth are strongly developed, with two on the left valve and one tooth located on the right valve, pseudocardinal teeth have a rough or grooved surface. Lateral teeth are strongly developed with two on the left valve and one on the right valve. Considering the arched umbo, there is a correspondingly deep umbo cavity. Additionally, the ligament is typically cylindrical, brown, and thickened along the dorsal margin (Figs. 134, 135) (Williams et al., 2008).

Gross Anatomical Features of the Mantle Cavity

*Fusconaia cerina* were collected in shoal habitats in gravel or sandy substratum. Incurrent and excurrent apertures are lined with papillae and especially along the incurrent aperture, papillae are conical to branched (Fig. 136). The body of *F. cerina* is typically pallid, grey to white. However, the mantle, foot and gill may become red to orange during the spawning season. Inner and outer demibranchs are obliquely oriented posteriorly, as the valves of *F. cerina* are rhomboidal. Females are tetrageneric brooders, and each gill may be filled with linear or subcylindrical masses of embryos or conglutinates. Mature conglutinates may be red to orange and fill most of the vertical volume of the marsupial water tubes (Fig. 137). During the summer months, white ovigerous female *F. cerina* may be collected. Conglutinates from white females consist entirely of unfertilized ova, whereas mature, red to orange conglutinates consist of colored, developing embryos or glochidia adhered to unfertilized, white ova (Fig. 138). The mantle edge has a homogenous coloration, with a fine, brown line across the ventral margin. Papillae along the siphonal apertures are located along a truncated posterior margin and appear mottled, brown to white. The foot of *F. cerina* is broad, and the visceral mass is creamy white. The mantle isthmus is broadly rounded and extends deep into the cavity between the sinistral and dextral dorsal margins (Fig. 139).

Cellular Structure of Mantle

**Anterior Mantle Edge:** The anterior portion of the mantle edge (lateral to labial palps) of *Fusconaia cerina* bears the same cell types as the anterior mantle edge of *Villosa nebulosa*. Further research is needed to understand the cellular structure of *Fusconaia cerina*.
losa. However, there are unique morphological features of mantle edge epithelium of *F. cerina* not observed on *V. nebulosa*. The outer mantle edge epithelium spanning the distance between the pallial line and outer lobe is sinuous, and there are crypts along the ventral surface of the outer mantle lobe (Figs. 140, 141). The outer mantle lobe is branched with as many as three lobes, and the distal tip of each branch is bluntly rounded (Fig. 142). Each branch of the outer mantle lobe is pleated, and plicae are irregular such that each extension of the outer lobe is asymmetrical. The basal bulb of *F. cerina* is located on a triangular plication of columnar cells that is rounded posteriorly (Fig. 141). The middle mantle lobe of *F. cerina* is bulbous with eosinophilic squamous cells extending approximately half the length of the ventral surface before abruptly becoming columnar. Given the abrupt transition from squamous to columnar, and considering how columnar cells maintain the same cytological characteristics as the squamous cells, these two cell types may differ only in orientation (Fig. 143). Inner mantle lobe of *F. cerina* is triangular and plicated, but unlike *V. nebulosa*, the columnar cells along the inner surface of the inner mantle lobe appear to lack brown intracellular granules (Fig. 144). Inner lobe epithelium consists of irregularly shaped plicae, becoming teardrop-shaped along the dorsal surface (Fig. 145). The columnar epithelium along the ventral surface of the mantle edge is wavy, and isolated goblet cells may be interspersed among epithelial cells (Fig. 146).

**Posterior Mantle Edge, Middle Mantle and Mantle Isthmus:** Posterior mantle edge was sampled adjacent to the posterior adductor. Posterior mantle edge of *F. cerina* maintains the same cell types from the anterior, but the shape of the mantle lobes is different (Fig. 147). Outer lobe is forked with minor epithelial undulations along the dorsal and ventral branches. The middle lobe is cylindrical and pleated along its dorsal surface, and the inner lobe is broader at its base than it is anteriorly. Papillae along the posterior margin of *F. cerina* mantle edge primarily differ from *V. nebulosa* in appearing bluntly rounded to branched (Fig. 148). The columnar epithelium and musculature of *F. cerina* mantle edge papillae is indistinguishable from that of *V. nebulosa* (Fig. 149).

The middle mantle of *F. cerina* has an outer columnar epithelium, inner cuboidal epithelium, and subepithelial strata consisting of loose connective tissue fibers and hemolymph (Fig. 150). The cellular composition of the middle mantle is not different from that of *V. nebulosa*.

Mantle isthmus of *F. cerina* is a well-defined median extension of mantle tissue. The dorsal epithelium around the visceral mass consists of pale eosinophilic columnar epithelial cells similar to the columnar epithelium of middle mantle. Isthmus epithelium is cuboidal along the lateral surfaces and basophilic columnar cells are localized to the dorsal margin (Fig. 151). Additionally, isthmus epithelium features sparsely distributed goblet cells and a wavy subepithelium of connective tissue fibers and hemolymph.
Cellular Structure of Gill

Non-Marsupial Demibranch: Ctenidia of *Fusconaia cerina* feature cylindrical filaments across each ascending and descending lamella, united by septa and vertical water tubes (Fig. 152). Tissue structure of each ctenidial face consists of cylindrical filaments with frontal, frontal-lateral, and lateral groups of ciliated cells across the apical surface. Filaments are occasionally separated by ostia, communicating with medial water tubes, and each filament has a medially located hemolymph sinus. Inner and outer lamellae of each demibranch are joined by a septum, and septa possess teardrop-shaped goblet
Marsupial Demibranch: Female Fusconaia cerina utilize both pairs of inner and outer demibranchs for larval incubation. Fertilized and unfertilized ova are packaged into strings or conglutinates and transported into the ctenidial water tubes. Conglutinates may be allocated to nearly all water tubes anterior to posterior and may occupy the entire vertical length of each water tube. Marsupial gills feature enlarged water tubes and interbranchial septa as compared to male demibranchs (Fig. 160). Filaments, ostia and ctenidial vasculature of a marsupium are structurally similar to such structures of male ctenidia (Fig. 161). In gravid female F. cerina collected from Cahaba River (Shelby Co., Alabama) in May 2011, water tubes contained clusters of unfertilized ova and spherical, early-stage embryos (Figs. 160–162). Morphologically, the unfertilized ova and early stage embryos are spherical and possess a series of sinuous branches extending from the surface of the ova (Fig. 162).

The main cytological difference between male and female gill is the thickened septa of a marsupium. Septa have a broad, triangular base and generally become narrow at the water tube midline (Figs. 160, 161). Septum epithelium is pleated and typically there is a prominent, median fold extending into the water tube lumen. The epithelium of interbranchial septa consists of teardrop-shaped columnar cells and goblet cells, and septa contain an irregular subepithelium of fibrous tissue and a pale, eosinophilic ground substance (Fig. 163). Branches have a pale, basophilic pigmentation and typically have a sharply defined border similar to the appearance of a ribbon of periostracum. Embryos are typically spherical, while unfertilized ova tend to have an irregular shape in histological sections. Unfertilized ova have an eosino-
philic, homogenously granular composition, while early stage embryos are multinucleated, but lacking apparent cell membranes. Nuclei of early-stage embryos are spherical and a small nucleolus may be observed in some cells. In female *F. cerina* collected from Cahaba River in August 2011, conglutinates had been shed, but the marsupia maintained the same histological characteristics as a filled ctenidium (Fig. 164).

**Cellular Structure of Foot and Associated Tissues**

**Pedal Musculature and Byssal Gland:** At low magnification, and in a transverse plane of section, pedal tissue of *Fusconaia cerina* is characterized by a median triangle of somatic musculature flanked by strongly basophilic regions of chromatocytes (Fig. 165). The ventral margin has dark-staining and pale-staining basophilic chromatocytes intertwined among myofibers (Fig. 166). Granulocytes continue dorsally to the terminus of type-three epithelium. Pedal musculature consists of a ventral irregular or woven region with distinct horizontal and vertically oriented myofibers (Fig. 167), while the base of the foot consists of well-defined layers of vertical and horizontally oriented musculature (Fig. 168).

*Fusconaia cerina* has a diamond-shaped byssal gland located posterior to the pedal ganglion. The luminal surface of the byssal gland is ciliated with an emarginated epithelium and a densely fibrous subepithelium (Figs. 169, 170). There are a series of small, spherical ciliated structures in an oblique, linear path between the dorsal base of the foot, where the hemocoel joins the pedal musculature and the posterior margin of the pedal integument. Byssal canal remnants consist of a ciliated, columnar epithelium surrounding a lumen filled with debris. Luminal contents of byssal canal may appear to be a shapeless eosinophilic mass material, or a darkened, granular material (Fig. 171).

**Pedal Integument and Mesentery:** Pedal integment of *F. cerina* consists of five regions from ventral to dorsal. The most distinguishing cytological characteristic of *F. cerina* is the preponderance of violet granulocytes in the ventral portion of the foot. The abundance of granulocytes is so high that histological sections of foot have a distinctive violet tinge (Fig. 172). Type one epithelium is densely ciliated consisting of irregular to rectangular plicae. Granulocytes within the subepithelium of type one plicae appear dorsoventrally compressed. Chromatocytes underlying type one plicae of *F. cerina* are so densely packed that it is difficult to determine the boundaries of some cells. Tall rectangular plicae become narrower and diminish in height between integumentary regions two and three (Figs. 173, 174). Plicae diminish to a simple columnar epithelium featuring subtle undulations in integumentary region four (Fig. 175). Columnar cells of integument four are ciliated, and numerous goblet cells are present. Type four integument of *F. cerina* has a distinct subepithelium of connective tissue.

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FIGS. 140−146. Anterior mantle edge of *Fusconaia cerina*. FIG. 140: Transverse section of anterior mantle edge showing the position of the outer lobe (OL), middle lobe (ML), and inner lobe (IL); FIG. 141: Transverse section of mantle edge emphasizing columnar epithelium (CE) of the outer lobe, basal bulb (BB), periostracal groove (PG), squamous epithelium (SE) of the middle lobe, and periostracum (PE); FIG. 142: Transverse section of the distal margin of outer mantle lobe revealing a basophilic columnar epithelium (CE) along branched plicae (PL) and a dense subepithelium of fibrous tissue (FT); FIG. 143: Transverse section of middle mantle lobe revealing a periostracum ribbon (PE) and squamous epithelium (SE), violet cells (VC), characterizing the dorsal margin consisting of a folded columnar epithelium (CE); FIG. 144: Transverse section of the inner mantle lobe edge portraying an irregular epithelium consisting of teardrop-shaped to flattened plicae (PL); FIG. 145: Transverse section of inner epithelium of proximal mantle edge defined by teardrop-shaped plicae (PL), granulocytes (GC), and a well-defined stratum of myofibers (MF); FIG. 146: Transverse section of the outer epithelium of proximal mantle edge characterized by a wavy columnar epithelium (CE), isolated goblet cells (GC), a horizontal lamina of myofibers (MF).
FIGS. 147−151. Posterior mantle edge, middle mantle, and mantle isthmus of *Fusconaia cerina*. FIG. 147: Transverse section of posterior mantle edge showing a forked outer lobe (OL), conical middle lobe (ML), and triangular inner lobe (IL) with distinct plications (PL); FIG. 148: Sagittal section of posterior mantle edge characterized by conical papillae (PA) and oblique plications (PL) along the surface; FIG. 149: Sagittal section of a papilla displaying brown intracellular granules (BG) of the columnar epithelium and wandering hemocytes (HC) interspersed among epithelial cells, and a subepithelium endowed with connective tissue fibers (CT); FIG. 150: Transverse section of middle mantle displaying columnar cells of outer epithelium (OE), squamous cells of inner epithelium (IE) and a fibrous subepithelial strata of connective tissue (CT), and hemocytes (HC); FIG. 151: Transverse section of mantle isthmus revealing a well-organized basophilic epithelium (BE), goblet cells (GC), and sinuous connective tissue fibers (CT).
portion of the foot, as seen in sagittal sections, is highly rugose and irregular (Fig. 177). Histological sections of visceral mass reveal numerous horizontally to obliquely oriented bands of mesentery spanning the hemocoel (Fig. 178). Bundles of mesentery are characterized by a series of eosinophilic filaments with slender, basophilic nuclei. Sagittal sections of coelom reveal a preponderance of mesentery fascicles throughout the hemocoel (Fig. 179).

**Labial Palps:** Labial palps of *Fusconaia cerina* feature plicae along the inner surface and a smooth outer surface (Fig. 180). Plicae of the inner surface are bulbous at the distal margin and have two small epithelial folds along the stem. Ciliated columnar cells represent the main constituent of the inner palp epithelium and extend along the palp interior until the distal margin of each lip. Ciliated columnar cells of the labial palps have an eosinophilic cytoplasm, and an elliptical nucleus with no visible nucleolus (Fig. 181). The distal margin of the palp features prominent goblet cells as the tissue transitions to a flattened epithelium. Additionally, there appears to be two types of goblet cells, many have pale, white intracellular vesicles, while the second type features eosinophilic vesicles (Fig. 182). The outer surface of each palp features a flattened epithelium with teardrop-shaped columnar cells. Columnar cells have a minimal amount of cytoplasm with a slender, elliptical nucleus and no apparent nucleolus. Additionally, goblet cells are interspersed among columnar cells (Fig. 183). The subepithelium of the labial palps consists of eosinophilic fibers, a pale ground substance, and hemolymph.

**Anterior Adductor and Anterior Pedal Retractors:** Adductor muscle features large fascicles and conspicuous perimysium containing capillaries (Fig. 184). Myofibers of the anterior muscle group consists of spindle-shaped cells with cylindrical nuclei. Nuclei are cylindrical, pale and granular. Fascicles are well organized, and in sagittal sections, the musculature has a woven appearance. Myofibers of the anterior protractors have a dark eosinophilic composition, and the tissue features distinct fascicles (Fig. 185).

**Oral Groove and Esophagus:** The oral groove of *F. cerina* resembles a deep cavity located posteriorly to the anterior adductor. At the ventral entrance to the oral groove, there are two cylindrical extensions of tissue histologically resembling labial palp. The epithelium consists of teardrop-shaped columnar cells along the ventral surface and a pseudostratified, ciliated epithelium along the dorsal surface. The tip of each cylindrical extension of oral groove entrance consists of a preponderance of goblet cells. Subepithelium of the cylindrical extensions consists of muscle fibers and hemolymph sinuses (Fig. 186). Lateral to the cylindrical extensions, the walls of the oral groove curve dorsally and become vertically oriented. The curved portion of the oral groove wall is pleated, while the straight or vertically oriented extent of the walls consists of uniform line of ciliated columnar cells (Figs. 187, 188). Ciliated columnar cells of oral groove walls feature an eosinophilic cytoplasm and a homogenous, basophilic nucleus. The dorsal floor of the oral groove features ciliated plicae. Plicae of the dorsal aspect of the oral groove are histologically similar to the plications along the curved portion of the walls located at the ventral side of the organ.

The esophagus is an oval tube characterized by deep sinusous folds extending into the...
FIGS. 160–164. Marsupium, and conglutinates of *Fusconaia cerina*. FIG. 160: Transverse section of a marsupial ctenidium containing a conglutinate (CO), within a distended water tube (WT), supported by thickened septa (SE); FIG. 161: Transverse section of the lateral margin of a filled marsupium portraying filaments (FI), an ostium (OS), hemolymph vessels (HV), and a conglutinate consisting of early stage embryos (EE), and unfertilized ova (UO); FIG. 162: Transverse section of a marsupium portraying part of a conglutinate consisting of unfertilized ova (UO), early stage embryos (EE), both featuring basophilic apical branches (BR); FIG. 163: Transverse section of the marsupial septum consisting of a columnar epithelial cells with transparent vesicles (VE), and a subepithelium consisting of fibrous connective tissue (CT), and hemocytes (HC); FIG. 164: Transverse section of an empty marsupium illustrating how the histological composition of filaments (FI), water tube (WT) and septa (SE) exhibit minimal change long after conglutinates are shed.
lumen from the dorsal and ventral surfaces (Fig. 189). The esophagus has a well-defined lamina propria consisting of loose connective tissue fibers and a pale ground substance. Esophageal epithelium consists of ciliated columnar cells arranged along tall, rectangular to "Y"-shaped plicae (Fig. 190).

**Cellular Structure of the Alimentary Canal**

**Digestive Diverticulum:** The esophagus enters the stomach on the left side of the body. The esophageal-stomach junction is characterized by a median typhlosole along the ventral surface of the esophagus. The typhlosole has a flattened, rectangular tip and a pleated stem. The ventral wall of the esophagus bends ventrally creating a rounded, sinistral fold of rounded plicae (Fig. 191). Rounded plicae feature ciliated columnar cells and such plicae are a repetitive feature of the stomach walls (Fig. 192). In contrast, the dorsal and lateral walls of the esophageal-stomach junction largely lack epithelial folds. Subepithelium of the stomach possesses a well-defined lamina propria.

*Fusconaia cerina* has primary, secondary and tertiary digestive tubules. The ciliated primary tubules feature thin, ciliated plicae extending to the midpoint of the lumen (Fig. 193). Ciliated columnar cells of primary digestive tubules are densely packed and teardrop-shaped. Additionally, cytoplasm of ciliated columnar cells lining the plicae has a strong eosinophilic characteristic (Fig. 194). Secondary tubules feature columnar cells with an eosinophilic and vesiculated cytoplasm and apical microvilli (Fig. 195). However, columnar cells of secondary tubules tend to curl or become teardrop-shaped at the junction of tertiary tubules. The morphology of secondary tubule cells creates the appearance of cycle-shaped valves extending into the lumen of tertiary tubules (Fig. 196). Tertiary tubules have a vesiculated and eosinophilic cytoplasm. The nuclei of tertiary tubule cells have a strongly basophilic, triangular region containing a spherical nucleus, typically with a distinct nucleolus (Fig. 197).

**Stomach:** The stomach is a dorsoventrally elongated chamber with ciliated plicae along the gastric walls and a large, forked typhlosole extending medially into the lumen from the sinistral wall (Fig. 198). The ventral epithelium has an eosinophilic cuticle or gastric shield along the sinistral portion of the stomach. The cuticle is relatively uniform across the surface, but may occasionally form a point as it may be whipped or pulled by ciliary action (Fig. 199). At a higher magnification, the cuticle appears gelatinous and layered with fine lines. The cuticle is attached directly to the cilia along the apical surface of the columnar epithelium. Columnar cells have an eosinophilic cytoplasm and an ovular nucleus with no visible nucleolus (Fig. 200). The medial portion of the ventral wall has a deep groove or furrow that is rectangular (Fig. 201). Dextral to the medial groove is a ciliated and plicated epithelium. Plicae are generally square-shaped, and they are similar in size (Fig. 202). The dorsal wall of the stomach has a series of tall, cylindrical plicae extending into the lumen. Plicae are ciliated and may also have a secretory function since the tissue appears to be bubbling (Fig. 203). A key characteristic of the stomach is the large, medially extending typhlosole located along the sinistral wall. The typhlosole has a forked epithelium with a gelatinous cuticle (Fig. 204).
Crystalline Style Sac: The crystalline style sac begins as a ventral outpouching of the stomach. The style sac consists of a large spherical chamber or style sac and a horizontal stem canal or midgut (Fig. 205). The crystalline style is an eosinophilic rod within the style sac extending anterior to posterior throughout the length of the style sac. Style sac epithelium consists of three types of ciliated columnar cells, herein referred to as types one, two and three. Type one epithelium lines the majority of the style sac and is represented by dark-staining, eosinophilic columnar cells with straight cilia (Fig. 206). Type one columnar cells are brightly eosinophilic with a basally located nucleus and a distinct apical cell membrane. The midgut contains the second type of columnar epithelium. Type-two cells range in size from tall columnar cells along the dorsal and ventral surfaces to shorter columnar cells along the lateral aspect. Type two cells differ from type one epithelium because the cytoplasm has a pale staining characteristic and the cilia are wispy (Fig. 207). Type three epithelium is restricted to a small region of the ventral style sac wall representing a junction between epithelium type one and type two epithelium of the stem canal. Type three epithelium is pseudostratified consisting of thin, basophilic, ciliated columnar cells (Fig. 208). Crystalline style consists of a dense eosinophilic material that tends to form wrinkles during microtomy. The style appears to be layered with concentric, dark, granular lines. The central layers may contain small particulates and appear broken, with an irregular space surrounding such particulate matter (Fig. 209).

Intestine: The crystalline style sac extends posteriorly through the visceral mass, bends dorsally and represents the first intestinal limb. The walls of the first intestinal limb are pleated and deeply sinuous (Fig. 210). Subepithelium of first intestinal limb consists of a well-defined lamina propria supporting the plicae and becomes thinner around the straightened portions of the intestinal walls. Intestinal epithelium alternates between style sac epithelium types one, two and three and creates a heterogeneous luminal surface (Fig. 211).

Ventral to the crystalline style sac are a pair of elliptical ducts, which constitute the second and third intestinal limbs. In contrast to the sinuous epithelium comprising first intestinal limb, the second limb lacks deep epithelial folds (Figs. 212, 213). The ascending second intestinal limb consists of ciliated columnar cells with eosinophilic, granular vesicles. Cilia are thin and filamentous, similar to cilia of style sac type-three epithelium (Fig. 213). Transverse sections of second intestine reveal a generally flattened luminal surface, while sagittal sections portray the intestine as a tube with bends characterized by a small inclination. High magnification shows the intestinal epithelium has two types of columnar...
FIGS. 180–185. Labial palps, anterior pedal protractor, and anterior adductor of *Fusconaia cerina*. FIG. 180: Transverse section of labial palps revealing plicae (PL) along the inner epithelium (IE), and flattened outer epithelium (OE). FIG. 181: Transverse section of the inner palp epithelium featuring ciliated plicae (PL), and connective tissue fibers (CT) of the subepithelium. FIG. 182: Transverse section of distal palp margin showing cilia (CI) and goblet cells (GC). FIG. 183: Transverse section of outer epithelium emphasizing teardrop-shaped columnar cells (TC), and subepithelial connective tissue (CT). FIG. 184: Sagittal section of anterior adductor portraying myofibers (MF), myocyte nuclei (MN), and a pale eosinophilic perimysium (PM). FIG. 185: Transverse section of anterior pedal protractor illustrating densely packed myofibers (MF), scattered myocyte nuclei (MN) and perimysium (PM).
FIGS. 186–190. Oral groove, and esophagus of *Fusconaia cerina*. FIG. 186: Transverse section of the ventral portion of the oral groove featuring a muscular extension (MU) of ciliated columnar cells (CC), and goblet cells (GC); FIG. 187: Transverse section of ventral oral groove wall showing an extension of cilia (CI) along the surface of plicae (PL) and a subepithelium of connective tissue fibers (CT); FIG. 188: Transverse section of lateral oral groove epithelium emphasizing flattened columnar epithelial cells endowed with cilia (CI); FIG. 189: Transverse section of the dorsal aspect of anterior visceral mass portraying an ovoid esophagus (ES) with a distinct lamina propria (LP); FIG. 190: Transverse section of the esophagus revealing plicae (PL) and a densely ciliated epithelium (CI).
epithelium. There are ciliated columnar cells with eosinophilic intracellular granules, suggesting that these cells may have a secretory function (Fig. 214). The second columnar cell type has a homogenous eosinophilic cytoplasm that lacks intracellular granules (Fig. 215).

The fourth and fifth intestinal limbs are each characterized by a large typhlosole occupying a large proportion of the lumen. The typhlosole of the fourth intestinal limb extends ventrally into the lumen (Fig. 216). Ventral epithelium of the fourth intestinal limb is pleated around the lateral portion and smooth along the medial aspect. Epithelial cells of the ventral wall are ciliated and columnar with elliptical eosinophilic, granular inclusions (Fig. 217). Dorsal epithelium has a smooth surface, consisting of simple ciliated columnar cells (Fig. 218). Subepithelium of intestinal limb four is comprised of a distinct lamina propria containing a series of hemolymph sinuses. Lamina propria supporting dorsal epithelium is expansive, while marginal connective tissue of ventral epithelium is thin.

Fifth intestinal limb is analogous to intestinal limb four as the alimentary canal bends dorsally and continues along the hinge towards the posterior adductor. The typhlosole of the fifth intestinal limb projects dorsally from the visceral mass (Fig. 219). Ventral epithelium lining the typhlosole is pleated, with tall ciliated columnar cells (Fig. 220). Columnar cells of the dorsal epithelium are shorter than typhlosole cells. Dorsal epithelium is pleated around the lateral margins, but generally smooth in the median (Fig. 221). Unlike the fourth intestinal limb, dorsal epithelium lacks elliptical eosinophilic inclusions.

Cellular Structure of Cardiovascular System Tissues

Heart: The most prominent feature of the heart is the thickened, muscular ventricle surrounding the intestine (Fig. 222). The ventricle consists of an epicardium, and a thickened myocardium of irregular muscle fibers. Epicardial surface is sinuous with teardrop-shaped cells, some of which have a transparent apical vesicle (Fig. 223). Epicardium and myocardium feature red-staining hemocytes between epithelial cells of the epicardium and juxtaposed among myofibers. Cardiac myocytes are spindle-shaped, and form rectangular bands of musculature generally oriented anterior to posterior or dorsal to ventral (Fig. 224).

Auricles are thin-walled, tubular structures uniting the pericardial wall with the ventricle. Auricles are composed of an outer squamous epithelium, and inner myocardial fibers (Fig. 225). Each auricle has a ventral and a dorsal branch and the auricular branches may be closely apposed to the squamous pericardium. The heart has a pair of auriculoventricular valves, and each set of valves is located along the lateral margin of the ventricle at the auricular junction. Therefore, hemolymph flow is regulated as it travels from the auricles to the ventricle. Valves consist of an outer squamous epithelium covering the dark-staining myocardial and pale-staining connective tissue fibers (Fig. 226).

Arteries, Veins, Capillaries and Pericardial Gland: Throughout the body there are a series of hemolymph vessels resembling arteries, veins and capillaries based upon the overall

FIGS. 191–197. Digestive diverticulum of Fusconaia cerina. FIG. 191: Transverse section of the junction of esophagus (ES) and digestive diverticulum (DD) showing the position of plicae (PL) in relation to the typhlosole (TY) and the non-plicated dorsal wall (DW); FIG. 192: Transverse section of digestive diverticulum, ciliated plicae (PL) with a distinct lamina propria (LP); FIG. 193: Transverse section of digestive diverticulum portraying the deeply infolding plicae (PL) of a primary tubule; FIG. 194: Transverse section of a primary digestive diverticulum tubule illustrating cilia (CI) extending from conical plicae (PL); FIG. 195: Transverse section of secondary digestive diverticulum tubule emphasizing vesiculated epithelium (VE), apical microvilli (MV), and a thin subepithelium of eosinophilic connective tissue fibers (EF); FIG. 196: Transverse section of digestive diverticulum showing the valve-like (VA) appearance of the secondary tubules (ST) as they unite with tertiary tubules (TT); FIG. 197: Transverse section of tertiary digestive diverticulum tubules emphasizing the characteristic basophil cells (BC) and vesiculated digestive cells (DC).
size of the tissue and its cellular constituents. Possible arteries consist of several layers of muscle encircling emarginated nuclei of endothelium. A good example of a potential artery is the medial hemolymph vessel located at the ventral margin of the hemocoel where it may regulate pedal circulation (Fig. 227). The muscular walls have a pale, eosinophilic ground substance with darker, red fibers interspersed throughout the arterial wall. Arteries and veins may be similar in size, but possible veins consist of a lesser-developed muscular wall encircling an endothelium (Fig. 228). Such vessels are abundant within the coelom, especially between the digestive diverticulum and gonad. Capillaries are small circular to ovular tubes consisting of endothelial cells surrounded by a delicate, loose connective tissue matrix. Capillaries are best observed within the perimysium of the adductors, pedal protractor, pedal retractors and mantle edge (Fig. 229). The pericardial gland is a large latticework of adipocyte-like cells surrounding the pericardial cavity. Hemocytes are typically present within the lumen and there are emarginated hemocytes that appear to be attached to the lumen or incorporated within a narrow space between emarginated pericardial gland cells (Fig. 230).

Cellular Structure of Renal System Tissues

Anterior Nephridium: Nephridium is located dorsally to ctenidia and visceral mass. Nephridium has a ventral, convoluted limb, while the dorsal limb has fewer epithelial folds extending into the lumen (Fig. 231). Anteriorly, ventral nephridium features numerous convoluted plicae restricting the lumen to a series of irregular, narrow spaces. Columnar cells of ventral epithelium have a vesiculated, eosinophilic cytoplasm. Ventral nephridial cells have a pale cytoplasm, but plicae appear mottled at a low magnification since wandering hemocytes are scattered throughout the plicated epithelium (Fig. 232). Hemocytes are distinguished from epithelial cells by a red-staining cytoplasm and a spherical nucleus. Subepithelium of nephridial plicae consists of connective tissue, endothelial cells and hemolymph. Plicae of the dorsal nephridium are more sparsely distributed and may be distinguished from ventral plicae in having a convoluted surface, whereas ventral nephridial plicae are smoother. Epithelium of dorsal plicae may be distinguished from ventral epithelium in having brown intracellular granules (Fig. 233). A characteristic feature of dorsal nephridium is the presence of teardrop-shaped columnar cells with a transparent apical vesicle. Teardrop cells are distributed along the lateral and dorsal surfaces of dorsal nephridium and contain brown intracellular granules (Fig. 234).

Anterior nephridium with its extensively folded luminal branches is located anterior to the heart and pericardium. Pericardium unites with the ventral nephridial limb at the anterior-posterior midpoint of the visceral mass. The most distinguishing feature of the anterior nephridium is the presence of the renopericardial canal and urethra (Figs. 235–237). The renopericardial canal is a pleated tubule derived from the pericardial cavity. The renopericardial canal is lined with narrow, rounded plicae containing ciliated columnar cells and elliptical goblet cells (Fig. 237). The renopericardial canal is adjacent to the urethra representing the excurrent aperture of the dorsal nephridium. Urethra

FIGS. 198–204. Stomach of Fusconaia cerina. FIG. 198: Transverse section through visceral mass showing the morphology of the stomach (ST) and surrounding digestive diverticulum (DD); FIG. 199: Transverse section of the dextral portion of the ventral stomach wall showing a cuticle (CU) attached to the columnar epithelium (CE) and the underlying connective tissue (CT) of the subepithelium; FIG. 200: Transverse section of the dextral portion of the ventral stomach wall revealing a layered cuticle (CU) along the surface of the columnar epithelium (CE); FIG. 201: Transverse section of the medial portion of the ventral stomach wall showing a columnar epithelium (CE), medial groove (MG), and underlying connective tissue (CT) of the subepithelium; FIG. 202: Transverse section of the sinistral portion of the ventral stomach wall revealing ciliated plicae (CI), and connective tissue (CT); FIG. 203: Transverse section of the dorsal stomach wall showing plicae (PL), and chyme (CH); FIG. 204: Transverse section of dextral stomach wall showing a rectangular and forked typhlosole (TY), with an attached cuticle (CU).
has a columnar epithelium with goblet cells lining the dorsal half of the tissue and a densely ciliated epithelium within the ventral half (Fig. 236). Goblet cells reappear along the ventral epithelium between the ctenidia and urethra. Some goblet cells in the urethra have eosinophilic contents.

**Posterior Nephridium**: Between the posterior terminus of the visceral mass and the posterior adductor, the nephridial branches become elongated and the lumen greatly expands (Fig. 238). Nephridial epithelium encircles the posterior pedal retractors, and nephridial branches resemble a series of stems and loops. Branches of posterior nephridium consist of columnar cells with pale, eosinophilic intracellular vesicles. The cell types of posterior nephridium appear morphologically similar to each other all around the organ. However, at high magnification, cells of the nephridial branches typically have an eosinophilic, wispy material at the apical surface (Fig. 239). Cells lining the walls of the posterior nephridium sometimes lack a wispy residue (Fig. 240). Additionally, the hemolymph vessels underlying columnar cells of epithelial branches are wider than in the anterior nephridium.

**Posterior Adductor and Posterior Pedal Retractors**: Posterior pedal retractors unite with the posterior margin of the visceral mass and extend ventrally into the foot. Transverse histological sections of the posterior region of *F. cerina* reveal groups of retractor fascicles with a distinct perimysium and endomysium (Fig. 241). Additionally there are thin, elliptical capillaries located between fascicles. Posterior adductor, as seen in a sagittal plane, consists of filamentous myofibers with an elliptical nucleus and a distinct nucleolus (Fig. 242). Myofilaments overlap and at high magnification and striations are not evident.

**Cellular Structure of Nervous System Tissues**

**Pedal Ganglion, Cerebral Ganglia and Visceral Ganglion**: The ganglia of *Fusconaia cerina* have similar cytological characteristics, and the description of the pedal ganglion is representative of the anterior and posterior ganglia. Pedal ganglion of *F. cerina* consists of a pair of vertically oval lobes. Each lobe has a distinct outer cortex containing neuron cell bodies with axons extending into the inner medulla. Pedal ganglion has a distinct epineurium, dorsal and ventral fissures, with commissures uniting the two lobes (Fig. 243). Neuron cell bodies are polygonal, with a basophilic cytoplasm and a spherical, heterochromatic nucleus. Neurons have a homogenous cytoplasm and brown intracellular inclusions. Additionally, epineurium fibers appear granular and the tissue has a distinct filamentous appearance (Fig. 244). Axons are darkened and well defined as they extend from the neuron cell body into the medulla. Nerve fibers of pedal ganglion of *F. cerina* are distinctively filamentous (Figs. 245, 246). Glial cells of the medulla have a darkened, homogenous nucleus with a minimal amount of cytoplasm. Ganglionic roots are large bundles of axons extending dorsolaterally and ventral-laterally from each ganglionic lobe (Fig. 247).

Anterior ganglia are paired spherical masses located between the anterior adductor and labial palps (Fig. 248). The posterior ganglion is cylindrical, located anteriorly along the ventral surface of the adductor (Fig. 249). A horizontal band of connective tissue overlays the posterior ganglion and consists of fibrous tissue and hemolymph. Ventral to the hemolymph sinus is an apical layer of columnar cells and goblet cells (Fig. 250). The posterior ganglion extends into the base of the inner ctenidia and may appear as an oval mass of nervous tissue at the base of the gill (Fig. 251).

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FIGS. 205−209. Crystalline style sac of *Fusconaia cerina*. FIG. 205: Transverse section of the crystalline style sac showing the style sac (SS), style (ST), midgut (MG), style sac epithelium type 1 (E1), epithelium type 2 (E2), epithelium type 3 (E3), and underlying lamina propria (LP); FIG. 206: Transverse section of style sac showing the straightened cilia (CI) of type one epithelium and the close proximity of cilia to the style (ST); FIG. 207: Transverse section of the midgut showing the slender cilia (CI) of the columnar epithelium; FIG. 208: Transverse section of the style sac showing basophilic columnar cells of type-three epithelium and its apical cilia (CI); FIG. 209: Transverse section of the crystalline style (ST) revealing darkened concentric layers (CL).
Nerves and Statocysts: Nerves are distributed throughout the visceral mass, foot, nephridium and mantle edge. Transverse sections of nerves reveal a thickened, eosinophilic epineurium encircling an irregular filamentous core of axons (Fig. 252). A sagittal view of a nerve illustrates how the tissue resembles a wavy band of filaments (Fig. 253). Additionally, there is a sinistral and dextral statocyst lateral to the pedal ganglion near the dorsal margin of the coelom. Statocysts appear stratified with ciliated columnar cells lining a spherical lumen (Fig. 254). Statocysts contain a spherical mass or statolith in the median of the lumen.

Cellular Structure of Reproductive System Tissues: Gonadal tissue consists of testicular and ovarian acini, ciliated gonadal ducts throughout the visceral mass, and a pair of ciliated gonadal pores located at the base of the visceral mass at the anterior end of the nephridium. Female and male *Fusconaia cerina* in the Cahaba River (Bibb, Shelby Co., Alabama) exhibit peak gametogenesis in May, and females may bear glochidia during June. Males and females collected in May 2011 exhibited peak gametogenesis, while considerably less gametogenesis was observed in individuals collected in August 2011. The luminal contents of stage 1 ovaries mainly consisted of an eosinophilic granular material with minimal input of oocytes (Fig. 255). Walls of stage 1 ovarian acini are lined with squamous cells with basophilic oocytes adhered to the luminal surface. Developing oocytes initially possess a violet cytoplasm and may appear to be attached to the acinus wall by a stem. Oocytes appear to grow and separate from the acinus wall. Separated oocytes have an irregular cell membrane, with a pale, basophilic margin. Immature oocytes feature a red, granular cytoplasm with a basophilic focus consisting of an irregular violet nuclear membrane and a spherical, black nucleolus (Fig. 256). Ovarian acini also produce a series of spherical, red, granular cells with a small basophilic nucleus. Given the small size and undifferentiated appearance, it is likely that the red cells are polar bodies. The lumen of stage 1 ovarian acini also appears to contain the remnants of polar bodies, considering the presence of an irregular, red, granular material and a series of nuclei lacking surrounding cytoplasm. Mature oocytes are enlarged and densely packed within the acinus (Fig. 257). Mature oocytes have a pale basophilic nucleus with a distinct nucleolus within a red, granular cytoplasm. Oocytes released from the acinus wall feature a pale, basophilic membrane surrounding the cell body. Developing oocytes are attached to the acinus wall via a basophilic stem. *Fusconaia cerina* males predominantly exhibited stage 1 acini in August 2011. Stage 1 exhibited a preponderance of blackened single-celled spermatocytes and multicellular sperm morula (Fig. 258). Spermatozoa are present in some acini, but restricted to the center of the lumen. Primary spermatocytes are typically emarginated along the acinus wall and have a pale, basophilic cytoplasm. Violet spermatocytes appear to differentiate into sperm morula and cells of sperm morula become spermatozoa (Fig. 259). Additionally, stage 1 testicular acini contain a series of small eosinophilic granules, possibly representing remnants of degenerative spermatocytes or fluid. A high level of spermatogenesis was evident in stage 3 testicular acini collected in May 2011. Sperm morula as seen in stage 1 testes were less conspicuous within stage 3 testes. Violet spermatocytes along...
FIGS. 216−221. Fourth intestinal limb, and fifth intestinal limb of *Fusconaia cerina*. FIG. 216: Transverse section through the fourth intestinal limb emphasizing a large median typhlosole (TY), lamina propria (LP), and surrounding adipose tissue (AT); FIG. 217: Transverse section of the fourth intestinal limb showing eosinophilic columnar cells (EC) and cilia (CI) along the ventral wall, and a fibrous lamina propria (LP) and subepithelial hemocytes (HC); FIG. 218: Transverse section of fourth intestinal limb portraying the dense cilia (CI) of the typhlosole, hemocytes (HC) juxtaposed among epithelial cells as well as within sinuses of the lamina propria (LP); FIG. 219: Transverse section of the fifth intestinal limb showing a large median typhlosole (TY) with an expansive lamina propria (LP); FIG. 220: Transverse section of the dorsal epithelium surrounding the typhlosole consisting of ciliated columnar cells (CI), plicae (PL), and a distinct lamina propria (LP); FIG. 221: Transverse section of dorsal intestinal wall featuring cilia (CI), lamina propria (LP) and wandering hemocytes (HC).
the acinus wall appear to differentiate into darkened, spherical cells. Darkened testicular cells have such a small size that it is difficult to determine whether these cells represent clusters of spermatocytes or single-celled spermatocytes (Fig. 260).

Gonadal ducts of *Fusconaia cerina* consist of pale, eosinophilic, ciliated cells with an ovular nucleus. Spermatozoa were observed in the testicular ducts of stage 3 male *F. cerina* collected in May 2011 (Fig. 261). Gonadal ducts merge dorsally and extend along the lateral margins of the coelom to the base of the visceral mass. The gonadal pores of *F. cerina* feature a bulbous ventral ledge and a pleated, ciliated epithelium (Fig. 262).

FIGS. 222–226. Heart, and pericardial cavity of *Fusconaia cerina*. FIG. 222: Transverse section through the dorsal portion of the visceral mass revealing branching myofibers of the ventricle (VE) surrounding the intestine (IN); FIG. 223: Transverse section of the ventricle revealing the epicardium (EC) and myocardium (MC); FIG. 224: Transverse section of the ventricle illustrating horizontal and vertical myofibers (MF); FIG. 225: Transverse section of the auricle (AU) emphasizing squamous epithelium (SE), cardiac muscle (CM), and adjacent pericardium (PC); FIG. 226: Transverse section of the ventricle focusing on an auriculoventricular valve (AV), endowed with connective tissue fibers (CT), and cardiac muscle (CM).
FIGS. 227–230. Hemolymph vessels of *Fusconaia cerina*. FIG. 227: Transverse section of the base of the foot showing a potential artery containing a thick muscular wall (MU), emarginated nuclei of endothelial cells (EN), and surrounding connective tissue (CT); FIG. 228: Transverse section of digestive diverticulum revealing a potential vein consisting of a thin muscular wall (MU), emarginated nuclei of endothelial cells (EN), and supporting adipocytes (AC); FIG. 229: Sagittal view of adductor muscle portraying a capillary and its endothelium (EN) within the perimysium (PM); FIG. 230: Transverse section of the pericardial gland displaying hemocytes (HC), emarginated hemocytes (EH), and emarginated nuclei (EN) of connective tissue cells.

**Strophitus connsaugaensis**

Shell Morphology

Valves of *Strophitus connsaugaensis* are elongated, and ovular to rhomboidal. The shell is characterized by a light-to-dark brown periostracum, and there may be thin to thick black lines along the posterior surface. The ventral margin of each valve is approximately double the length of the dorsal margin or hinge line. The ventral shell margin may be...
FIGS. 238–242. Posterior nephridium of Fusconaia cerina. FIG. 238: Transverse section of the posterior nephridium featuring nephridial branches (NB) and posterior pedal retractor (PR); FIG. 239: Transverse section of posterior nephridium detailing columnar epithelium (CE), and fibrous material (FM) of the apical surface, and underlying hemolymph vessels (HV); FIG. 240: Transverse section of posterior nephridium consisting of a nerve fascicle (NE), and a smooth surfaced columnar epithelium (CE); FIG. 241: Transverse section of posterior retractor illustrating myofibers (MF), myocyte nuclei (MN), and a capillary (CA); FIG. 242: Sagittal view of posterior adductor portraying spindles of myofibers (MF) and cylindrical myocyte nuclei (MN).
The inside of the shell has a lustrous, silver nacre. The pallial line is a scar forming an incomplete ellipse around the ventral margin of the nacre between the anterior adductor scar and posterior adductor scar. The anterior adductor is a triangular depression between the anterior shell margin and interdentinum. The posterior adductor scar is broad, ovular and flattened with a smaller, circular marking positioned anteriorly demarking the posterior pedal retractor scar. Pseudocardinal teeth are reduced to small, rounded triangular extensions of the shell at the anterior end of the shell.

FIGS. 243–247. Pedal ganglion of *Fusconaia cerina*. FIG. 243: Transverse section of pedal ganglion portraying neural cortex (CO), inner medulla (ME), central commissures (CC), dorsal fissure (DF) and ventral fissure (VF); FIG. 244: Transverse section of pedal ganglion showing neuron cell bodies (CB), and fibrous epineurium (EN); FIG. 245: Transverse section of the median of the pedal ganglion showing central commissures (CC), and dorsal fissure (DF); FIG. 246: Transverse section of the pedal ganglion medulla emphasizing the irregular meshwork of axons (AX) and cell bodies (CB); FIG. 247: Transverse section of pedal ganglion revealing axons (AX) bundled within a root (RO) and surrounded by membranous connective tissue of the epineurium (EN).
hinge line between the interdentum and ligament. Lateral teeth are virtually absent and appear as low, rounded ridgeline ventral to the ligament. The umbo cavity is small, forming a narrow cavity that curves dorsally and anteriorly behind the interdentum (Figs. 265, 266) (Williams et al., 2008).

Gross Anatomical Features of the Mantle Cavity

The posterior aspect of the mantle edge forms a short, incomplete siphon when the sinistral and dextral mantle edges are cupped.

FIGS. 248–251. Anterior ganglion, and posterior ganglion of Fusconaia cerina. FIG. 248: Transverse section of anterior ganglion revealing the neural cortex (CO), neural medulla (ME) and a large nerve root (RO); FIG. 249: Transverse section of the ventral surface of the posterior ganglion revealing cell bodies (CB) of the neural cortex, and a thick layer of hemolymph (HE) between neural tissue and columnar epithelial cells (CE) facing the suprabranchial cavity; FIG. 250: Transverse section of posterior ganglion showing the neural cortex (CO), neural medulla (ME), and a nerve root (RO); FIG. 251: Transverse section of the base of the ctenidia showing an anterior nerve (NE) branch of the posterior ganglion juxtaposed with columnar epithelial cells (CE), and a muscle fascicle (MU).
The apertures of *S. connasaugaensis* are grey, uniformly pigmented externally, but with irregular transverse lines along the internal surface. When mantle edges are cupped, incident and excurrent apertures or openings are formed. The incident and excurrent apertures are not divided by a septum, but rather mantle tissue is curled medially and forms trapezoidal flaps that may meet medially or overlap. The incident aperture is papillose and papillae are uniramous. Papillae are reduced or absent along the excurrent aperture (Fig. 267).

The mantle edge is orange, flattened and pigmentation continues posteriorly to the blackened siphon, just posterior to the posterior adductor. The middle mantle is white and somewhat translucent, spanning the nacre. A translucent, trapezoidal keel of mantle tissue, the isthmus, extends dorsally into a cleft between the left and right halves of the shell. The ctenidia are paired, consisting of elongated inner and outer demibranchs (Fig. 268). Ovigerous females brood using the outer demibranchs and red lines of glochidia are present in marsupia of gravid females (Fig. 269). Removing the mantle and gill on one side exposes the foot and visceral mass. The foot of *S. connasaugaensis* is orange, forming a right triangle with a vertical anterior face and a posterior slope. The visceral mass is white and extends dorsally to the base of the ctenidia. Labial palps are translucent lips located anterolaterally between the foot and visceral mass. The anterior adductor is pale, white to orange, ovular in outline and oriented vertically between the anterior shell margin and foot. The posterior adductor is grossly identical to the anterior adductor, but is parallel with the posterior shell margin (Fig. 270).

**Cellular Structure of the Mantle**

**Anterior Mantle Edge:** Anterior mantle edge of *Strophitus connasaugaensis* is dorsoventrally compressed with outer, middle and inner lobes nearly equidistant from the base of the mantle edge (Fig. 271). The outer mantle lobe is generally flattened, except for a few trapezoidal plications along the ventral margin (Figs. 271–273), and pleated distal end (Figs. 271, 273). The outer lobe has a simple columnar epithelium, with deeply basophilic cells ventrally located along the medial half of the lobe. Outer lobe columnar cells feature an ovular, monochromatic nucleus, obliquely oriented in relation to the horizontal plane of the outer lobe, and the cytoplasm may contain a single, apical, transparent vesicle. In contrast, dorsal epithelium of the outer lobe is largely flattened and columnar cells characterizing dorsal epithelium have an eosinophilic cytoplasm, but are otherwise indistinguishable from ventral columnar cells (Figs. 272, 273). Dorsal epithelium of the outer lobe terminates at the small, cleft-like
basal bulb and abruptly transitions to simple, squamous epithelium (Fig. 272). The middle lobe is flattened along the ventral surface, and pleated along the dorsal margin. Middle lobe cell types include a simple squamous epithelium along the ventral margin and a simple columnar epithelium lining the dorsal surface. Squamous cells of the ventral surface consist of an eosinophilic cytoplasm and a monochromatic nucleus. Secreted periostracum is sometimes observed in histological sections of outer and middle lobes, appearing as an eosinophilic filament, and may be closely apposed to the ventral surface. Columnar cells of the dorsal margin are eosinophilic with an oval, monochromatic nucleus. Plications of the dorsal surface of the middle lobe give an irregularity to the spacing of columnar cells such that some cells appear more laterally compressed than others (Fig. 274). The inner mantle lobe is triangular with thin, teardrop-shaped plicae along the dorsal and ventral surfaces. Columnar cells of the inner lobe are indistinguishable from those of the dorsal surface of the middle lobe (Fig. 275). The dorsal bend to the middle lobe and the ventral bend to the inner lobe may represent fixation artifacts. Teardrop-shaped plicae with columnar epithelial cells extend medially towards the base of the mantle edge and are replaced by plicae endowed with goblet cells. Goblet cells may contain a single transparent vesicle or a series of vesicles, and goblet cell nuclei are oval and emarginated (Fig. 276). Dorsal epithelium of anterior mantle edge is flattened, with a simple columnar epithelium, and the tissue maintains a flattened surface along the medial aspect of the tissue until the base of the outer mantle lobe. Columnar cells of dorsal mantle epithelium have a densely staining, basophilic cytoplasm and an oval, monochromatic nucleus (Fig. 277).

Muscle tissue is a large constituent of the mantle edge (Fig. 271). The subepithelium of mantle lobes consists of linear tracts of muscle fibers. Myofibers are wavy and organized into dense bands in the outer and middle lobes (Figs. 272–274). Myofibers of the inner lobe are arranged in an irregular meshwork (Fig. 275). Other tissue components of the mantle edge include irregular sinuses containing blackened hemolymph, nerve fibers (Fig. 276), and blue to violet granular cells beneath the epithelium of the inner mantle lobe (Fig. 275).

**Posterior Mantle Edge, Middle Mantle and Mantle Isthmus:** Mantle lobe morphology differs from anterior mantle edge in several respects. The outer and middle lobes become a forked extension of mantle tissue, each lobe equidistant from its base. Inner mantle lobe is reduced in comparison to the outer and middle lobes. Outer and middle lobes each feature flattened ventral and dorsal epithelia, while epithelium surrounding the inner lobe is plicated. The distal tip of the outer lobe is rectangular and there is a single obliquely oriented crease near the dorsal margin. Inner mantle lobe is reduced and does not extend to the margins of the outer and middle lobes. The epithelium of the outer lobe and middle
lobe is flattened, while plicae extend along the inner lobe (Fig. 278). Papillae are conical extensions of the mantle edge along the narrow, posterior shell margin. Papillae are darkened, consisting of a simple columnar epithelium containing brown to black intracellular granules and an ovular, monochromatic nucleus.

Subepithelium is fibrous and irregular, consisting of somatic musculature, hemolymph sinuses and nerve fibers. The epithelium and subepithelium of the papillae is wavy, giving the surface a folded texture with sharply defined crypts (Fig. 279). Darkened granules in posterior mantle edge are located in the
epithelium and subepithelial musculature, and the preponderance of granules conspicuously blackens the tissue (Fig. 280).

Middle mantle is a thin integument between mantle edge and visceral mass. An outer epithelium of columnar cells and goblet cells is adjacent to the nacre. Inner epithelium consists of cuboidal cells, and there is a fibrous subepithelium of connective tissue between the outer and inner epithelial layers. Connective tissue of the middle mantle consists of wavy fibers interspersed among hemocytes (Fig. 281).

The isthmus is a keel-like structure extending dorsally from the base of the visceral mass. Mantle isthmus originates anteriorly at the beginning of the digestive diverticulum and terminates posterior to the posterior adductor. Isthmus epithelium is simple columnar with isolated goblet cells and appears to have a flattened to irregular. Isthmus epithelial cells have a pale, eosinophilic cytoplasm and a monochromatic, oval nucleus, but columnar cells along the dorsal margin are strongly basophilic. Mantle isthmus has a subepithelium consisting of connective tissue fibers and hemocytes (Fig. 282).

Cellular Structure of Gill

Non-Marsupial Demibranch: Gill demibranchs are non-plicate consisting of ascending and descending lamellae with a series of horizontally oriented filaments. Ascending and descending gill lamellae are joined by a regular arrangement of septa. Cylindrical, vertical water tubes are located between ascending and descending lamellae (Fig. 283). Branchial filaments are cylindrical with three groups of cilia (lateral, latero-frontal, and frontal) along the lateral and distal surfaces. Filaments are each supported by a pair of basophilic skeletal rods and a pale, eosinophilic matrix of connective tissue. The medial aspect of gill filaments consists of a simple squamous epithelium comprising hemolymph vessels. Groups of two or more ctenidial filaments are separated by horizontal pores or ostia. Water is admitted into the water tubes through a series of ostia distributed throughout the gill (Fig. 284). The structure of the inner and outer gills is functionally identical (except for marsupial demibranchs, see below) until the ventral margin. Outer demibranchs have a convex ventral margin (Fig. 285), and the ventral margin of an inner demibranch is furrowed to allow particles captured along the lateral surfaces to accumulate in the median (Fig. 286).

Inner demibranchs and outer demibranchs also differ in the way gill tissue is joined to connective tissue dorsally. Ascending lamellae of the inner demibranchs are united with the dorsolateral portion of the visceral mass until the anterior-posterior midline of the visceral mass near the gonopores. The ascending lamella of the inner gill becomes separated from the body at the anterior margin of the nephridium near the urethra (Fig. 287). Descending lamellae of the inner demibranchs

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FIGS. 263–270. Shell morphology, and gross anatomical features of the mantle cavity of *Strophitus connasaugaensis*. FIG. 263: Lateral view of right valve showing the anteriorly directed umbo (UM), dorsally located ligament (LI), posterior slope (PS), and a well-defined posterior ridge (PR); FIG. 264: Lateral view of left valve showing the anteriorly directed umbo (UM), dorsally located ligament (LI), posterior slope (PS), and a well-defined posterior ridge (PR); FIG. 265: Medial view of the right valve showing the leading edge of the periostracum (PE), nacre (NA), pallial line (PL), anterior adductor scar (AS), posterior adductor scar (PS), pseudocardinal tooth (PT), interdentum (ID), umbo cavity (UC), and ligament (LI); FIG. 266: Medial view of the left valve showing the leading edge of the periostracum (PE), nacre (NA), pallial line (PL), anterior adductor scar (AS), posterior adductor scar (PS), pseudocardinal tooth (PT), interdentum (ID), umbo cavity (UC), and ligament (LI). FIG. 267: Ventrolateral view of *Strophitus connasaugaensis* (ca. 80 mm shell length) buried in sand at Shoal Creek with its mantle cupped forming an incurrent aperture (IA) and ecurrent aperture (EA); FIG. 268: Medial view of the mantle cavity with the right valve and right mantle removed to show the position of the mantle edge (ME), middle mantle (MM), foot (FO), anterior adductor (AA), labial palps (LP), inner demibranch (ID), outer demibranch (OD), posterior adductor (PA), mantle isthmus (MI), and supraanal aperture (SA); FIG. 269: Medial view of the mantle cavity with the right valve, right mantle, and right gills removed to show the position of the mantle edge (ME), middle mantle (MM), foot (FO), labial palps (LP), visceral mass (VM), anterior adductor (AA), left inner demibranch (ID), posterior adductor (PA), mantle isthmus (MI), and supraanal aperture (SA).
are connected to connective tissue located ventrally to the nephridia. At the dorsal end of the inner demibranch paired fascicles span the length of the gill (Fig. 288). Descending lamellae of the outer demibranchs are joined to the abdomen by connective tissue, and muscle fascicles are absent from the dorsal end of the outer demibranch (Fig. 289). Outer lamellae of outer demibranch joins a narrow region of connective tissue near the middle mantle (Fig. 290).

**Marsupial Demibranch**: Female *S. connasaugaensis* brood their glochidia larva in the outer demibranch, and virtually the whole gill is used for larval incubation (Fig. 291). A marsupial demibranch is expanded and includes a cylindrical mass of connective tissue, hemolymph and a sac containing glochidia. Overall, the marsupium of *S. connasaugaensis* is moderately enlarged. The ventral margin of the marsupium is convex and thickened with loose connective tissue and hemolymph (Fig. 292). Interbranchial septum consists of numerous spherical, goblet cells with a basophilic cytoplasm, and a vacuole containing pale, basophilic fibers (Fig. 293). Glochidia within a filled marsupium appear to be held together by strands of fibrous tissue, possibly representing a binding substance of a conglutinate of embryos. Glochidia are semicircular with a prominent, median adductor. Two tissue components are evident in regard to the valves; there is an eosinophilic, granular constituent and a basophilic material with a homogenous staining character (Fig. 294). Glochidia of *S. connasaugaensis* attach to fish skin and glochidial valves have spines along the ventral margin of the valves (Fig. 295). A collection of *S. connasaugaensis* from May 2011 presented an opportunity to study the outer gills of a female without glochidia. The marsupium was laterally expanded in comparison to the inner gill, and there was a medial core of connective tissue and hemolymph, which may represent a contracted placenta within the central water tube (Fig. 296).

**Cellular Structure of Foot and Associated Tissues**

**Pedal Musculature and Byssal Gland**: The ventral margin of the foot features a triangular array of overlapping muscle fibers in the median, subepithelial granulocytes and a highly folded integument (Fig. 297). Violet staining granulocytes are located directly beneath the pedal epithelium, while pale, blue-staining granulocytes are medial in respect to violet cells (Fig. 298). Foot muscle is composed of regions of irregular and regular muscle fibers. Musculature in the ventral region of the foot has a woven appearance, with fibers arranged into a vertical and horizontal plane (Fig. 299). The somatic muscle at the base of the foot is organized with distinct vertical and horizontal layers (Fig. 300). The base of the foot is broadly triangular, with an ovular byssal gland located medially at the ventral margin of the coelom, posterior to the pedal ganglion (Fig. 301). The byssal gland is supported by concentric connective tissue fibers and musculature. The lumen is lined with a simple, ciliated columnar epithelium with occasional, pale, basophilic goblet cells. Goblet

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FIGS. 271~277. Anterior mantle edge of *Strophitus connasaugaensis*. FIG. 271: Transverse section of the mantle edge portraying the position of the outer lobe (OL), middle lobe (ML), and inner lobe (IL); FIG. 272: Transverse view of the base of the outer lobe showing columnar epithelium (CE) and basal bulb (BB) of the outer lobe, squamous epithelium (SE) of the middle lobe, periostracal groove (PG) and periostracum ribbon (PE); FIG. 273: Transverse section of the free distal tip of the outer mantle lobe featuring columnar epithelium (CE), plicae (PL), and fibrous tissue (FT) in the subepithelium; FIG. 274: Transverse section of the middle mantle lobe revealing squamous epithelium (SE) and periostracum (PE) along the ventral surface, and columnar epithelial cells (CE) of the dorsal surface; FIG. 275: Transverse section of the inner mantle lobe featuring narrow plicae (PL), violet cells (VC), and irregular fibrous tissue (FT); FIG. 276: Transverse section of the dorsal epithelium of the mantle edge featuring plicae (PL), goblet cells (GC), and a subepithelium consisting of myofibers (MF), hemolymph sinuses (HS), and nerves (NE); FIG. 277: Transverse section of the base of the mantle edge revealing a deeply basophilic columnar epithelium (CE), and myofibers (MF) in the subepithelium.
FIGS. 278–282. Posterior mantle edge, middle mantle, and mantle isthmus of *Strophitus connasaugaensis*. FIG. 278: Transverse section of posterior mantle edge revealing thin outer lobe (OL) and middle lobe (ML) and a triangular inner lobe (IL) featuring plicae (PL) along the dorsal and ventral surfaces; FIG. 279: Sagittal section of posterior mantle edge characterized by conical papillae (PA) with brown-pigmented epithelium (BE), and a subepithelium consisting of hemolymph sinuses (HS), nerves (NE) and myofibers (MF); FIG. 280: Sagittal section of posterior mantle edge emphasizing brown intracellular granules (BG) present in columnar epithelium (CE), and subepithelial musculature (MU); FIG. 281: Transverse section of middle mantle displaying an outer epithelium (OE) of columnar cells bearing cilia (CI), goblet cells (GC) inner epithelium (IE), connective tissue (CT), and hemocytes (HC); FIG. 282: Transverse section of mantle isthmus emphasizing columnar epithelium (CE), goblet cells (GC), and irregular connective tissue (CT) and hemocytes (HC) in the subepithelium.
cells are interspersed among columnar cells in the lumen, and the contents of goblet cells appear granular to wispy. Byssal gland goblet cells are similar in some respects to goblet cells of marsupial septa (see Fig. 293). The vestiges of the byssus is represented by a spherical, mass containing a pale to darkened eosinophilic material. The byssal mass occupies the center of the byssal gland lumen and is surrounded by an irregular, eosinophilic mass, likely constituting a fluid (Fig. 302). Vestiges of the byssal canal were not observed in histological sections of the foot.

**Pedal Integument and Mesentery:** There are five epithelium types surrounding the foot and visceral mass. From ventral to dorsal, the integument is initially pleated with deep folds, and the folds become shorter in height and narrower in width. In the first two regions, plicae are tall and thickened with subepithelial musculature. The first region possesses ciliated, columnar cells with a subepithelium consisting of irregular myofibers and violet granulocytes. Ciliated cells have an eosinophilic cytoplasm, similar to the coloration of the underlying muscle tissue, and cilia are short and nearly straight. Nuclei of type one epithelium are oval and have a homogeneous staining character (Fig. 303). Epithelial region two consists of tall plicae with a more conspicuous space between the folds. Columnar cells largely lack cilia and have an oval, monochromatic nucleus. Numerous goblet cells are present and closely juxtaposed, giving the tissue the appearance of large, oval to teardrop-shaped mucus pores. Subepithelial tissue of integument type two consists of horizontally arranged myofibers (Fig. 304). Plicae are noticeably reduced in height and width in region three. Cells in the second region have similar cytological characteristics as region one, however ciliated cells are sparsely distributed. The third integumentary region has two layers of musculature, including a horizontally and a vertically oriented layer (Fig. 305). Integument four consists of a squamous epithelium around rectangular folds of connective tissue. There is a prominent space between the epithelium and pedal musculature of region four. The space possibly represents a hemolymph sinus (Fig. 306). The fifth integumentary region of the foot is flattened, with squamous cells and goblet cells. Only a subtle waviness of the tissue layer is reminiscent of the plications further down the length of the foot. Musculature under the fifth epithelium is thinner and is separated from the overlying epithelium except for occasional fibrous, connective tissue linkages (Fig. 307). While the pedal integument is pleated, the folds occur in an irregular manner and sagittal sections reveal an irregular series of furrows (Fig. 308).

The sinistral and dextral halves of the visceral mass are joined by mesentery fascicles distributed throughout the coelom (Fig. 309). Fascicles are composed of eosinophilic fibrils with a pale to darkened cytoplasm, and fibrils are quadrangular to polygonal. Furthermore, there does not appear to be an obvious pattern of fibril arrangement within a fascicle, and the mesentery bundles are histologically identical to pedal muscle fibers (Fig. 310).

**Labial Palps, Oral Groove and Esophagus:** Labial palps of *S. connasaugaensis* have an inner surface lined with ciliated, rectangular plicae and a smooth outer surface (Fig. 311). Plicae of the inner palp surface are rectangular with a flattened dorsal margin and a pleated ventral surface. Ciliated columnar cells represent the main constituent of the inner palp epithelium and extend along the palp interior up to the distal margin of each lip. Ciliated cells of the inner palp surface have a pale, basophilic cytoplasm and an oval, monochromatic nucleus. The surface of the epithelium is densely ciliated and cilia are wispy (Fig. 312). The free distal palp tip transitions from a pleated, ciliated epithelium to a non-ciliated mucosa characterized by oval goblet cells (Fig. 313). The external palp surface is flattened, with non-ciliated cuboidal cells with a monochromatic nucleus (Fig. 314). Subepithelial tissue of the labial palp is composed of loose connective tissue and hemolymph sinuses.

**Anterior Adductor and Anterior Pedal Retractors:** Anterior adductor consists of bands of eosinophilic myofilaments longitudinally oriented across the viscera. Fibers are eosinophilic and typically appear wavy in histological sections. Myocytes have emarginated and
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FIGS. 283−290. Outer demibranch and inner demibranch of *Strophitus connasaugaensis*. FIG. 283: Transverse section of inner gill displaying horizontally oriented filaments (FI), a septum (SE) linking the inner and outer lamella, and a median water tube (WT); FIG. 284: Transverse section of the inner lamella of inner gill showing the main tissue components of gill including frontal cilia (FC), frontal-lateral cilia (FL), lateral cilia (LC), ostia (OS), hemolymph vessels (HV), hemocytes (HC) skeletal rods (SR), and a water tube (WT); FIG. 285: Transverse section of the distal tip of outer gill characterized by a rounded distal margin (DM), resembling merged branchial filaments (FI); FIG. 286: Transverse section of the base of the gill where muscle fascicles (MU) are aligned anterior to posterior; FIG. 287: Transverse section of the base of the gill showing the junction of the outer lamina of the inner gill (OL), and inner lamella of the outer gill (IL), and loose connective tissue; FIG. 288: Transverse section of the base of the inner gill where muscle fascicles (MU) are aligned anterior to posterior; FIG. 289: Transverse section of the base of the gill showing the junction between the outer lamina of outer gill (OG) and middle mantle (MM).

Oral Groove and Esophagus: Oral groove is a derivation of labial palp wrapping around the anterior visceral mass. In transverse sections of visceral mass, the oral groove appears as a large chamber posterior to the anterior adductor. The ventral wall of the oral groove resembles the distal margin of a labial palp. The ventral epithelium of the lip resembles the flattened epithelium along the outer surface of the palp, while the elongated, rectangular plicae appear to be a continuation of inner palpal epithelium. Additionally, the plicae contain elliptical, basophilic goblet cells (Fig. 317). Note the similarity between the columnar epithelium of the oral groove ledge with the epithelia along the inner surfaces of the palps, located at the dorsal end of the palps (Fig. 311). The lateral walls of the oral groove consist of cuboidal cells bearing cilia, and a loose matrix of connective tissue fibers comprises the subepithelium (Fig. 318). The dorsal epithelium is flattened with ciliated columnar cells (Fig. 319).

Elongated nuclei with granular chromatin. Perimysium consists of thin wispy connective tissue fibers and form ellipsoid chambers that may contain hemolymph. Additionally, the underlying space of perimysium consists of black granules (Fig. 315). The anterior pedal retractors are located between the labial palps and mantle, and fascicles are arranged in a transverse orientation when the visceral mass is sectioned transversely. The epimysium, perimysium and endomysium are present, but these structures are delicate and thin (Fig. 316).

Cellular Structure of the Alimentary Canal

Digestive Diverticulum: The esophagus opens dextrally into the digestive diverticulum and where there is a conspicuous "L"-shaped chamber. The walls of the vestibular chamber feature cylindrical plicae similar to the constituents of the esophageal-digestive diverticulum junction of *F. cerina*. The lumen of the vestibular chamber contains irregular patches of an eosinophilic fluid, possibly

The esophagus is a tubular continuation of the palps, since the walls feature, tall, rectangular plicae. The esophagus is oval, with concentric connective tissue fibers forming a distinct lamina propria (Fig. 320). The esophagus is located at the base of the visceral mass, located between digestive gland tubules and the mantle isthmus. Ciliated, rectangular folds extend from the dorsal and ventral esophageal walls. Epithelial cells are pseudostratified and ciliated, with an eosinophilic cytoplasm and an elliptical, monochromatic nucleus. Cilia along the apical surface of esophageal cells are short and generally straight. The lumen of the esophagus may contain a shapeless, eosinophilic mass, which may represent mucus and ingested substances. Additionally, the esophageal epithelium is supported by concentric layers pink and red eosinophilic fibers, creating a heterogeneous lamina propria. The esophagus is lined with plicae featuring ciliated columnar cells, and there is a subtle, median furrow to the surface of each plication (Fig. 321).
mucus (Fig. 322). Plicae of the vestibule are cylindrical and ciliated, and the subepithelium features a well-defined connective tissue support (Fig. 323). The cell types of the vestibule resemble digestive diverticulum type one epithelium. Cells are ciliated and columnar with a conspicuously eosinophilic cytoplasm and contain a granular nucleus with a small nucleolus. Plicae of type one tubules are flattened to rounded, and some plicae have a single, median furrow (Fig. 324). Secondary tubules have a homogenous, eosinophilic character and a minute brush border membrane (Fig. 325). Furthermore, type two tubules of S. connasaugaensis do not appear vesiculated (Fig. 326). Tertiary tubules of S. connasaugaensis consist of columnar digestive cells with an eosinophilic, vesiculated cytoplasm, and triangular, dark-staining basophil cells. A small, spherical nucleus may be located at the base of basophilic cells and the nucleus has a darker staining character than the surrounding cytoplasm (Fig. 326).

**Stomach:** The stomach is somewhat square-shaped, located between the digestive diverticulum and crystalline style sac. The gastric walls are pleated nearly on all sides, and there is a prominent typhlosole along the dextral wall (Fig. 327). The ventral wall has a distinctive cuticle (Fig. 328). The cuticle is attached to cilia of the columnar epithelium and it has linear fibers embedded within it. The cytoplasm of these columnar cells is pale, eosinophilic with brown intracellular granules. The nucleus is ovular and contains a distinct nucleolus (Fig. 329). The medial portion of the ventral gastric wall features a furrow and the cuticle is absent from the sinistral portion of the gastric epithelium (Fig. 330). The sinistral portion of the ventral stomach wall consists of a series of rounded, ciliated plicae (Fig. 331). The dorsal margin of the stomach features low, broadly rounded plicae with ciliated columnar cells (Fig. 332). The typhlosole also has a ciliated columnar epithelium, and the distal margin of the typhlosole is forked. Additionally, there is an eosinophilic cuticle surrounding the typhlosole, and it is dense such that microtomy dislodges it slightly from the apical surface of the epithelium (Fig. 333).

**Crystalline Style Sac:** The crystalline style sac is an ovoid chamber spanning the length of the body from the stomach to the posterior margin of the visceral mass. The style is a pale, eosinophilic rod located sinistrally, within the circular aspect of the organ. Extending dextrally from the style sac is a horizontal chamber known as the midgut. The style sac consists of three different types of epithelium based on cell size, staining characteristics and cilium morphology (Fig. 334).

The style sac is lined with rectangular, eosinophilic columnar cells with thick, rigid cilia and a conspicuous cell membrane. Nuclei of type-one cells are ovular and contain a distinct nucleolus (Fig. 335). Type-two epithelium is located along the ventral surface of the midgut, dextral to the median fold. There are a series of short plicae along the dextral wall of the midgut and they are subtle in the anterior portion of the style sac, but become more prominent posteriorly. Columnar cells of type-two epithelium have a slightly more basophilic and vesiculated cytoplasm. Cilia of type-two epithelium are thinner than type-one cilia and appear correspondingly more flexible given their wispy appearance. Nuclei of type-two cells have an ovular nucleus and a median nucleolus (Fig. 336). Type-two epithelium represents the main constituent of the midgut.
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Figures 297–302. Pedal musculature, and byssal gland of Strophitus connasaugensis. Figure 297: Transverse section of the ventral margin of the foot characterized by an inner core of somatic musculature (SM), and peripheral, basophilic granulocytes (GC). Figure 298: Transverse section of pedal subepithelium characterized by myofibers (MF) pale, blue granulocytes (BC) in the median, and violet-staining granulocytes (VC) between the blue cells and the epithelium. Figure 299: Transverse section of ventral pedal musculature featuring an irregular meshwork longitudinal fibers (LF), and transverse fibers (TF). Figure 300: Transverse section of dorso-lateral foot musculature characterized by transverse myofibers (TF) and type-two pedal epithelium (E2). Figure 301: Transverse section of the byssal gland (BG) showing its close proximity to ovarian acini (OA), hemolymph (HL), and mesentery (ME) characterizing the ventral margin of the coelom. Figure 302: Transverse section of the byssal gland displaying ciliated columnar cells (CC), goblet cells (GC), byssus (BY) and an outer wrapping of fibrous tissue (FT).

Intestine: The style sac represents the first limb of the intestine and it extends dorsally through the posterior visceral mass with an anteriorly directed curvature (Fig. 339). Sagittal sections of the posterior visceral mass reveal a complex epithelial structure at the posterior margin of the visceral mass. Style sac epithelium is consistent through the descending portion of its length, however each type of epithelium may be located on either the anterior or posterior intestinal wall, which creates a complex structure (Fig. 340). Transverse sections of visceral mass reveal two limbs of the intestine ventral to the crystalline style sac in a sinistral and dextral position (Fig. 341). The sinistral limb comprises the ascending second intestinal limb, while the dextral limb represents the third, descending portion of the intestine. The second intestinal limb is characterized by tall and short plicae, and in a sagittal plane, the lumen has a slightly undulating appearance (Figs. 341, 342). There are two types of epithelium comprising the lining of the second intestinal limb. The first type of epithelium is located at the beginning of the second intestinal limb as it begins to bend along the posterior visceral margin. Type-one cells are columnar, with a pale, eosinophilic and vesiculated cytoplasm. Additionally, there are transparent bubbles located at the ciliated surface, possibly representing mucus secretions. Nuclei of type-one cells are ovular with a distinct nucleolus (Fig. 343). Secondly, there are ciliated columnar cells that are more widely distributed throughout the second and third intestinal limbs. Ciliated columnar cells are distinguished by a darker eosinophilic cytoplasm and the lack of vesicles. Nuclei of type-two epithelial cells are ovular and contain a distinct nucleolus (Fig. 344). The second intestinal limb travels anteriorly to the mid-point of the visceral mass and becomes the third intestinal limb when it bends to extend posteriorly. Epithelial tissue of the third intestinal limb is not different from the second intestinal limb. Connective tissue surrounding the second and third intestinal limbs is different from the supporting tissue surrounding the first intestinal limb. The tissue has a darker eosinophilic character, and nuclei are larger and more spherical than connective tissue surrounding the style sac.

The fourth limb of the intestine is characterized by a prominent, ventrally extending typhlosole. The typhlosole is a large extension of the dorsal intestinal wall consisting of pale, eosinophilic, columnar cells. The ventral wall of the fourth intestine is pleated with short, rounded to flattened plicae (Fig. 345). The ventral and dorsal epithelia are both ciliated with short cilia. Ventral epithelium consists of darkened, basophilic columnar cells. The nucleus of ventral epithelial cells is compressed and

Note: The text provided includes a table of figures but the specific details of the table are not included in the natural text representation.
teardrop-shaped, and there is a slightly more eosinophilic granular region in the apical portion of the cell. Cytoplasm of ventral epithelial cells is thin and most conspicuous at the apical and lateral portions of plicae (Fig. 346). Nuclei of typhlosole columnar cells are oval and contain a distinct nucleolus. Epithelial cells of the typhlosole are well defined in comparison to the darkened basophilic cells. Additionally, typhlosole epithelium contains isolated goblet cells. The nuclei of dorsal epithelial cells are oval with a distinct nucleolus (Fig. 347). Connective tissue supporting the typhlosole consists of a latticework of loose connective tissue and spindle-shaped nuclei.

The fifth limb of the intestine is located medially and runs parallel to the hinge line. The intestine is enclosed within the heart, and there is a ring of cardiac muscle surrounding the anterior portion of the intestine. The most prominent feature of the fifth intestinal limb is the large typhlosole extending dorsally into the lumen, and the dorsal wall has numerous, narrow plicae (Fig. 348). Epithelium of the typhlosole is ciliated and columnar, with a pale, eosinophilic cytoplasm and an oval nucleus with a distinct nucleolus (Fig. 349). Plications around the dorsal intestinal wall consist of pale, eosinophilic columnar cells bearing cilia. Columnar cells of dorsal and ventral epithelium of intestinal limb five bear a close resemblance to each other (Fig. 350). Connective tissue comprising lamina propria of the fifth intestinal limb consists of a lattice of fibers within a pale ground substance and spindle-shaped nuclei. However, there is a thin, darkened layer of fibrous tissue, possibly representing musculature, surrounding the intestine.

Cellular Structure of Cardiovascular System Tissues

**Heart:** The heart is located dorsally in relation to the visceral mass, and it begins at the anterior margin of the nephridium and terminates between the posterior margin of the visceral mass and posterior pedal retractors. Ventricular cardiac muscle begins as a thin wrapping around the intestine and progressively becomes thicker posteriorly. The ventricle is large, bulbous and medially located, while the auricles are thin, irregular extensions of cardiac tissue extending obliquely and medially into the ventricle from the lateral pericardium (Fig. 351). The ventricle consists of a thin epicardium and a thickened myocardium. Epicardium is a pale, eosinophilic, simple squamous epithelium enclosing the underlying myocardium and epicardium is highly irregular (Fig. 352). Myocardium consists of thick bands of myofibers oriented in a transverse and longitudinal plane. Cardiac myocytes have a dark, red, eosinophilic composition with spherical to oval, monochromatic nuclei (Fig. 353). Auricles are thin, incorporate a small amount of cardiac muscle, and have an irregular epicardium. Auricles may be closely positioned to the pericardial sac, and pericardium is constructed

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FIGS. 303–310. Pedal integument, and mesentery of *Strophitus connasaugaensis*. FIG. 303: Transverse section of pedal integument type-one, emphasizing tall irregular plicae (PL) with densely packed cilia (CI) overlying a subepithelium of irregular fibers (IF), and basophilic granulocytes (BG); FIG. 304: Transverse section of pedal integument type-two represented by tall plicae (PL), columnar cells (CC), goblet cells (GC), and transverse myofibers (TF); FIG. 305: Transverse section of pedal integument type-three, characterized by shorter, thinner plicae (PL) bearing goblet cells (GC), cilia (CI), and a distinct subepithelium of connective tissue (CT), and underlying strata of transverse myofibers (TF); FIG. 306: Transverse section of pedal integument type-four, characterized by a sinuous, but more flattened integument of cuboidal epithelium (CE), cilia (CI), goblet cells (GC), and a subepithelium of hemolymph sinues (HS), and transverse myofibers (TF); FIG. 307: Transverse section of pedal integument type-five, revealing the flattened squamous epithelium (SE), cilia (CI), goblet cells (GC), and longitudinal myofibers (LF); FIG. 308: Sagittal section of pedal integument featuring irregular plicae (PL) giving the integument a rugose structure; FIG. 309: Transverse section of the lateral coelomic margin emphasizing longitudinal myofibers (LF), and struts of musculature forming mesentery (ME); FIG. 310: Sagittal section through the coelom revealing a mesentery fascicle (FA) surrounded by adipocytes (AC), and hemolymph (HL).
from squamous epithelial cells (Fig. 354). Hemolymph flow traveling into the ventricle is regulated on the sinistral and dextral sides of the ventricle by the auriculoventricular valves. The auriculoventricular valves are composed of squamous cells and cardiac muscle. Inner and outer valve surfaces feature squamous cells, and there is an inner layer of muscle tissue (Fig. 355).

Arteries, Veins, Capillaries and Pericardial Gland: Hemolymph is located throughout the viscera and mantle of S. connasaugaensis. Adipose tissue represents a significant portion of the connective tissue of the coelom and mantle, and there is a preponderance of black, ink-like granules located within the interstitial spaces of adipose tissue. Additionally, there are isolated hemocytes located within interstitial spaces of coelom and mantle (e.g., Figs. 281, 312, 357). However, the circulatory system has numerous circular to oval tubes representing hemolymph vessels. Hemolymph vessels consisting of thickened musculature may comprise arteries (Fig. 356). Hemolymph vessels that incorporate less muscle tissue are potential veins and veins have a more irregular shape (Fig. 357). Capillaries are also apparent, especially within adductor muscle and mantle edge. Capillaries are characterized by a ring of squamous cells and correspondingly have a small diameter (Fig. 358).

The dorsal aspect of the visceral mass consists of an extensive fibrous tissue surrounding the pericardium. The fibrous tissue represents the pericardial gland and it is characterized by pale, eosinophilic fibers and hemocytes. Pericardial gland cells are irregularly oriented and possess an emarginated nucleus. Hemocytes are located freely within the lumen of the pericardial gland, and some hemocytes seem to adhere to the pericardial gland cells (Fig. 359).

Cellular Structure of Renal System Tissues

Anterior Nephridium: The nephridium is a large tubular organ located dorsally to the left and right of the visceral mass. Nephridium extends down the length of the body from the midpoint of the visceral mass to the posterior adductor. Nephridium consists of distinct ventral and dorsal limbs, and the morphology of each limb changes posterior to the visceral mass. At its anterior extent, the ventral nephridium has a convoluted epithelium, and the dorsal limb is a simple cavity underlying the ventral region (Fig. 360). Ventral nephridium features a simple columnar epithelium consisting of strongly eosinophilic, vesiculated cells. The surface of the ventral nephridial cells is smooth, seemingly lacking cilia or microvilli. Nuclei are spherical to oval without a distinct nucleolus. The ventral limb has irregular branches of epithelial tissue extending into the nephridial lumen. The subepithelium of ventral nephridium consists of a pale, eosinophilic layer of squamous cells comprising the endothelium (Fig. 361). The dorsal nephridium consists of cuboidal cells with a pale, eosinophilic cytoplasm and a spherical, monochromatic nucleus. Dorsal nephridium has isolated regions of columnar cells containing a pallid median vesicle. Some vesiculated columnar cells are teardrop shaped with an apical vesicle (Fig. 362). Dorsal nephridium largely lacks branches, but a small number of reduced plicae are
FIGS. 317–321. Oral groove, and esophagus of *Strophitus connasaugaensis*. FIG. 317: Transverse section of oral groove showing a cylindrical shelf consisting of musculature (MU), goblet cells (GC), and a ciliated columnar epithelium (CC); FIG. 318: Transverse section of the lateral oral groove wall displaying ciliated columnar cells (CC) and a subepithelium of loose connective tissue (CT); FIG. 319: Transverse section of the dorsal oral groove wall, displaying cilia (CI), and loose connective tissue (CT); FIG. 320: Transverse section through dorsal aspect of visceral mass showing the esophagus (ES) lined with plicae (PL), enclosed within a distinct lamina propria (LP), and surrounded by tubules of the digestive diverticulum (DD); FIG. 321: Transverse section of esophagus emphasizing plicae (PL) consisting of ciliated columnar cells (CC) supported by a dense matrix of fibrous connective tissue (CT).
FIGS. 322−326. Digestive diverticulum of *Strophitus connasaugaensis*. FIG. 322: Transverse section of the junction between the esophagus (ES) and digestive diverticulum (DD) revealing a large vestibular chamber characterized by ciliated plicae (PL); FIG. 323: Transverse section of the junction between the esophagus and digestive diverticulum, emphasizing conical plicae (PL) bearing cilia (CI), and underlying lamina propria (LP); FIG. 324: Transverse section of digestive diverticulum portraying plicae (PL) and cilia (CI) of primary digestive tubules; FIG. 325: Transverse section of digestive diverticulum revealing the minute structure of microvilli (MV) of secondary tubules (ST) and the contrasting cellular features of tertiary tubules (TT); FIG. 326: Transverse section of the digestive diverticulum focusing on tertiary tubule structure including eosinophilic vesicles of digestive cells (DC), and violet-staining basophil cells (BC).
present (Fig. 363). Fluid from the pericardial cavity is flushed into the ventral nephridium through a ciliated duct or renopericardial canal at the anterior end of the nephridium. The renopericardial canal merges with ventral nephridium at the lateral margin of the ventral nephridium, but appears to be a circular duct at its midpoint. Transverse histological sections of the nephridium show a circular duct representing the midpoint of the renal-pericardial canal and the adjacent, laterally positioned urethra (Fig. 364). Epithelial tissue comprising the renopericardial canal features ciliated columnar cells, and goblet cells organized into conical plicae enclosed within a thickened circumferential lamina propria (Fig. 365). The histological composition of the urethra resembles the dorsal nephridial chamber until the distal-most portion near the communication between the nephridium and suprabranchial cavity. Darkened, basophilic columnar cells bearing cilia characterize the urethra of *S. connasaugaensis*. Cilia are short and densely distributed across the surface of the epithelium towards the distal end of the urethra (Fig. 366).

**Posterior Nephridium:** Nephridial branches expand, and the tissue becomes enlarged between the posterior margin of the visceral mass and posterior adductor. Nephridial epithelium is convoluted, consisting of epithelial folds with a repeating stem-loop configuration (Fig. 367). Posterior nephridial branches are spaced farther apart from each other than in the anterior nephridium. Epithelial cells are cuboidal to columnar with a pale eosinophilic and granular cytoplasm. Cytoplasm of nephridial cells may contain brown granules, while the apical surface of some cells has a filamentous, eosinophilic residue, possibly representing a secretion. Each half of a nephridial branch is united by filamentous, connective tissue septa. The medial portion of a nephridial branch constitutes a hemolymph vessel and emarginated nuclei of endothelial cells are present (Fig. 368). The ventral margin of the posterior nephridium features a pair of large nerves. Each nerve consists of an outer epineurium and an inner, granular focus of axons (Fig. 369).

**Posterior Adductor and Posterior Pedal Retractors:** Posterior pedal retractors are located within the nephridial septum, between the posterior adductor and visceral mass. Pedal retractors have a distinct epimysium surrounding a series of fascicles. Connective tissue of pedal retractors is delicate, especially the epimysium and perimysium. Endomysium consists of a pale, eosinophilic membranous material between myofibers. Myofibers are polygonal in a transverse orientation, and have a dark, eosinophilic cytoplasm (Fig. 370). The posterior adductor is located posterior to the retractors and is oriented longitudinally when mussels are transversely cut. Adductor myofibers are organized into large fascicles with a simple, squamous epimysium and a delicate, fibrous perimysium. Myofibers of the posterior adductor are long, eosinophilic filaments with elliptical, heterochromatic nuclei and a distinct nucleolus (Fig. 371). Perimysium is thin, membranous and contains a series of ellipsoid capillaries.

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FIGS. 327–333. Stomach of *Strophitus connasaugaensis*. FIG. 327: Transverse section through visceral mass showing the morphology of the stomach (ST) and surrounding digestive diverticulum (DD); FIG. 328: Transverse section of the dextral portion of the ventral stomach wall showing a thin cuticle (CU) adhered to the columnar epithelium (CE) with fibrous underlying connective tissue (CT); FIG. 329: Transverse section of the dextral portion of the ventral stomach wall showing a dense cuticle (CU), vesiculated columnar epithelium (CE), and connective tissue (CT) of the subepithelium; FIG. 330: Transverse section of the medial portion of the ventral stomach wall showing a columnar epithelium (CE), medial groove (MG), and underlying connective tissue (CT) of the subepithelium; FIG. 331: Transverse section of the sinistral portion of the ventral stomach wall revealing broadly rounded plicae (PL), connective tissue (CT), and tertiary digestive tubules (TT); FIG. 332: Transverse section of the dorsal stomach wall showing short plicae (PL), and a dense mat of cilia (CI); FIG. 333: Transverse section of dextral stomach wall showing a rectangular and forked typhlosole (TY), with an attached cuticle (CU).
Pedal Ganglion, Cerebral Ganglia and Visceral Ganglion: Nervous system of *S. connasaugaensis* consists of four ganglia, each with a series of nerve fibers extending throughout the body. A pair of cerebral ganglia is located anteriorly; each ganglion is positioned laterally along the body between the anterior adductor and labial palp. The third ganglion is referred to as the pedal ganglion since it is located at the ventral margin of the hemocoel between the digestive diverticulum and gonad, dorsal to the foot. Finally, the pleural ganglion is located on the ventral surface of the posterior adductor. Each ganglion has a distinct outer cortex of neuron cell bodies, and a fibrous inner medulla. Furthermore, there is not an obvious difference between the cellular characteristics of all ganglia, and therefore the foregoing description of the neural cortex and medulla of the pedal ganglion is representative of the anterior and posterior ganglia.

Pedal ganglion has two lobes, and each hemisphere is surrounded by a distinct epineurium and separated by a median fissure. Central commissures oriented horizontally represent communications between the hemispheres (Fig. 372). Neuron cell bodies in the cortex are conical to polygonal, with the apex extending towards the medulla neuron. Cell bodies have a pale, basophilic cytoplasm and a spherical nucleus, and a nucleolus may be observed in some cells (Fig. 373). Fissures separating the hemispheres are fibrous with a pale, eosinophilic character. The medulla has an irregular array of axons and isolated cell bodies. Commissures represent a continuation of the medulla, joining each ganglionic lobe by means of horizontal bundles of axons (Fig. 374). Cell bodies of the medulla have a spherical to spindle shaped nucleus and only a small amount of darkened, eosinophilic cytoplasm is visible (Fig. 375). Axons leave the hemispheres at lateral extensions of the medulla at dorsal and ventral margins of the hemispheres. Roots along the lateral margins of the pedal ganglion consist of large bundles of axons and supporting cells (Fig. 376). Nerves derived from the ventral aspect of each hemisphere extend ventrally through the pedal musculature. Pedal nerves from dorsal roots span the vertical length of the visceral mass.

The cerebral ganglia are spherical to ovoidal, located within connective tissue between the palp, anterior pedal retractor and adductor (Fig. 377). Nerves derived from these anterior ganglia extend into the anterior adductor, retractor, foot and mantle edge. The visceral ganglion is cylindrical, and closely applied to underlying myofibers of the posterior adductor. The ventral surface of the visceral ganglion consists of a thin layer of connective tissue and an epithelium represented by simple columnar cells and goblet cells. Additionally, columnar cells contain brown intracellular granules and the entire cytoplasm is darkened (Figs. 378, 379). The inner gill reunites with the body at the anterior margin of the posterior adductor. At ctenidial-adductor junction, ganglionic tissue extends ventrally into gill tissue (Fig. 380).

Nerves and Statocysts: Nerves may be distinguished from connective tissue by the pale eosinophilic membrane or perineurium, surrounding nerve fascicles. The perineurium
is thickened with a homogenous staining character, while groups of axons have small, irregularly by polygonal spaces between each other. Transverse sections show how the contents of a bundle of nerves are irregular or vesiculated mass of fibers (Fig. 381). Longitudinal sections of nerves portray bundles of axons as sinusuous ribbons of fine, eosinophilic fibers (Fig. 382). Additionally, nerves contain a series of nuclei; cylindrical to emarginated nuclei located within a nerve appear to be neuron cell bodies. Nuclei of neuron cell bodies have a small perimeter of basophilic cytoplasm. Given the above, the smallness of nerve cells precludes definitive identification with hematoxylin and eosin-stained tissue sections.

Statocysts represent accessory structures of the nervous system associated with the pedal ganglion. A statocyst is an ovular capsule located at the ventral margin of the coelom, lateral to the pedal ganglion. Statocyst epithelium consists of ciliated columnar cells lining the lumen and a basal layer of cuboidal cells. Columnar cells of a statocyst have a pale eosinophilic cytoplasm and a spherical, basophilic nucleus containing a small nucleolus. The lumen of the statocyst contains a dark, spherical mass called the statolith. The statolith has a dark, violet character and may appear fragmented. A thickened, pale, eosinophilic capsule surrounds the statocyst and connective tissue becomes continuous with coelomic connective tissue associated with the pedal ganglion (Fig. 383).

Cellular Structure of Reproductive System Tissues

Ovarian and testicular tissues are organized into ovular acini. Ovarian and testicular acini exhibited a low-level of gametogenesis during May 2011 from individuals collected in Shoal Creek while peak gametogenesis was evident in one male and one female S. connasaugaensis collected from South Fork Terrapin Creek in August 2011. Ovarian acini contained a large concentration of eosinophilic matter consisting of small, spherical granules and larger cells, possibly representing polar bodies. The eosinophilic granules may represent the remains of polar bodies and apoptotic oocytes. Oocytes were spherical to ovular, basophilic, with a distinct, spherical nucleus, and a series of transparent cytoplasmic vesicles. Some oocytes appeared to be free within the lumen or attached to the acinus wall by a pellicle (Fig. 385).

A higher level of gametogenesis was evident in August. Acini were enlarged with correspondingly enlarged mature oocytes. Mature oocytes typically exhibited a well-defined perimeter of eosinophilic granules and small irregular nuclei. Mature oocytes appear to begin as a small basophilic cell with a distinct nucleus and nucleolus. Oocytes are attached to the acinus wall by a pellicle and eventually separate from the wall when they reach a certain size. As oocytes mature, they become larger and eosinophilic cytoplasm becomes more prominent. Mature
FIGS. 345–350. Fourth intestinal limb, and fifth intestinal limb of *Strophitus connasaugaensis*. FIG. 345: Transverse section of the fourth intestinal limb featuring a large, ventrally extending typhlosole (TY), with a correspondingly large lamina propria (LP), and plicae along the lateral-dorsal wall (PL); FIG. 346: Transverse section of ventral epithelium of the fourth intestinal limb focusing on basophilic cells (BC) containing vesicles (VE) and a thin lamina propria (LP); FIG. 347: Transverse section of dorsal epithelium of the fourth intestinal limb featuring ciliated columnar cells (CC), and the underlying lamina propria (LP); FIG. 348: Transverse section of the fifth intestinal limb revealing a large, dorsally extending typhlosole (TY), with an extensive lamina propria (LP), plications (PL) along the dorsal wall, and thin tissue layers comprising the ventricle (VE) and pericardium (PC); FIG. 349: Transverse section of the ventral epithelium of the typhlosole emphasizing ciliated columnar cells (CC), containing vesicles (VE), and extensive lamina propria (LP); FIG. 350: Transverse section of dorsal epithelium portraying conical plicae (PL), bearing cilia (CI), and underlying tissues including a thin lamina propria (LP) and rim of myofibers (MF).
HISTOLOGICAL ATLAS OF FRESHWATER MUSSELS

FIGS. 351−355. Heart, and pericardium of *Strophitus connasaugaensis*. FIG. 351: Transverse section the lateral portion of the heart showing the fifth intestinal limb (IN), ventricle (VE), auricle (AU), auriculoventricular valve (AV), and pericardial gland (PG). FIG. 352: Transverse section through the ventricle focusing on the thin epicardium (EC), and underlying myocardium (MC). FIG. 353: Transverse section of the ventricle emphasizing transverse and longitudinal myofibers (MF), and interspersed hemocytes (HC). FIG. 354: Transverse section of the auricle (AU) showing epicardium (EC), cardiac muscle (CM), pericardial sac (PC), hemocytes (HC), and pericardial gland (PG). FIG. 355: Transverse section of an auriculoventricular valve showing cardiac muscle (CM), enveloped by a thin endothelium (EN).

Oocytes of *S. connasaugaensis* have a red, granular cytoplasm and a pale, basophilic nucleus with dark patches of chromatin. The membrane of mature oocytes is irregular, with a pale staining character. However, the distal margin of the vitelline membrane appears to be more condensed and fibrous. Mature oocytes are greatly enlarged, occupying the majority of the lumen of the acinus (Fig. 386).
FIGS. 356–359. Hemolymph vessels, and pericardial gland of *Strophitus connasaugaensis*. FIG. 356: Transverse section of visceral mass showing a possible artery featuring an endothelium (EN), and thick muscular wrapping (MU), and surrounding pockets of hemolymph (HL); FIG. 357: Transverse section through the visceral mass featuring a possible vein demarcated by its endothelium (EN), and surrounded by adipocytes (AC) and pockets of hemolymph (HL); FIG. 358: Sagittal section through posterior adductor emphasizing longitudinal myofibers (MF), thin perimysium (PM), and a capillary consisting of endothelium (EN); FIG. 359: Transverse section of the pericardial gland showing emarginated nuclei of pericardial gland cells (EN), and hemocytes (HC).

FIGS. 360–366. Anterior nephridium of *Strophitus connasaugaensis*. FIG. 360: Transverse section of the dorsal aspect of the visceral mass (VM) showing the branched ventral nephridium (VN), dorsal nephridium (DN), pericardium (PC) and ventricle (VE); FIG. 361: Transverse section of the ventral nephridium emphasizing the sinuous nephridial lumen (NL), eosinophilic intracellular granules (EG) of nephridial epithelium and subepithelial hemolymph vessels (HV), and endothelium (EN); FIG. 362: Transverse section of dorsal nephridium consisting of teardrop-shaped columnar cells (TC), an expansive nephridial lumen (NL), and adjacent hemolymph (HL) and ventral nephridium (VN); FIG. 363: Transverse section of ventral nephridium focusing on the flattened, columnar epithelium (CE) of the dorsal wall, short, rounded plicae (PL) along the ventral surface, and hemocytes (HC); FIG. 364: Transverse section of the lateral margin of the nephridium showing the ventral nephridial branches (VN), urethra (UR), ciliated renopericardial canal (RC), and hemolymph (HL); FIG. 365: Transverse section of the renopericardial canal featuring ciliated columnar cells (CC), goblet cells (GC), and an encircling lamina propria (LP); FIG. 366: Transverse section of the urethra showing basophilic columnar cells bearing cilia (CI), goblet cells (GC), and lamina propria (LP).
Stage 1 testicular acini are small and positioned within a matrix of adipocytes and ciliated ducts (Fig. 387). Low gametogenesis in testicular acini was characterized by the presence of sperm morula appearing as clusters of small, spherical cells. Spermatocytes undergo a cell division process resulting in a series of smaller, closely spaced cells. Spermatocytes of sperm morula appear to largely lack cytoplasm and consist of a darkened cell body. Small
eosinophilic granules are interspersed among sperm morula, possibly representing a fluid or the remains of broken down spermatocytes. A small quantity of spermatozoa may be present within the center of the acinus (Fig. 388).

Testicular acini in a male collected in August were conspicuously enlarged and distinguished by a plethora of spermatozoa. The luminal contents contain such a large quantity of actively dividing spermatocytes and spermatozoa that...
FIGS. 377–380. Cerebral ganglia, and visceral ganglion of *Strophitus connasugaensis*. FIG. 377: Transverse section through cerebral ganglion showing neural cortex (CO), and medulla (ME); FIG. 378: Transverse section of visceral ganglion showing axons (AX), neuron cell bodies (CB), and a ventral layer of columnar epithelium (CE) containing brown intracellular granules; FIG. 379: Transverse section of visceral ganglion showing the cortex (CO), medulla (ME), and longitudinal myofibers of the posterior adductor (PA); FIG. 380: Transverse section of the base of the ctenidia near the posterior visceral mass showing goblet cells (GC), musculature (MU), and cell bodies (CB) and axons (AX) of a ventral extension of visceral ganglion.
virtually the entire acinus is darkened. Dividing spermatocytes are spherical and have a pale basophilic character. Sperm morula are also present, but less abundant than in stage 1 testicular acini. As in stage 1 testicular acini, the small size and dark staining character of spermatocytes and sperm morula make observations of specific meiotic phases difficult (Fig. 389). When acini are mature, ciliated gonadal ducts carry sperm and ova from acini to the gonopores. Presently spermatocytes and mature ova were observed descending to the gonopores (Fig. 390). The gonopores are located at the dorsal end of the visceral mass, ventral and anterior to the nephridium. Gonopores appear C-shaped in histological sections, and the lining consists of ciliated columnar cells and goblet cells. Goblet cells are more numerous just outside of the gonopores (Fig. 391).

DISCUSSION

Overall, 13 tissues were structurally conserved between V. nebulosa, F. cerina and S. connasaugaensis, and there were 11 tissue types in which we observed interspecific morphological variation (Table 5). We have further summarized the morphological differences among these 11 tissue types in Tables 6–11. Given the above, we provide a detailed synthesis of the functional morphology of each tissue type. Noteworthy is that while there are many synoptical anatomical references for bivalves, few provide such details about how tissue morphology or cellular structure corresponds to bivalve physiology (Galtsoff, 1964; Yokley, 1968; Lasee, 1991; Norton & Jones, 1992; Eble, 2001; Grizel, 2003).

Tissue-Specific Conclusions

Mantle

Mantle of unionids has been described by various workers concerned with specific cells or functions of the mantle: Raßbach (1912; mantle edge and periostracum secretion in Anodonta cygnea (as Anodonta cellensis), Siebert (1913; cellular characteristics of mantle edge
Fig. 384: Transverse section of visceral mass revealing stage 1 ovarian acini (OA) and adjacent ciliated gonadal duct (CD); Fig. 385: Transverse section of an ovarian acinus emphasizing emarginated nuclei (EN) constituting the acinus wall, developing oocytes (OC), and cell bodies (CB); Fig. 386: Transverse section of a stage 3 ovarian acinus showing a developing oocyte (DO) arising from a pellicle (PE), larger, mature oocytes (MO), and an irregular vitelline membrane (VM); Fig. 387: Transverse section of stage 1 testicular acini (TA), and an adjacent ciliated gonadal duct (CD); Fig. 388: Transverse section of an stage 1 testicular acinus revealing emarginated nuclei (EN) constituting the acinus wall, primary spermatocytes (PS), and sperm morula (SM); Fig. 389: Transverse section of a stage 3 testicular acinus showing prodigious spermatogenesis (SG), and spermatozoa (SZ) occupying the lumen; Fig. 390: Transverse section of a gonadal duct emphasizing ciliated columnar cells (CC), and preponderant spermatozoa (SZ) in the lumen; Fig. 391: Transverse section of a gonopore showing ciliated columnar cells (CC), and goblet cells (GC).
TABLE 6. Summary of structural differences in anterior mantle edge of Alabama rainbow (*Villosa nebulosa*), Gulf pigtoe (*Fusconaia cerina*) and Alabama creekmussel (*Strophitus connasaugaensis*).

<table>
<thead>
<tr>
<th>Species</th>
<th>Anterior mantle edge</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outer lobe</td>
<td>Middle lobe</td>
</tr>
<tr>
<td><em>Villosa nebulosa</em></td>
<td>Dorsoventrally</td>
<td>Dorsoventrally</td>
</tr>
<tr>
<td></td>
<td>compressed,</td>
<td>compressed,</td>
</tr>
<tr>
<td></td>
<td>tapering distally</td>
<td>tapering distally</td>
</tr>
<tr>
<td>Ventral epithelium</td>
<td>flattened</td>
<td>flattened</td>
</tr>
<tr>
<td>with plicae along</td>
<td></td>
<td></td>
</tr>
<tr>
<td>distal end of lobe</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fusconaia cerina</em></td>
<td>Branched and bulbous</td>
<td>Rounded distally</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventral epithelium</td>
<td>with plicae</td>
<td>flattened</td>
</tr>
<tr>
<td>with plicae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal epithelium</td>
<td>with plicae</td>
<td>flattened</td>
</tr>
<tr>
<td><em>Strophitus connasaugaensis</em></td>
<td>Dorsoventrally</td>
<td>Dorsoventrally</td>
</tr>
<tr>
<td></td>
<td>compressed,</td>
<td>compressed,</td>
</tr>
<tr>
<td></td>
<td>tapering distally</td>
<td>tapering distally</td>
</tr>
<tr>
<td>Ventral epithelium</td>
<td>flattened</td>
<td>flattened</td>
</tr>
<tr>
<td>Dorsal epithelium</td>
<td>with reduced,</td>
<td>flattened</td>
</tr>
<tr>
<td></td>
<td>sparsely distributed plicae</td>
<td></td>
</tr>
</tbody>
</table>

crystals from the columnar epithelium located along the ventral surface of the outer mantle lobe (Petit et al., 1979).

Morphological differences in mantle lobe shape and epithelial topography were observed between *V. nebulosa*, *F. cerina*, and *S. connasaugaensis* at the anterior and posterior ends (Tables 6, 7). Potentially, the morphology of the mantle edge may correspond to shell geometry, thickness and or sculpture. *Villosa nebulosa* and *S. connasaugaensis* have an ovular shell of a moderate thickness and height (Figs. 1–4, 263–266). In contrast, the shell of *F. cerina* is circular to rhomboidal, considerably thicker and higher than the shells of *V. nebulosa* and *S. connasaugaensis* (Figs. 132–135) (also see Williams et al., 2008). The outer mantle lobe of *F. cerina* is branched and pleated, and the epithelium features numerous basophilic cells (Figs. 140–142). Given the high surface area of a branched mantle lobe with a pleated epithelium, the outer lobe of *F. cerina* may exhibit a high-level of protein production. In contrast, *V. nebulosa* and *S. connasaugaensis* both have a uniramous outer mantle lobe, and the epithelium features fewer plicae (Figs. 9–11, 271–273). Furthermore, plicae along the surface of the outer mantle lobe of these two species were shorter. Additionally, the columnar cells around the outer lobe of *F. cerina* appear to be taller (Figs. 140, 142) than outer lobe cells of *V. nebulosa* and *S. connasaugaensis* (Figs. 10, 11, 272, 273). Electron microscopy of the outer, and middle mantle lobes of *Amblema plicata* indicated additional substrate is added to the periostracum as it travels down the length of the mantle lobes (Petit et al., 1978). Although no secretions were observed between the outer and middle lobes of *V. nebulosa*, *F. cerina*, and *S. connasaugaensis*, the basophilic epithelium of the outer lobe may secrete additional substrate into the periostracum ribbon. The basophilic columnar epithelium located on the ventral surface of the outer mantle lobe is the site of nacre production (Figs. 9, 15, 140, 146, 271, 277). Considering the above, *F. cerina*...
**TABLE 7. Summary of structural differences in posterior mantle edge of Alabama rainbow (Villosa nebulosa), Gulf pigtoe (Fusconaia cerina) and Alabama creekmussel (Strophitus connasaugaensis).**

<table>
<thead>
<tr>
<th>Species</th>
<th>Outer lobe</th>
<th>Middle lobe</th>
<th>Inner lobe</th>
<th>Papillae</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Villosa nebulosa</em></td>
<td>Dorsoventrally compressed, tapering distally, extending from an elongated base</td>
<td>Dorsoventrally compressed, tapering distally, extending from an elongated base</td>
<td>Bulbous, enlarged, extending to outer and middle lobes</td>
<td>Finely conical</td>
</tr>
<tr>
<td></td>
<td>Ventral epithelium flattened</td>
<td>Ventral epithelium flattened</td>
<td>Epithelial cells with conspicuous black or brown, intracellular granules</td>
<td></td>
</tr>
<tr>
<td><em>Fusconaia cerina</em></td>
<td>Outer lobe branched with two triangular lobes</td>
<td>Middle lobe straightened, not rounded distally</td>
<td>Triangular, compressed, not extending past outer, middle lobes</td>
<td>Subtriangular, branched</td>
</tr>
<tr>
<td></td>
<td>Ventral epithelium flattened</td>
<td>Ventral epithelium flattened</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Strophitus connasaugaensis</em></td>
<td>Dorsoventrally compressed</td>
<td>Dorsoventrally compressed</td>
<td>Triangular, short, not extending beyond outer and middle lobes</td>
<td>Conical</td>
</tr>
<tr>
<td></td>
<td>Flattened epithelium</td>
<td>Flattened epithelium</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

May have a greater capacity to produce precursory shell material than either *V. nebulosa* or *S. connasaugaensis*, and this ability might explain why *F. cerina* has a thicker shell.

Although a mechanism for shell formation has been proposed for Unionidae, noteworthy is that shell formation has been studied from a limited range of species. Additionally, cellular structure of the mantle edge has been studied using different methods. Members of Unionidae are known for exhibiting a high degree of interspecific variation in shell geometry, height, thickness and sculpture (knobs, ridges or projections on the exterior surface) (Williams et al., 2008). From a sample of histological literature on mantle lobes of bivalves there is variation in the shape of the lobes, number plicae present, and size of plicae across different orders (Morton, 1987; Morrison, 1993; Eble, 2001; Colville & Lim, 2003). It seems likely that mantle lobe morphology is related to structural differences in bivalve shells, but this topic is understudied.

An additional consideration for shell formation should be mantle lobe musculature. The mantle edge is rich in somatic musculature, and myofibers extend into the mantle lobes. A recent theory regarding the formation of sculpture patterns on the shells of marine bivalves suggests muscle contractions may cause periostracum to build up unevenly. Extension, retraction and lateral movements of the mantle edge may cause periostracum to accumulate in such a way as to form shell ribs (Checa, 2002). The outer mantle lobe of *F. cerina* is branched and the flexure of one or more of these branches may allow periostracum to thicken.

The role of the triangular and pleated inner mantle lobe is indeterminate; the inner lobe plausibly can extend and retract given its highly muscular composition (Figs. 9, 13, 140, 144, 271, 275). Possibly the inner mantle lobe plays a role in initial settlement of juveniles considering the highly pleated surface and basophilic, subepithelial cells. Perhaps the mantle is glandular during the juvenile stage.
and produces an anchoring substance similar to a byssus. Pediveligers of Ostrea edulis have a glandular integument that secretes a cement to allow settlement (Cranfield, 1973a, b). Irregular, basophilic cells with a granular cytoplasm were located in the subepithelial tissue of the middle lobe and inner lobe of V. nebulosa, F. cerina and S. connasauagensis. Such cells were also located in the subepithelium of the foot (see below). Possibly, these cells produce an adhesive substance needed for attachment to substrate, but ducts leading from the cell body to the apical surface of the mantle lobe epithelium were not observed. Therefore, the basophilic cells may be connected to ducts during an earlier life history stage. Beedham (1958) referred to the inner mantle lobe as the “sensory lobe,” possibly because the inner lobe of scallops (Pecten spp.) is manifested as a series of tentacles that have a sensory function. However, axons observed in the lateral aspect of the mantle edge were not observed in the inner mantle lobe of V. nebulosa, F. cerina and S. connasauagensis (e.g., Fig. 279). Furthermore, sensory cells such as ciliated olfactory receptors were not observed in histological sections of the inner lobe.

The base of the mantle edge between the pallial line and mantle lobes consists of adipose tissue and musculature. Adipocytes were observed in the subepithelial tissue of the base of the mantle edge (Figs. 14, 15, 140, 146, 271), and in the middle mantle and have been referred to as vesicular cells or Leydig cells. Leydig cells are located throughout the visceral mass and they may represent a source of energy in the form of glycogen (Colville & Lim, 2003). The dorsal surface of the mantle edge between the pallial line and the inner mantle lobe consists of a region of glandular plicae (Figs. 14, 145, 276). It is not certain what the true function of these structures is, but it seems likely that the plicae are associated with water currents that run parallel with the mantle cavity. The half-shell photographs of the mantle cavity of V. nebulosa, F. cerina and S. connasauagensis show that the mantle edge lines up with the incumbent aperture (Figs. 6, 137, 268). Therefore, the plicae may be associated with water currents entering the mantle cavity, and the mucus produced by the extensive network of goblet cells may bind particles suspended in local water currents, and allow them to be collected by cilia along the gill filaments.

Papillae around the apertures were sectioned from V. nebulosa, F. cerina and S. connasauagensis with the intent of revealing sensory receptor cells and associated nerves. While divisions of the pallial nerve extended from the base of the mantle edge to the main axis of each papilla, sensory neurons were not observed on epithelium surrounding each papilla (Figs. 17, 18, 148, 149, 279). Sensory receptor neurons have been described from mantle tentacles of Lima hians (Owen & McCrae, 1979) and from siphon papillae of Eurytellina lineata (as Tellina lineata) and Macoma biota (Vitonis et al., 2012). Owen & McCrae (1979) reported that there are three types of sensory receptors, two multi-ciliated receptors, and a third receptor featuring a single kinocilium surrounded by stereocilia. Ciliated receptors consist of types A, and B. Type A receptors consist of a dense tuft of 35–40 cilia distributed across 4–6 cells. Type B receptors have 17–20 cilia on the apical surface of one cell. Based on transmission, and scanning electron microscopy the authors were not able to determine whether the ciliated receptors respond to chemical or mechanical stimuli (Owen & McCrae, 1979). Vitonis et al. (2012) estimated that siphon papillae may contain as many as 10/100 µm² ciliated receptors, and from siphon papillae of Eurytellina lineata (as Tellina lineata) and Macoma biota (Vitonis et al., 2012) estimated that siphon papillae may contain as many as 10/100 µm² ciliated receptors while the epithelium facing the lumen has more sparsely distributed receptors (20/10,000 µm²). Furthermore, papillae and lumenal epithelia have different types of ciliated receptors based on cilia density and cilium height. However, the status of ciliated receptors as either chemosensory or mechanoreceptory has yet to be determined.

Females of Villosa have a papillose mantle edge that functions as a lure to attract potential host fishes (Fig. 7; Haag et al., 1999; Williams et al., 2008). Species of Lampsilis and Villosa and have modified mantle flaps to attract host fishes (Ortmann 1910a; Haag & Warren, 1999; Haag et al., 1999; Williams et al., 2008). Kraemer (1967) observed a ganglion in the mantle edge of Lampsilis ventricosa (as Lampsilis cardium) and proposed that it may regulate mantle flap movements. However, no ganglia were observed in the mantle edge of gravid female V. nebulosa.

The middle mantle is a thin, delicate skin-like tissue and serves to exchange hemolymph between the mantle edge and viscera (Fig. 19, 143, 281). Illustrations of the mantle cavity of freshwater mussels suggest that there are a series of anterior to posterior currents along the surface of the middle mantle (Kellogg, 1915). Petit et al. (1978) observed ciliated cells along the inner surface of the middle mantle of Am-
blema plicata perplicata (as Amblyea plicata). Herein, the presence of ciliated cells along the inner mantle surface can be confirmed from V. nebulosa, F. cerina, and S. connasaugaensis. The darkened, basophilic columnar cells located at the dorsal margin of the isthmus may secrete periostracum (Figs. 20, 21, 144, 282). The ligament is composed of periostracum and represents a combined product from secretions of the isthmus and dorsal aspect of the mantle edge; mantle lobes produce the outer layer of the ligament while the inner layer is secreted by the isthmus (Beedham, 1958). Petit et al. (1978) observed thread-like secretions uniting the isthmus and ligament, and Beedham (1958) reported similar staining characteristics of the ligament while the inner layer is secreted by the isthmus (Beedham, 1958).

Non-Marsupial Gill

Non-marsupial demibranchs in unionids have been described using light microscopy and SEM, to elucidate the functional morphology of the gill as both a feeding and respiratory organ: Posner (1875; histology of gill in Anodonta anatina and Unio pictorum), Peck (1877; cellular nature of gill in Anodonta sp.), Ridewood (1903; basic cellular structure of gill in Anodonta cygne a, Monocondylaea sp. (Unionidae), Velesunio ambigu us (as Unio ambigu us), Unio pictorum (Unionidae), and Etheria elliptica (as E etheria plumbea), Pseudomulleria dalyi (as Mulleria dalyi) (Etheriidae)), Ortmann (1911a, b; comparative morphology of inner and outer gills plus marsupial and non-marsupial gills of various unionids), Allen (1914; functional morphology of inner and outer gill plus ciliary currents for particle capture in Pyganodon grandis (as Anodonta grandis), Potamilus alatus (as Lampsis alatus), Lampsis fasciola (as Lampsis ligamentinus), Actinonaias ligamentina (as Lampsis luteolus), Ligumia recta (as Lampsis rectus), Ligumia subrostrata (as Lampsis subrostratus), Fusconaia flava (as Quadrula rubiginosa), Elliptio dilatata (as Unio gibbosus)), Atkins (1937; particle capture on gill in Anodonta anatina), Kellogg (1915; functional morphology of ctenidial currents in Elliptio complanata (as Unio complanatus)), Stasek (1963; ctenidial currents in Anodonta californiensis), Smith (1988; histology of fibrous tissue in gill of Margaritifera hembeli), Gardiner et al. (1991; SEM of ostium pores and water tubes in Py ganodon grandis (as Anodonta grandis), and Ligumia subrostrata), Kovitvadhi et al. (2007; ontogeny of gill in Hyriopsis myersiana (as Hyriopsis (Limnoscapha) myersiana).

Based on the above literature gill structure of V. nebulosa, F. cerina and S. connasaugaensis is similar to what has been reported previously on unionoids. There was no apparent difference in the structure of gill filaments between each species studied herein (Figs. 22, 23, 152, 153, 283, 284). Galbraith et al. (2009) reported marginally significant differences in the density of cirral plates between Actinonaias ligamentina, Amblyea plicata, Fusconaia flava and Obli quaria reflexa and intraspecific and interspecific variation in the number of cilia per cirrus. Considering the observations of Galbraith et al. (2009) were derived from SEM preparations of ctenidia, there may be differences in cirral plates and density of cilia between V. nebulosa, F. cerina and S. connasaugaensis at the ultrastructure level.

We observed interspecific morphological differences in fascicle structure located at the base of the inner and outer ctenidia (Table 8). The fascicles located at the base of the gill possibly represent supporting structures that allow attachment of gill filaments to dorsal connective tissue fibers near the nephridia. Alternatively, the fascicles may represent musculature that allows movement of the entire gill (Figs. 27, 28, 157, 158, 288). Longitudinal and transverse muscle groups in gill described may control water circulation by altering the diameter of ostia and water tubes. However, the description of musculature was based on myofibers that are incorporated into the water tubes (Gardiner et al., 1991). Given the above, it is uncertain whether transverse musculature located at the base of the gill would play a role in water circulation or if muscular contractions generated by this tissue would facilitate the transport of ova into marsupia.

Marsupial Gill

Early anatomical descriptions of unionid marsupial gill detailed gross anatomy and included illustrations of cells lining the gill, but later works focused on sexual dimorphism and reproductive biology of the structure and relationship with glochidia: Lefevre & Curtis (1910, illustrations of cells comprising the marsupial gill water tubes of Pyganodon cataracta (as Anodonta cataracta), Alasmidonta marginata (as Alasmidonta truncata) and Lasmigona com planata (as Symphynota complanata); see also Ortmann [1910b]), Ortmann (1911a, b; sexual
TABLE 8. Summary of structural differences in gill of Alabama rainbow (Villosa nebulosa), Gulf pigtoe (Fusconaia cerina) and Alabama creekmussel (Strophitus connasaugaensis).

<table>
<thead>
<tr>
<th>Species</th>
<th>Gill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outer gill base</td>
</tr>
<tr>
<td><strong>Villosa nebulosa</strong></td>
<td>Two conspicuous fascicles spanning anterior-posterior</td>
</tr>
<tr>
<td></td>
<td>Water tubes thickened with fibrous tissue</td>
</tr>
<tr>
<td></td>
<td>Small, ciliated plicae lining water tubes</td>
</tr>
<tr>
<td></td>
<td>Glochidia enclosed in a thin membrane</td>
</tr>
<tr>
<td><strong>Fusconaia cerina</strong></td>
<td>Well developed fascicles between posterior third of visceral mass and posterior adductor</td>
</tr>
<tr>
<td></td>
<td>Laterally distended, branchial width is the same dorsal to ventral</td>
</tr>
<tr>
<td></td>
<td>Water tubes lined with squamous epithelium</td>
</tr>
<tr>
<td></td>
<td>Septa with irregular, vesiculated plicae, plicae become tall in the median</td>
</tr>
<tr>
<td></td>
<td>Glochidia and unfertilized ova linked together in conglutinate</td>
</tr>
<tr>
<td><strong>Strophitus connasaugaensis</strong></td>
<td>Reduced fascicles between posterior third of visceral mass and posterior adductor</td>
</tr>
<tr>
<td></td>
<td>Fascicles absent</td>
</tr>
<tr>
<td></td>
<td>Water tubes lined with squamous epithelium</td>
</tr>
<tr>
<td></td>
<td>Septa with spherical, basophilic mucus cells</td>
</tr>
<tr>
<td></td>
<td>Glochidia contained within a cylindrical placenta</td>
</tr>
</tbody>
</table>

We observed distinct differences in the morphology and cell types of the marsupium of *V. nebulosa*, *F. cerina*, and *S. connasaugaensis* (Table 8), and we feel the specific structural characteristics are a reflection of the dispersal strategy of each species. Species of *Villosa*,...
such as *V. nebulosa*, brood using the posterior portion of the outer gill. A marsupium of *V. nebulosa* is a grossly enlarged group of water tubes that become distended when filled with glochidia (Fig. 7) (Ortmann, 1911a). The walls of the water tubes are strengthened with connective tissue fibers to maintain a strong connection between the outer and inner faces of a marsupium as it expands to accommodate ova. The increasing diameter of the water tubes from dorsal to ventral may be reflective of the mechanism of glochidial release (Fig. 29). Glochidia are released from the ventral margin of a marsupium following the appropriate stimulus from a potential host fish (Fig. 30). Host fishes may approach a displaying mussel and bite or come into direct contact with flapping mantle tissue. Following a fish strike, a mussel may rapidly expel the contents of one or both marsupia (Haag et al., 1999).

The interbranchial septa and vertical water tube walls of the marsupia of *V. nebulosa* were lined with ciliated columnar cells (Figs. 31–37). Cilia may occur on the septa or along the walls of water tubes of non-marsupial gills but appeared to be localized near the ostia, most notably in *F. cerina* (Fig. 153). However, water currents through the vertical water tubes of unionid gills may be regulated by the muscular contractions of horizontal and vertical muscle fibers (Gardiner et al., 1991). The function of cilia within the marsupial chambers of *V. nebulosa* could serve to exchange oxygen rich and oxygen poor water currents (Figs. 32, 33). However, it is unclear if or how cilia inside of a marsupium function. Richard et al. (1991) demonstrated that water does not enter the water tubes of a marsupium in *Pyganodon grandis* (as *Anodonta grandis*), *Ligumia subrostrata* and *Toxolasma texasiensis* (as *Carunculina parva texasiensis*) when filled with glochidia. Therefore, we may infer that cilia may be inactive in a filled marsupium or perhaps cilia aid in the delivery of calcium ions or nutrients to glochidia (Silverman et al., 1987; Schwartz & Dimock, Jr., 2001).

Glochidia contained within the marsupial water tubes were loosely organized within the lumen. Histological sections of the ventral margin of filled marsupia revealed the presence of a wispy, fibrous material possibly representing a sac that surrounds the entire mass of glochidia (Fig. 30). However, the contents of the gill of *V. nebulosa* were delicate, and the glochidia and embryos observed in the lumen of the water tube were often fragmented or displayed separation artifacts, making it difficult to study a membrane surrounding the mass of glochidia. Mature glochidia were mixed with early stage embryos judging by the presence of spherical clusters of undifferentiated cells (Fig. 34). Considering these observations were made from mussels collected in May and that spawning occurs during the late summer, these observations may indicate that not all fertilized ova mature into glochidia. Furthermore, there may be a limit to the total number of glochidia that could form from a set of fertilized ova. Glochidia of *V. nebulosa* have discoid valves that lack hooks along the ventral margin. Hookless glochidia are typically gill parasites that attach to the delicate gill lamellae of fishes. Based on laboratory infections, *V. nebulosa* is considered to be host specific and transform on species of *Micropterus* (Neves et al., 1985).

Species of *Fusconaia* incubate their larvae using all four gills and are therefore referred to as tetragenous brooders (Fuller, 1973). The marsupia of *F. cerina* are uniformly thickened dorsal to ventral, and virtually the entire gill becomes incorporated with glochidia. Like the marsupial gill of *V. nebulosa*, the water tubes are strengthened with fibrous tissue. Hookless glochidia of *Fusconaia* spp. are packaged into cylindrical masses called conglutinates (Fuller, 1973; Bruenderman & Neves, 1993; Haag & Warren, 2003). A single, mature conglutinate consists of layers of ova and each layer contains a linear arrangement of peach-colored glochidia, attached to red, unfertilized ova (Figs. 152, 154). Glochidia mature in a conglutinate while attached to unfertilized ova, and together, glochidia and unfertilized ova are released dorsally, through the suprabranchial cavity, as masses of conglutinates. Exactly how glochidia of *Fusconaia* spp. are transferred to a host fish is unknown, but possibly mature conglutinates, when released from a female mussel, are perceived as aquatic insect larvae and a fish strike may separate some glochidia from a conglutinate and attach to gill tissue of a potential host (Bruenderman & Neves, 1993; Haag & Warren, 2003). This seems plausible given that conglutinates consist of spherical bodies arranged into a cylindrical unit and appear segmented like insect larvae (DeWalt et al., 2010). Observations of released conglutinates of *F. cerina* indicated that conglutinates are buoyant, and cyprinids will closely approach (Haag & Warren, 2003). Laboratory infections have indicated that members of Cyprinidae may be suitable hosts for species of *Fusconaia*.
Anodontoides ferussacianus

The structural features of the marsupia of S. connasaugaensis generally resembled marsupia of F. cerina (Figs. 160, 291). Strophitus connasaugaensis brood using the outer demibranchs and masses of glochidia may be contained within an entire demibranch. Species of Strophitus are generally believed to disperse glochidia within a matrix of mucus (Ortmann, 1911a; Lefèvre & Curtis, 1912; Haag & Warren, 1997; Hove, 1995; Watters, 2002). Strophitus undulatus may produce a gelatinous and cylindrical conglutinate (Ortmann, 1911a; Lefèvre & Curtis, 1912; Watters, 2002). Haag & Warren (1997) reported that glochidia of Strophitus subvexus may be released within a copious mucus matrix that may indiscriminately entangle fish, however no specific details regarding the exact nature of the mucus was described. Furthermore, it may be implied from Haag & Warren (1997) that the mucus matrix of Strophitus subvexus conglutinates resembles a web of fibers similar to conglutinate of Anodonta cygnea and Anodontoides ferussacianus. Herein, we observed a placenta-like sac containing glochidia in gills of S. connasaugaensis (Fig. 291), and the core of placenta tissue appears to contract following the release of glochidia (Fig. 296). This cylindrical mass may represent a mold for the formation of a mucoid conglutinate similar to conglutinates described by Watters (2002). The interbranchial septa of the marsupium of S. connasaugaensis are distinctively basophilic, and the spherical cells containing wispy cytoplasmic figures may be responsible for the formation of a gel (Fig. 293). According to Watters (2002), the glochidia larva may be attached to the exterior of the conglutinate following its release into stream water and that osmotic pressure may drive internalized glochidia out of this gelatinous rod. Glochidia may become anchored to the exterior of the conglutinate by means of a filament extending from interior of the valves. Following the release of conglutinates from the gill, the opening and closing of glochidial valves may cause the whole conglutinate to move in a worm-like fashion and mimic a prey item of a host fish (Watters, 2002). Based on photographs of released conglutinates of Strophitus undulatus, it is possible that a fish might perceive conglutinates to be a worm (Barnhart, 2008). Glochidia of Strophitus spp. have hooks (Fig. 295), can attach to skin, and have proven to be host generalists capable of transforming off a phylogenetically broad spectrum of fish species in a laboratory setting (Watters, 2002). Glochidia of S. undulatus may transform on fish gill in a laboratory setting (Hove et al., 1997), but it is not certain where they typically attach in the wild. Lefèvre & Curtis (1912) reported that some Strophitus undulatus (as Strophitus edentulus) larvae passed the glochidium stage while remaining within the mucoid conglutinate, since a small-number of newly transformed juveniles were observed and juveniles exhibited a protractible foot. Considering the above-mentioned adaptations for parasitism, the possibility that glochidia would forego the parasitic stage of their lifecycle and transform within the water tubes of its parent seems remote.

The conglutinates observed in demibranchs of S. connasaugaensis resembled a placenta with inner and outer walls of squamous epithelial cells and a subepithelium consisting of loose connective tissue and hemolymph (Fig. 291). Moreover, the placenta resembled a derivation of tissue from the interbranchial septum and water tube walls (Figs. 291, 296). Histological sections of the placenta revealed a loosely organized group of spinous glochidia, but it was indeterminate as to whether there was a gelatinous rod surrounding the glochidia and located within the placenta. However, the abundant, basophilic goblet cells along the dorsal and ventral surfaces of gill septa of S. connasaugaensis may extrude mucus into the lumen of the water tube and create a gelatinous rod prior to the release of a conglutinate (Fig. 293).

The possibility that glochidia receive nutrients by means of a placenta-like connection to the surrounding gill tissue has been suggested in Schwartz & Dimock, Jr. (2001). Herein, we have only observed a placenta-like structure in the marsupial gills of S. connasaugaensis. Conglutinates of F. cerina did not appear to be attached to the vertical walls of the water tube. Embryos and glochidia of V. nebulosa appeared to be enclosed within a sac, but the separation artifacts made it difficult to determine whether such a sac was united with the surrounding gill tissue. However, gill tissue was sectioned in a transverse orientation, and if the conglutinates of F. cerina or the sac encasing glochidia were attached to interbranchial septa, it may not be apparent from this orientation. Possibly, coronal sections through marsupia could provide insight into whether there is a connection between glochidia and gill tissue. Although no physical attachment of glochidia to gill tissue was observed, the abundance of
goblet cells on the interbranchial septa of *F. cerina* and *S. connasaugaensis* could support the hypothesis that gill tissue somehow delivers nutrients to developing glochidia. Perhaps these cells secrete sugars derived from glyco-gen deposits in the septa.

**Foot**

The foot of unionids has been detailed by Brück (1914), pedal musculature in distinctive vertical and horizontal planes and the rugose pedal epithelium in *Anodonta cygnea* (as *Anodonta cellensis*), Yokley (1968); pedal musculature, extensive folds of pedal epithelium, with subepithelium comprised of basophilic cells in *Pleurobema cordatum*, Araujo et al. (2002; SEM showing dense mat of cilia covering the pedal integument in juvenile *Margaritifera auricularia*, Lasee (1991, used SEM and LM to show development of the foot in *Lampsilis cardium* (as *Lampsilis ventricosa*) [see also Lima et al. (2006)], Kovitvadhi et al. (2007; SEM of pedal cilia on dorsal and ventral surface of foot in juvenile *Hyriopsis myersiana* (as *Hyriopsis (Limnoschapa) myersiana*).

Relative to the anatomical literature of foot, our observations dovetail with those of Brück (1914). The musculature in *V. nebulosa, F. cerina* and *S. connasaugaensis* consisted of dense, regular chords of muscle fibers that become irregular within the ventral tip (Figs. 36, 38, 165, 167, 297, 299). Whereas we describe the cellular structure of pedal musculature, Brück (1914) documented gross anatomy. Pedal musculature forms a median, triangular hemocoel that is likely filled with hemolymph and may be regulated by vasculature (Brand, 1972). Freshwater mussels burrow into sediment with the muscular foot, and the foot is a hydrostatic organ capable of great distension. For example, Trueman (1968) revealed that the foot of *Margaritifera margaritifera* can be flattened, and blade-like, and later expand ventrally and laterally, exhibiting a distended, spherical shape. The work of Brand (1972) indicated that distension of the foot is achieved by the rapid filling of pedal hemolymph sinuses with hemolymph. The irregular array of myofibers in the ventral tip of the foot may enable movement in many directions, and possibly maintains tensile strength when the foot is enlarged.

Secondly in regard to Brück (1914), we confirm that pedal epithelium of unionoids is pleated. Overall there are conspicuous epithelial folds at the ventral margin of the foot and these folds became progressively shorter towards the gill-visceral mass junction (Table 9). The pedal epithelium of *V. nebulosa, F. cerina* and *S. connasaugaensis* is rugose with deep, irregular crypts along the surface of the integument around the ventral portion of the foot (types 1–3 epithelium). Considering that the foot may be used as an anchor, it seems that the high surface area created by the folded epithelium surrounding the foot would create a strong frictional force and allow a burrowing bivalve to remain embedded (Figs. 43–45, 172–174, 303–305). The plethora of mucus cells within pedal epithelium may create a lubricant that would minimize cutaneous damage from abrasion when the foot is inserted into fine sand. Based on histological sections of the three-unionid species, it seems that grooves around the foot were deeper on *F. cerina* than on *V. nebulosa or S. connasaugaensis*. This observation could be an indication of an adaptation exhibited by mussels in large rivers, such as the Cahaba River. Pedal grooves may enable mussels to stay firmly embedded in the substratum minimize the possibility of becoming dislodged by strong water currents. Likewise, plicae around the foot of *V. nebulosa* and *S. connasaugaensis* were shorter, and the small size of plicae may reflect adaptation to headwater streams, such as Terrapin Creek and Shoal Creek, where a smaller channel size would accommodate less water volume than a larger river such as the Cahaba. Since the historic ranges of *V. nebulosa, F. cerina* and *S. connasaugaensis* may have overlapped, perhaps there are differences in the depth of the pedal grooves that may reflect local adaptation (Williams et al., 2008).

The ventral-most region of the foot has a highly ciliated surface. Cilia may be present further down the vertical length of the foot and visceral mass, but ciliated cells were indistinct in epithelial regions 2–5. Juvenile unionids have minute gill buds and may employ pedal feeding during the weeks following transformation from the glochidium stage (Lasee, 1991; Yeager et al., 1994). During pedal feeding, particulate matter may be collected by a combination of foot scraping and ciliary action. Scanning electron microscopy of the foot of juvenile unionoids has revealed a densely ciliated pedal surface (Lasee, 1991; Araujo et al., 2002; Lima et al., 2006).

Basophilic granulocytes were distributed within the subepithelium underlying pedal epi-
TABLE 9. Summary of structural differences in pedal epithelium of Alabama rainbow (*Villosa nebulosa*), Gulf pigtoe (*Fusconaia cerina*) and Alabama creekmussel (*Strophitus connasaugaensis*).

<table>
<thead>
<tr>
<th>Species</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4</th>
<th>Type 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Villosa nebulosa</em></td>
<td>Tall, irregular plicae</td>
<td>Medium-sized, irregular plicae</td>
<td>Short, irregular plicae</td>
<td>Epithelium plicated to papillose, folds becoming reduced</td>
<td>Epithelium papillose with teardrop-shaped columnar cells and convex squamous cells</td>
</tr>
<tr>
<td></td>
<td>Violet basophilic granulocytes moderately abundant</td>
<td>Violet basophilic granulocytes sparsely distributed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fusconaia cerina</em></td>
<td>Tall, rectangular plicae</td>
<td>Tall, irregular plicae, may appear coalescing</td>
<td>Short, teardrop-shaped plicae, irregular shape</td>
<td>Columnar epithelium, epithelium is furrowed</td>
<td>Cuboidal epithelium, epithelium flattened</td>
</tr>
<tr>
<td></td>
<td>Basophilic granulocytes very abundant</td>
<td>Basophilic granulocytes sparsely distributed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Strophitus connasaugaensis</em></td>
<td>Tall, irregular, branched plicae</td>
<td>Tall, irregular, branched plicae</td>
<td>Short, thin, irregular plicae</td>
<td>Squamous epithelium, epithelium is furrowed</td>
<td>Squamous epithelium, epithelium flattened</td>
</tr>
<tr>
<td></td>
<td>Basophilic granulocytes moderately abundant</td>
<td>Subepithelial glands, abundant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
glochidium to juvenile. Pediveligers of
produce mucus or an adhesive that may assist
ously violet. Alternatively, granulocytes may
that histological sections of foot were conspicu-
high number of basophilic granulocytes such
are somewhat pallid, and females de-
velop a temporary red to orange pigmentation
when gravid (Fig. 137; Bruenderman & Neves,
1993). Fusconaia cerina had a significantly
h in the compos ition of the byssus (Figs. 40, 41.
169, 170, 301, 302), but such differences are
 presently indeterminate. Smith (2000) observed
s of ducts leading into the byssal gland of
juvenile Elliptio complanata and Lampsilis radiata. Such ducts were not observed in V.
nebulosa, F. cerina and S. connasaugaensis, and
the byssal gland of each species consisted of
a spherical chamber with tightly associated
columnar cells and a well-defined capsule of
fibrous tissue. Whether or not byssal gland
ducts are present in juveniles is indeterminate,
and therefore there is a need to understand
how a potentially non-glandular byssal gland
and a more glandular byssal gland would
functionally differ. For example, a glandular
byssal gland may produce a stronger or larger
filament extending from the foot. Smith (2000)
proposed that unionids living in “low energy”
environments lack a byssal gland; whereas,
mussels in “high energy” environments have
one. The present results show that representa-
tive species from three unionid lineages have
a byssal gland. Secondly, Terrapin Creek and
Shoal Creek may constitute low energy envi-
ronments in comparison to the Cahaba River
for reasons stated previously. Considering V.
nebulosa and S. connasaugaensis were
collected in small streams, and F. cerina was
collected in a larger river, the “low energy-high
energy” hypothesis, in terms of byssal glands,
may be rejected.

Adductors, Pedal Retractors and Mesentery

In the treatise of the musculature of Anodonta
cygnea (as Anodonta cellensis), Brück (1914)
described the adductor, including details of
myofiber organization, myocytes, perimysium
and motor endplate nodes. Smith (1983) de-
scribed columnar epithelial cells and fibrous

Byssal Gland

To our knowledge Smith (2000) is the only his-
torical reference on byssal gland of unionids.
Smith (2000) focused on byssal gland struc-
ture, formation, substrate affinity, morphology,
and rigidity in Elliptio complanata, Lampsilis
radiata, Leptodea ochracea and Alasmidonta
undulata.
tissue representing the mantle-shell junction of *Margaritifera margaritifera*

Musculature of the foot, adductors, pedal retractor and mesentery fascicles all have similar cellular characteristics (Figs. 38, 50, 55, 56, 167, 179, 184, 185, 299, 309, 315, 316). Each type of fibrous tissue consists of long, filamentous cells with a dark, eosinophilic cytoplasm and a distinct, basophilic nucleus. Our observations differ from Brück (1914) in that we did not observe striations or a star-shaped pattern of chromatin within the nucleus of myocytes. Transverse sections through a whole mussel provide a longitudinal view of the adductors and pedal musculature. According to Morrison (1996), the adductor of *Crassostrea virginica* has translucent and opaque regions with the translucent portion representing the majority of the tissue volume. Translucent muscle has a fast contractile ability and consists of elongated, ribbons of myofibers approximately 3–4 µm thick. Opaque muscle fibers are round, 10–20 µm in diameter with a slow contractile ability (Morrison, 1996). Opaque and translucent muscle is best shown in Figures 6–8. However, the adductor muscles studied herein were sectioned such that myofibers were studied longitudinally. Possible differences between opaque and translucent muscle were not addressed herein.

Mesentery fascicles were observed throughout the visceral mass of *V. nebulosa*, *F. cerina* and *S. connasaugaensis*. These bands of fibrous tissue extend medially into the hemocoel from a layer of vertically oriented fibers along the inner surface of the hemocoel. The muscle fibers extending ventrally into the foot from the dorsal aspect of the visceral mass have similar staining characteristics to the mesentery based on hematoxylin and eosin-stained tissue sections (Figs. 49, 50, 178, 179, 309, 310). Considering that the mesentery fibers extend across the hemocoel, it seems their function is to hold the sinistral and dextral portions of the visceral mass together. Alternatively, if the mesentery fibers stained positively for muscle proteins using Masson’s trichrome stain (Luna, 1968), possibly the fibrous bands could also serve to circulate hemolymph in the hemocoel.

_Digestive System_

Gutheil (1912) and Yokley (1968) previously comprised the most in depth treatments of the digestive system of unionids. Gutheil (1912) described all organs and cell types of the digestive tract of *Anodonta cygnea* (as *Anodonta cellensis*), and although the reference is useful towards understanding general morphological features, the illustrations of observed cell types may be inaccurate considering how much intracellular detail is depicted. For example, Gutheil (1912: figs. 22–24) shows cilia embedded deep into the cytoplasm, a series of distinct, and well-separated granules, and patches of chromatin that are linked together within the nucleus.

The digestive system consists of the labial palps, oral groove, esophagus, digestive diverticulum, stomach, crystalline style sac and intestine. Particles gathering in the ventral food groove of the inner gill travel anteriorly by means of ciliary action. The palps are positioned close to the anterior curvature of the inner demibranch such that food groove particles may be dispelled and captured by the palps. Particular matter that does not get captured by the palps may travel dorsally and may be transported posteriorly towards the excurrent aperture by means of cilia. Cilia located on the middle mantle, and lateral portion of the foot may facilitate the departure of rejected particles (Kellogg, 1915).

_Labial Palps and Oral Groove_

Kellogg (1915) diagrammed the labial palps, including cylindrical folds of ciliated tissue along the inner surface of each lip, and functional morphology of cilia on palps in *Elliptio complanata* (as *Unio complanatus*). Yokley (1968) detailed the palps and esophagus of *Pleurobema cordatum*, and Colville & Lim (2003) detailed the labial palps in *Velesunio ambiguus* and *Hyridella depressa* with LM and SEM, respectively.

Herein, we have shown that the labial palps are a soft tissue with a complex structure. The description of unionid palps herein may be key to understanding how mussels discriminate between particles that should be accepted or rejected. Palps are muscular, endowed with large hemolymph sinuses and connective tissue, which supports prior observations of their pliable and contractile nature (Figs. 51, 180, 311) (Kellogg, 1915). Particles captured by the palps may travel anteriorly, perpendicular to the length of the plicae, become incorporated into a mucoid mass traveling to the esophagus (Kellogg, 1915). No cellular differences in the cellular structure of labial palps were observed between *V. nebulosa*, *F. cerina* and *S. connasaugaensis*. However, based on the
scanning electron microscopy observations of unionid gills by Galbraith et al. (2009), it seems that there could be interspecific differences in the density of these bristle-like structures. Also, considering the observations of ciliated sensory receptors on mantle tentacles (Owen & McCrae, 1979) and on siphons (Vitonis et al., 2012), there may be ciliated, sensory receptors on the free distal tips of the palps. Given that there are different types of ciliated receptors, the density of receptors, and their specific morphological features (e.g., number of cilia, height of cilia) may provide insights into particle selection at the labial palps. Presently, sensory receptors were not observed on the palps, but perhaps they would be located more readily with scanning electron microscopy and we would expect that there are sensory receptors located at the free distal margin of the palps, and along the surface of the inner palp epithelium, and that sensory receptors would be absent along the outer surface of each palp, since the functional component of the labial palps comprises the ridges and troughs of the inner epithelium.

The oral groove represents a continuation of the labial palps and transfers ingested particles to the esophagus. Variation in the size of the oral groove may be reflective of differences in the quantity of matter ingested by each species. The oral groove of *F. cerina* and *S. connasaugaensis* resembled a cavity (Figs. 186–188, 317–319), whereas the oral groove of *V. nebulosa* was a more narrow tube (Fig. 57). Seemingly, a broad cavity would accommodate a larger volume of particulate matter than the narrower, tubular oral groove of *V. nebulosa* (Table 10).

The esophagus connects the oral groove with the digestive diverticulum (Figs. 60, 189, 320). The esophageal lining consists of conical plicae bearing ciliated columnar cells, and the deep grooves between the plicae may allow separation of small and large-sized particles (Yokley, 1968). Given the abundant ciliated cells, and concentric layers of connective tissue surrounding the lumen, it seems that ingested matter is delivered into digestive diverticulum by means of ciliary action, and not peristalsis (e.g., Figs. 61, 190, 321).

<table>
<thead>
<tr>
<th>Species</th>
<th>Oral groove</th>
<th>Entrance to digestive diverticulum</th>
<th>Digestive diverticulum tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Villosa nebulosa</em></td>
<td>Concave, tubular</td>
<td>Esophagus opens directly into sinistral and dextral tubules</td>
<td>Tubules with short, Columnar cells rectangular, rounded plicae, vesiculated, with microvilli</td>
</tr>
<tr>
<td><em>Fusconaia cerina</em></td>
<td>Deep cavity</td>
<td>Esophagus opens into a large sinistral chamber</td>
<td>Tubules with long, thin plicae, Tubules with teardrop-shaped columnar cells</td>
</tr>
<tr>
<td><em>Strophitus connasaugaensis</em></td>
<td>Deep cavity</td>
<td>Esophagus opens into a large sinistral chamber</td>
<td>Short, thin, irregular plicae, Columnar cells rectangular, vesiculated, with microvilli</td>
</tr>
</tbody>
</table>

TABLE 10. Summary of structural differences in oral groove, and digestive diverticulum of Alabama rainbow (*Villosa nebulosa*), Gulf pigtoe (*Fusconaia cerina*) and Alabama creekmussel (*Strophitus connasaugaensis*).
The shape of the oral groove was similar between *S. connasaugaensis* and *F. cerina*. Additionally, the morphology of the lumen of the digestive diverticulum at the point of merger with the esophagus was similar between *S. connasaugaensis* and *F. cerina*. Such features are suggestive of a closer phylogenetic relationship to each other than to *V. nebulosa*. Additionally, the large oral groove and digestive gland entrance featured by both species possibly indicates that a larger quantity of particulate matter enters the digestive gland than in *V. nebulosa*. An alternative hypothesis could be that the rate at which particles move between the labial palps and the anterior portion of the digestive gland are different. If so, perhaps the expansive oral groove chamber and the anterior digestive gland chamber would cause particles to “bounce” around and not move efficiently within a better defined tube. Observations of fluorescent dye-labeled particles traveling across the gill filaments of *Mytilus edulis* and *Arca zebra* for example, suggests that ciliary action may not cause particles to move within a perfectly vertical or horizontal, linear direction (Ward et al., 1998).

**Digestive Diverticulum**

The cell types of the digestive diverticulum have been described from *Anodonta cygnea* (as *Anodonta cellensis*) by Gutheil (1912), *Anodonta anatina* and *Unio tumidus* by Sumner (1966a, b), and *Pleurobema cordatum* by Yokley (1968). Feeding experiments conducted by Mansour & Zaki (1946) provided evidence that vesiculated cells of the digestive diverticulum of *Coelatura aegyptiaca* (as *Unio prasidens*) are specialized for intracellular digestion. Sumner (1966a, b) showed that there are two cell types in digestive tubules. Digestive cells are vesiculated columnar cells with an elaborate intracellular transport system involving vesicles that later fuse with lysosomes. Secondly, the conical, basophilic basiphil cells have an extensive rough endoplasmic reticulum and may therefore be specialized for protein synthesis and secretion. Therefore, it may be inferred from Sumner (1966a, b) that basiphil cells secrete lytic enzymes and perhaps these enzymes would digest algae and or bacteria, and the resulting chyme would be incorporated into digestive cells.

Our observations of digestive diverticulum of *V. nebulosa*, *F. cerina* and *S. connasaugaensis* support previous descriptions of digestive cells and basiphil cells from Sumner (1966a, b) and Yokley (1968). However, only scant details concerning primary and secondary tubules were presented in Yokley (1968). Digestive diverticulum is a large network of blind-ended tubules and constitutes one of the largest organs in a freshwater mussel. The openings of digestive tubules were mainly located between the esophagus and stomach (Figs. 62, 191, 322). A smaller subset of tubules communicated with the sinistral and dextral portions of the posteroventral aspect of the stomach. Together, there was no obvious histological difference between the anterior digestive diverticulum tubules and both the sinistral and dextral tubules leading into the posteroventral stomach chamber. Therefore, it would seem that each region of the digestive diverticulum is functionally identical. However, it is not clear why there is a separation of anterior and posterior digestive gland ducts. Seemingly these ducts take in particulate matter that escapes passage into the initial series of digestive gland ducts. A possible explanation for this could be that the anterior portion of the digestive gland could be filled to capacity, and an additional set of digestive ducts could handle excess food matter.

The key features of the digestive diverticulum that exhibited morphological variation include the primary and secondary tubules (Table 10). Based upon the tissue structure of *V. nebulosa*, *F. cerina* and *S. connasaugaensis*, it seems that ciliated primary tubules carry ingested particles down the length of a tubule, but it is not clear whether ingested matter travels into the secondary and tertiary portions of each tubule, since mussels were not fixed in the field and therefore, ingested matter that would normally be contained within the tubules was expelled. Some literature regarding ciliated digestive tubules suggests that cilia along the epithelial folds may create incurrent and excurrent pathways for particulate matter (Owen, 1955), but it is unclear whether cilia all around the lumen of primary tubules would create a synchronous ascending current and then later generate a synchronous descending current following a chemical change in the lumen. In contrast, Owen (1955) also stated that the subepithelium surrounding the digestive tubules contained myofibers that would create rhythmic contractions or peristalsis. Peristalsis seems unlikely considering the extensively developed ciliated epithelium, and although fibrous tissue was observed in the subepithelium of digestive...
tubules of V. nebulosa, F. cerina, and S. connasaugaensis, fibers more closely resembled thin, wispy connective tissue. Secondary tubules feature copious cytoplasmic vesicles, and microvilli, and the lumen may appear to be bubbling. Possibly secondary tubules produce mucus since tubule secretions were eosinophilic. Tertiary tubules constitute the longest and most conspicuous aspect of the organ. Given the affinity of hematoxylin to nucleic acids during hematoxylin and eosin staining, the conspicuously basophilic cytoplasm of basophil cells may be an indication that these cells have an elaborate rough endoplasmic reticulum (Stevens, 1982). Additionally, the vacuolated cytoplasm of digestive cells would be an indication of a cell specialized for intracellular transport. Based on our observations herein, and the work of Sumner (1966a, b), it seems that particulate matter may be carried into the digestive diverticulum to be chemically broken down, possibly by enzymes secreted by basophil cells (e.g., Fig. 326). Following digestion, the smaller endproducts may become incorporated into digestive cells via endocytosis. Additionally, considering the apical microvilli of secondary tubules, some absorption may occur in this region, although this tissue region is short (Figs. 65, 195, 325). Ciliary action in the primary tubules may transport some broken down particulate matter posteriorly into the stomach and some undigested matter into the stomach (Figs. 64, 194, 324). However, the digestive diverticulum literature presents competing hypotheses regarding possible functions of digestive tubules. Diverticulum cells may be absorptive considering observations India ink, iron or carmine particles in cytoplasm of tubule cells following experimental feeding trials (Yonge, 1926b; Owen, 1955). Furthermore, wet mounts or frozen sections of digestive tubules of recently collected animals contain green or brown intracellular pigments, while tubule cells appear colorless following a period of starvation (Mansour & Zaki, 1946). Alternatively, proteases, lipases, and enzymes capable of digesting carbohydrates have been isolated from digestive tubules of various marine bivalve species (Yonge, 1926b). Eble (1966) revealed the presence of acid phosphatase, alkaline phosphatases, and esterases on the apical surface of digestive tubules of Crassostrea virginica and reported that enzymes were most abundant during the summer and least abundant during the winter.

The long, finger-like plicae extending into the lumen of primary digestive gland tubules of F. cerina may be an indication that it feeds on algae or bacteria of a small size (Figs. 193, 194). The larger lumen of the primary tubules of V. nebulosa and S. connasaugaensis could represent adaptations for feeding on larger-sized particulate matter (Figs. 63, 64, 323, 324). Additionally, the long, slender plicae in the primary tubules of F. cerina may be indicative of foregut efficiency while the foregut of V. nebulosa and S. connasaugaensis is seemingly less efficient. Examinations of the second and third limbs of the intestine is needed (see below).

**Stomach**

Gutheil (1912), Purcheon (1958), Reid (1965), Dinamani (1967), Yokley (1968), Kat (1983) and Smith (1988) described general morphological characteristics of stomach, notably that the gastric walls have grooves and typhlosoles. Kat (1983) documented interspecific variation in stomach morphology based on a comparison of four species of Lampsilis. However, a pictorial representation of the morphology of the gastric lumen was not clearly presented in the above literature.

Our observations of the stomach of V. nebulosa, F. cerina and S. connasaugaensis support prior observations of rugose and ciliated gastric walls (Figs. 67, 70–72, 198, 201–203, 327, 330–332). Descriptions of the stomach of Anodonta have suggested that gastric grooves may transport particulate matter parallel to the grooves. Additionally, there may be circumferential currents that churn food matter (Purcheon, 1958; Dinamani, 1967). A cyclic arrangement of eosinophilic masses of fluid was observed in histological sections of the stomach of V. nebulosa, F. cerina and S. connasaugaensis. In addition to particle sorting, stomach epithelium may produce enzymes. For example, cellulase as well as dipeptidyl-aminopeptidase activity was detected in Crassostrea gigas, and N-acetylglucosaminidase was detected in Pecten maximus (Boucaud-Camou & Henry, 2003). The histological sections of freshwater mussel stomachs herein provide some evidence of secretory activity in the stomach (e.g., Fig. 203). Eosinophilic, spherical vesicles were observed in close proximity to the stomach epithelium of each species.

Additionally, the stomach epithelium, especially along the ventral wall possesses an
globular or spherical end and a linear (as F. cerina and S. connasaugaensis) lateral expansion. The midgut of V. nebulosa, F. cerina and S. connasaugaensis features wispy cilia and goblet cells, but it was not clear whether any of the cells secrete enzymes. The narrow, basophilic cells comprising the style sac produce a filamentous secretion, which may be an initial component of the style rod. Owen (1955) suggested that the style releases enzymes into the stomach, but provided no empirical evidence to support this idea. Boucaud-Camou & Henry (2003) observed enzymes in sections of the style, but it was not clear what cells secrete enzymes or whether enzymes become incorporated into style rod layers from an anterior source.

Although it is recognized that the style creates sufficient suction to draw ingested material from the stomach into the intestine, there are a series of hypotheses regarding the function of the style that seem questionable. In the literature review regarding crystalline style function, Nelson (1918) indicated that several prior anatomical works on bivalves had proposed competing hypothesis for the function of the style. Among these hypotheses, Nelson (1918) indicated that the style extends into the stomach, and rotates in close proximity to the stomach wall such that it would pulverize food matter like a mortar and pestle. Yonge (1926a) proposed that the rotation of the style causes cilia located along the stomach wall, to fuse together and form the gastric shield. Other anatomical works on the digestive tract of bivalves suggest the style grinds against the
The idea that the style rod is a grinding apparatus is questionable considering both the style and shield are soft. Nelson (1918) explained that the style is water soluble, and composed of mucus that is rolled up by ciliary action. Several others have pointed out that the style rod is soft and flexible (Owen, 1955; Lomte & Jadhav, 1980). A possible explanation for the grinding hypothesis could be that the style coagulates and becomes thickened when immersed in alcohol or boiling water (Nelson, 1918), and it would therefore appear to be a dense solid structure in a specimen preserved for anatomical study. Careful examination of Figures 74, 78, 205, 209, 334, and 338 provides evidence that the style may be a liquid or a soft, solid material. For example, creasing artifacts to the style indicate that the microtome knife had passed through a dense object. Secondly, the irregular composition of the style in Figures 334, 338 is evidence that the style may represent mucus that is being stirred. Concentric, semicircular rays were also observed in the stomach of all three species, but it seems likely that they represent a churning fluid (Figs. 69, 70, 73, 198, 204, 327, 333). Additionally, the gastric shield covers gastric epithelium in several locations including ventral surfaces where a pestle-like structure would never touch, and may support the hypothesis that it is an elaborate binding agent (Figs. 69, 73, 200, 204, 329, 333). Furthermore, if the style grinds particulate matter, it is unclear as to why grinding would occur between the stomach and intestine, especially considering the extensive digestive tubules located anterior to the stomach. It is also critical to point out that the illustration of the position of the crystalline style sac to the intestine and digestive diverticulum as depicted in Owen (1955) was proposed to be representative of many species of Eulamellibranchia. Based on serial sections of the digestive tract of V. nebulosa, F. cerina and S. connasaugaensis, the esophagus and digestive diverticulum are not located near the style sac and particles would not directly enter this structure from the esophagus. Nevertheless, the illustration in Owen (1955) appears in the more recent work of Boucaud-Camou & Henry (2003) and appears to have been accepted at face value. Considering the significant morphological differences in the crystalline style sac and stomach across different bivalve orders (Kato & Kubomura, 1954; Purcheon, 1958; Dinamani, 1967), morphological differences in the style sac of V. nebulosa, F. cerina and S. connasaugaensis regard the shape of the midgut (Table 11), but the significance of such differences remains unclear given that mussels were allowed to purge prior to being processed for histology. At best we may infer that there are differences in how food matter is processed by this organ. We speculate that the shape or density of the bolus may be different or the rate at which food matter travels through the style sac is different.

**Intestine**

At present, there is limited information regarding the cellular structure of the intestine of freshwater mussels. Gutheil (1912) described morphological and cellular features of the intestine, namely that the intestine has a crescent shaped lumen with a typhlosole. Yokley (1968) described tall basophilic cells and mucus secreting cells in anterior aspect of intestine of Pleurobema cordatum.

Herein we describe five intestinal limbs from and including the crystalline style sac to the so-called “rectum” (Simpson, 1884), which constitutes a straight region of the alimentary canal running parallel with the hinge. We distinguish each intestinal limb based on the location within the viscera, orientation, morphology of the lumen, and cellular structure. The intestine of V. nebulosa, F. cerina and S. connasaugaensis is a long tubular organ that extends through the medial and posterior portions of the visceral mass. The intestine bends several times to form five regions or limbs. Based on histological sections of the digestive tract, the direction of the alimentary canal of V. nebulosa, F. cerina and S. connasaugaensis was generally identical and resembles the layout described from Anodonta spp. (Simpson, 1884; Gutheil, 1912).
The crystalline style sac represents the first descending limb of the intestine (Figs. 79, 210, 339). The walls of the style sac feature numerous folds, and there was an eosinophilic mass typically associated with the ridges and troughs of the style sac walls. Since epithelial types 1–3 were sometimes seen on both the anterior and posterior walls of this intestinal limb, it seems that the style sac does not maintain its stem-loop configuration as it descends. Possibly, since style sac epithelium continues until the dorsal aspect of the visceral mass, circular mixing of food matter may continue to occur until the second intestinal limb. Even though the descending style sac appears to be significantly pleated, we think these folds are associated with mixing rather than absorption.

It is uncertain if absorption occurs in the crystalline style sac or if absorption begins in the second limb considering the bubbling observed in some areas and considering the conspicuous folding of the intestinal wall seen in *V. nebulosa* and *S. connasauagaensis* (Figs. 81, 82, 341, 342). In each species, the second and third intestinal limb featured numerous goblet cells and a dense, eosinophilic mass of chyme. In many areas, the ciliated cells along the intestinal wall appeared to be bubbling (Figs. 83, 214, 343), but it was not clear what these secretions might be. The second and third intestinal limbs appeared to have at least three cell types. The first cell type was ciliated with eosinophilic vesicles while the second cell type lacked vesicles. Goblet cells represented the third cell type, but these cells were sparsely distributed. Although microvillar cells are typically associated with the intestinal epithelium of a vertebrate (Ross et al., 2003), there was not any indication of such cells in the intestine of each unionid species. This could mean that either the ciliated cells of the intestine have a significant capacity for

### TABLE 11. Summary of structural differences in crystalline style sac, intestine, pedal ganglion and ovarian acini of Alabama rainbow (*Villosa nebulosa*), Gulf pigtoe (*Fusconaia cerina*), and Alabama creekmussel (*Strophitus connasauagaensis*).

<table>
<thead>
<tr>
<th>Species</th>
<th>Style sac</th>
<th>Intestinal limbs 2 and 3</th>
<th>Pedal ganglion</th>
<th>Ovarian acini</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Villosa nebulosa</em></td>
<td>Midgut forked, epithelium continuous throughout midgut</td>
<td>Presence of numerous plicae, lumen is folded</td>
<td>Axons fine, filamentous</td>
<td>Mature ova with a thin vitelline membrane</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Presence of numerous plicae, lumen is folded</td>
<td>Surrounding connective tissue is fine</td>
<td>Abundance of fluid between vitelline membrane and ova</td>
</tr>
<tr>
<td><em>Fusconaia cerina</em></td>
<td>Midgut not forked, tall columnar cells along dorsal and ventral surfaces, short columnar cells along lateral margin</td>
<td>Plicae largely absent, lumen is flattened</td>
<td>Axons thickened, conspicuous</td>
<td>Mature ova with thickened vitelline membrane</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Surrounding connective tissue is thickened, conspicuous</td>
</tr>
<tr>
<td><em>Strophitus connasauagaensis</em></td>
<td>Midgut with shallow fork, columnar cells along dorsal and ventral surfaces, short columnar cells along lateral margin</td>
<td>Presence of numerous plicae, lumen is folded</td>
<td>Axons fine, filamentous</td>
<td>Mature ova with a thin vitelline membrane</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Surrounding connective tissue is fine</td>
</tr>
</tbody>
</table>
absorption by themselves or microvilli were obscured from view.

The second and third intestinal limbs of *V. nebulosa* and *S. connasaugaensis* consisted of sinuous to deeply folded walls (Figs. 82, 344), but in *F. cerina*, the walls lacked folds or plicae (Figs. 212, 213; Table 11). A possible explanation for these differences could be that the deeply infolded walls of the ciliated digestive diverticulum tubules are more effective at delivering particles to cells that utilize intracellular digestion. A second explanation could be that *F. cerina* is taking in a greater volume of particulate matter than *V. nebulosa* or *S. connasaugaensis*.

The lumen of the fourth and fifth intestinal limbs was considerably larger than the lumen of the style sac, second and third intestinal limbs. In general, fourth and fifth intestinal limbs may have similar functions considering the morphological similarity of the typhlosole and surrounding intestinal wall (Figs. 85, 88, 216, 219, 345, 348). The primary distinction between the fourth and fifth intestinal limbs was the presence of granulocytes located along the ventral wall. Granulocytes reside in flattened plicae, have a teardrop-shape and contain eosinophilic to basophilic intracellular granules (Figs. 86, 217, 346). However, it is presently uncertain what the function of the granulocytes located within the plicae of the ventral wall of the fourth limb. There are two possible explanations, the strong staining character may be indicative of an apocrine cell that may produce enzymes or mucus. For example, aminopeptidase activity was detected in sections of the lower intestine of *Pecten maximus* (Boucaud-Camou & Henry, 2003). Goblet cells observed in histological sections of *V. nebulosa, F. cerina* and *S. connasaugaensis* are located in many places throughout the body and may be transparent to eosinophilic to basophilic. Granular mucus cells were most frequently observed in the labial palps (Figs. 53, 182, 313). Alternatively, the granules observed in ventral epithelial cells of the fourth intestinal limb may represent intracellular bacteria. Symbiotic intracellular bacteria are known from species of marine bivalves (Lucinidae, Thyasiridae, Vesicomyidae, Mytilidae) that live in sulfur-rich environments. In many cases energy is derived from the oxidation of hydrogen sulfide by intracellular clenidial bacteria (Cavanaugh et al., 2006). Intracellular bacteria are also known from the digestive tracts of herbivorous insects. Symbiotic bacteria inhabiting the digestive tract of such insects may allow insects to feed on diets that are rich in carbohydrates, but deficient in amino acids (Bauman et al., 2006). Transmission electron microscopy, bacterial stains such as Giemsa, or in situ hybridization using a universal bacterial DNA probe are needed to discern the nature of these granulocytes. Considering that the fourth and fifth intestinal limbs have a large typhlosole restricting the lumen and a perimeter of repeating plicae, it seems that absorption continues to occur. However, the morphological differences between the second, third, fourth and fifth limbs may be an indication that the absorptive processes occurring in each region are not identical. Scanning or transmission electron microscopy would provide a higher resolution images of the cell types characterizing each intestinal limb and could shed light on how or if absorption is different.

Histological sections of intestine sometimes reveals wandering hemocytes in the epithelium (e.g., Figs. 84, 218). Yokley (1968) observed wandering cells throughout the digestive tract and indicated that brown to green intracellular pigments may be evidence that these cells can engulf foreign bodies from digestive epithelium. Owen (1955) reported wandering cells in different places throughout the digestive tract. Owen (1955) suggested that hemocytes move into the digestive tract epithelium to engulf particles and that intracellular digestion by means of hemocytes is one of many ways in which bivalves derive nutrients from ingested matter. However, considering the sparse distribution of hemocytes in the epithelium of the intestine, it seems unlikely that phagocytic wandering cells would play a significant role in digestion. Alternatively, hemocytes may periodically move into the digestive epithelium to engulf invasive pathogens or repair local injuries.

Another curious matter regarding the function of the intestine stem from observations by Owen (1955) and Yokley (1968). Both authors reported that there are concentric bands of myofibers surrounding the intestine of bivalves. The supporting images in Owen (1955) are not informative about myofibers, and the micrographs appear to show dark-staining, thin filaments. Additionally, the micrographs of Sumner (1966a) do not show fibrous tissue between digestive ducts. Throughout the digestive tract of *V. nebulosa, F. cerina* and *S. connasaugaensis*, overlapping connective tissue fibers were typically observed supporting folded epithelial tissue (e.g., Figs. 70, 202, 336). In some cases, connective tissue ap-
peared to be densely compacted around the intestine similar to a serosa. Owen (1955) and Yokley (1968) suggested that muscle tissue in the digestive tract of a bivalve would create peristalsis. However, this seems unlikely given that the epithelium of the alimentary canal of each unionid species has extensively ciliated with numerous folds or grooves along the walls. Secondly, the crystalline style rotates and delivers particulate matter into the intestine, and this seems like a logical alternative to peristalsis in a suspension-feeding animal that does not consume a large solid mass of food.

Considering the histological differences in oral groove, digestive diverticulum, style sac and intestine, it seems that V. nebulosa, F. cerina and S. connasaugaensis have different feeding strategies. Overall, the digestive tract of V. nebulosa and S. connasaugaensis were most similar to each other, but different in comparison to F. cerina. It is difficult to speculate about how the feeding habits of each mussel could be different. Although V. nebulosa and S. connasaugaensis were collected in headwater streams, and F. cerina was obtained from a large river, the historic ranges of each species could have overlapped. Terrapin Creek and Shoal Creek may have less plankton biomass than the Cahaba considering that these areas have more shade. However, historically, V. nebulosa and S. connasaugaensis occurred in large rivers, such as the Cahaba, so it is likely that there is no relationship between the morphological features of the digestive tract of each species and available plankton (Williams et al., 2009).

Histology of field fixed mussels could reveal whether quantity of ingested matter, or specific species of algae or bacteria, but this could be problematic considering that routine tissue preparation for paraffin embedding or electron microscopy removes water-soluble liquids (Sims et al., 1991). Therefore, we may end up with an incomplete picture of the dietary composition.

Cardiovascular System

The gross and histological features of the heart were described in Krug (1922; Anodonta cygnea (as Anodonta cellulina)), Motley (1932; Tritogonia verrucosa, Amblema plicata (as Amblema peruviana), Lasmigona complanata (as Lasmagonia complanata), Elliptio crassidens, Fusconaia ebena, Actinonaias ligamentina (as Actinonaias carinata), Potamilus purpuratus (as Lamnulis purpurata) and Megalonaia nervosa (as Megalonaia giganta), Chaudhry & Narain (1972; Lamellidens corrianius), and Narain (1973, Lamellidens corrianius). An accessory pumping structure, the posterior aortic bulb, was described by Narain (1972; Lamellidens corrianius). Brand (1972) described the vascular structure of viscera, gill, and mantle of Anodonta anatina. Narain (1976) summarized knowledge about heart and cardiac output in unionids. Hemocytes were described in Dundee (1953; Amblema plicata (as Amblema costata), Quadrula quadrula, Unio merus tetralsmus, Tritogonia verrucosa, Toxolasma parvum (as Carunculina parva), Ligumia subrostrata, Potamilus alatus (as Proptera alata), Leptoidea fragilis, Lasmigona complanata, Lambsilis teres (as Lambsilis fallaciosa), and Pyganodon grandis (as Anodonta grandis), and Burhead et al. (2009; Quadrula sp.).

Our observations further support the notion of the histologically conserved nature of the auricles and ventricle among unionid species. The heart consists of a pair of laterally positioned auricles and a median muscular ventricle (e.g., Fig. 91). Overall cardiac structure of V. nebulosa, F. cerina, and S. connasaugaensis was very similar. Auricles and ventricle consisted of an epicardium of squamous cells, and a distinct myocardium of cardiac muscle. However, there was less investment of cardiac myofibers in the auricles than observed in the ventricle, possibly because the auricles are blood-receiving conduits that need to be distensible (Motley, 1932).

Andrews & Jennings (1993) reported that auricles of Anodonta sp. merge with the walls of the pericardium, and that fluid may leak out of the auricles into the surrounding pericardial gland. However, auricles of each mussel species studied herein extended freely into the pericardial cavity to merge with the ventricle at an oblique angle. Based on histological sections of three unionid species, it was not obvious whether the auricular epicardium would leak (Figs. 92, 223, 352). Examining the surface of the auricles of V. nebulosa, F. cerina and S. connasaugaensis may provide insight into whether there are gaps between epicardial cells that would be indicative of the passive transport of fluid into the surrounding pericardial cavity. Scanning electron microscopy of the heart of Crassostrea gigas has indicated that the ventricle and auricles each have a robust composition. Higher magnification images of the surface of the auricle and ventricle do not provide much information concerning the possibility of pores. Although Crassostrea gigas is
a marine bivalve, the morphological features of the auricles and ventricles seem to be morphologically comparable to the heart of a unionid and may be an indication that fluid does not leak out of the heart (Grizel, 2003).

There is a pair of muscular, auriculoventricular valves separating each sinistral and dextral auricle (Figs. 95, 226, 355), from the ventricle, to maintain proper tidal rhythm (Motley, 1932). Cardiac myofibers in the ventricle are significantly developed, with numerous longitudinal and transversely oriented myofibers. Considering the extensive development of longitudinal and transverse myofibers in the ventricle (Figs. 92, 223, 353), the orientation of myofibers would seemingly cause the ventricle to contract in a circular, and linear (posterior-to-anterior) fashion to drive hemolymph into anterior, and posterior aortic vessels (Brand, 1972). Additionally, as in the heart of a vertebrate (Ross et al., 2003) cardiac myofibers were spaced apart to facilitate constant expansion and contraction of the organ, and to maintain a supply of oxygen to the myofibers. Additionally, myofibers contained brown, granular inclusions and it is uncertain what this material represents or how it relates to cardiac function (e.g., Fig. 224). Furthermore, intracellular inclusions were not observed in any other muscle tissue in *V. nebulosa, F. cerina* or *S. connasaugaensis*.

Herein we observed distinct vessels resembling arteries, veins and capillaries throughout the body, but it was sometimes difficult to distinguish between arteries and veins (e.g., Figs. 96, 97). Our observations support the work of Brand (1972), who revealed the presence of vasculature by injecting the heart of *Anodonta anatina* with rubber-latex and removing the soft surrounding tissues. Arteries in vertebrates have a dense wrapping of smooth muscle with a circular lumen. Veins typically have thinner walls with an irregular somewhat flattened lumen (Ross et al., 2003). The aorta for example, would have a thick muscular wall, but histological sections of the anterior and posterior aorta of mussels revealed a large, oval lumen with a limited muscular wrapping. Thick musculature enclosed the lumen of arteries near the base of the foot, possibly to rapidly fill pedal hemolymph sinuses with hemolymph in order to facilitate expansion of the tissue (Trueman, 1966; Brand, 1972).

Andrews & Jennings (1993) suggested that there are a series of well-defined hemolymph vessels that converge at the base of the ctenidia, leading to the auricles. In transverse sections of the heart, a pale, violet, granular mass was observed at the base of the gill (e.g., Figs. 157, 158). Additionally, there was a network of loose connective tissue between the auricles and the gill. Well-defined hemolymph vessels leading from the base of the gills to the auricles were not observed. Possibly this region conveys hemolymph into the auricles in a diffuse manner considering the funnel-shaped configuration of unionid auricles.

Hemolymph consisted of distinct cellular and non-cellular components. Eosinophilic, granular hemocytes were observed throughout the viscera and mantle of *V. nebulosa, F. cerina* and *S. connasaugaensis* (e.g., Figs. 53, 86, 187, 196, 357, 363). Literature regarding hemocytes of unionids and marine bivalves suggests that there are distinct types of blood cells (Dundee, 1953). However, potential differences between hemocytes observed throughout the hemocoel and mantle are presently indeterminate. Hemolymph fluid appears in histological sections as a diffuse, black-pigmented material, throughout the body, typically within interstitial spaces (e.g., Figs. 18, 103, 150, 157, 341, 356). Considering that black granules were observed in the heart and interstitial spaces, it may represent plasma and interstitial fluid. Black granules may represent hematin, a pigmentation that occurs in blood-rich tissues when acidic formalin and hemoglobin react (Myers & McGavin, 2007). Clear examples demonstrating the appearance of hemolymph or interstitial fluid are lacking in the literature.

To our knowledge, the best micrograph of the pericardial gland of a bivalve appears in Norton & Jones (1992). The pericardial gland is a diffuse network of adipocyte-like cells located laterally and dorsally to the pericardial cavity (Figs. 99, 230, 359). Clearly this spongy tissue transmits hemolymph through the dorsum, and the high surface area of this tissue would be an indication that hemolymph travels slowly through this region. Prior studies on the pericardial gland have indicated that the pericardial gland plays a role in filtration (Morse, 1987; Andrews & Jennings, 1993). An additional possibility that bivalve researchers may have overlooked is hemopoiesis. In an effort to understand the host-parasite relationship between the infective larval stages of trematodes (*Fasciola hepatica*) and snails *Galba truncatula* (as *Lymnaea truncatula*), Rondelaud & Barthe (1981) observed hemocyte proliferation between the nephridium and pericardium following experimental infection of snails with...
infective trematode larval stages (miracidium). Given the above we may phylogenetically infer that the pericardial gland could play a role in hemopoiesis, since we observed emarginated, eosinophilic cells resembling hemocytes along the surface of the adipocyte-like pericardial gland cells. However, in *V. nebulosa*, we sometimes observed brown or otherwise discolored hemocytes, and we speculate that these cells may be senescent. Therefore, we are uncertain of whether the pericardial gland is associated with hemopoiesis, hemocyte senescence or both.

**Renal System**


Many of the morphological features and cell types of the nephridium of *V. nebulosa, F. cerina* and *S. connasaugensis* are consistent with the observations of Myers & Franzen (1970). However, the histological features of the nephridium of *V. nebulosa, F. cerina* and *S. connasaugensis* reveal new insights into renal physiology of bivalves. Overall nephridial structure was different from anterior to posterior (Figs. 100, 107, 231, 238, 360, 367). The ventral nephridial limb is compressed anteriorly because it is located adjacent to the visceral mass. The lumen of the anterior portion of the ventral nephridial limb is restricted by the numerous infoldings. Given the extensive network of branched plicae, it seems that reabsorption occurs in the anterior portion of the ventral nephridium (Figs. 101, 232, 361). Plicae become more widely spaced apart posteriorly between the posterior margin of the visceral mass and posterior adductor, and the overall lumen of the nephridium expands possibly to accommodate a larger volume of fluid. Epithelial folds have a stem-loop structure and the cells have a pale-staining cytoplasm (Figs. 108, 239, 368). Therefore, the posterior portion of the nephridium may function similar to the thin loop of Henle in a vertebrate (Ross et al., 2003). Dorsal nephridium resembles a sac and contains a small number of short, thin plicae. Considering the absorptive nature of the ventral nephridial limb, the function of dorsal nephridium is thought to be analogous to a bladder (Myers & Franzen, 1970; Andrews & Jennings, 1993). The structure of the nephridium of *V. nebulosa, F. cerina* and *S. connasaugensis* is similar to descriptions of unionid nephridia of *Pleurobema cordatum and Quadrula nodulata* (Yokley, 1968; Myers & Franzen, 1970).

Renal filtrate may be derived from hemolymph flowing through the pericardial gland. Podocytes are associated with the pericardial cavity and may be distinguished from other cells by the presence of branched cytoplasmic extensions of the basal part of the cell known as pedicels. Fluid derived from hemolymph may pass out of the pericardial gland and into the pericardial cavity (Meyhöfer et al., 1985; Morse, 1987). A more recent study indicated that podocytes of *A. cygnea* may be located anteriorly to the nephridium may allow passage of fluid into the renopericardial canal (Andrews & Jennings, 1993). A competing hypothesis regarding filtration suggests that fluid may pass through the pericardial wall from the pericardial gland (Yokley, 1968). Goblet cells located around the lumen of the renopericardial canal possibly secrete mucus into the renal lumen (Figs. 106, 237, 365), but the presence of mucigen has not been confirmed. Since these cells were located along the anterior wall of the pericardial sac, perhaps some of these cells represent podocytes. It is difficult to pinpoint exactly where podocytes occurred because there is no light microscopy reference point. Morse (1987), for example, illustrated two podocytes in a diagram portraying the path of fluid from the pericardial gland to the nephridium, but there is no information about the appearance of the surrounding tissue. Since podocytes were portrayed as teardrop-shaped columnar cells in Morse (1987), and since Andrews & Jennings (1993) stated that podocytes are located in the anterior region of the pericardial cavity, the columnar epithelium at the anterior portion of the pericardial sac at the entrance to the renopericardial canal may contain podocytes.

Ciliated cells in the renopericardial canal seemingly create the driving force needed to propel renal filtrate posteriorly through the nephridium. Cilia were conspicuous in the renopericardial canal (Figs. 106, 237, 365) and
urethra (Figs. 107, 238, 366), but there were no obvious indications of cilia throughout the ventral and dorsal nephridial limbs. Fine, wispy structures were observed on the apical surface of cuboidal cells representing the stem-loop structures of the posterior nephridium (Figs. 108, 239, 368). However, it seemed that these filamentous structures were the remnants of a fluid. Given the limited amount of cilia in the nephridium and the extensive volume of tissue throughout the entire ventral and dorsal nephridial limbs, and that unionid exhibit low cardiovascular pressure (Brand, 1972), it would seem that passage of filtrate through the nephridium occurs at a slow rate.

The columnar epithelium of the ventral nephridial limb of V. nebulaosa, F. cerina and S. connasaugaensis contained red to brown intracellular granules. Brown intracellular granules were most conspicuous in V. nebulaosa (Fig. 98), and the red granules were most conspicuous in S. connasaugaensis (Fig. 361). In most cases, the granular material resembled wandering hemocytes. The brown granules may represent either senescent hemocytes or hemocytes with engulfed material. In either case, the eosin dye poorly presented itself and suggests some chemical change occurred in the cytoplasm of wandering cells. It is unclear why the epithelium of S. connasaugaensis would have such a strong eosinophilic and granular staining character. An alternative explanation for the intracellular granules in the ventral nephridium could be that the columnar cells are phagocytic or absorb substances from the nephridial lumen via microvilli to form concrments. Concrements may consist of lipofuscin granules, membrane remnants or other substances that would appear to be electron dense when the tissue is studied with transmission electron microscopy. Concrements may later be expelled into the lumen once the intracellular vacuole-lysosome system is filled to capacity (Morse, 1987).

Teardrop cells with an apical, transparent vesicle (Figs. 103, 234, 362) have been observed in unionids such as Q. nodulata (Myers & Franzen, 1970) as well as marine bivalves such as C. virginica (Galtsoff, 1964). Teardrop cells with apical vesicles located in the bladder and have been previously suggested to pinch off the apical vesicle. The vesicle may contain an anionic substance based on a positive staining reaction with Heidenhain's Azan Stain (Myers & Franzen, 1970). Possibly, this anionic substance is ammonia.

The cuboidal cells of the posterior nephridium (e.g., Figs. 109, 368), and especially dorsal nephridial cells (both, dorsal and ventral) contained brown intracellular granules (Figs. 102, 233, 234, 368). Possibly this substance represents more accumulations of waste products that have become internalized within renal epithelium. The observation of plicae in the dorsal nephridium is somewhat surprising considering the extensive infoldings of the anterior portion of the ventral limb. The plicae in the dorsal nephridium would suggest that reabsorption continues to occur at a low level once filtrate enters the dorsal nephridium, which is thought to represent a bladder (Myers & Franzen, 1970; Andrews & Jennings, 1993).

Nervous System

Splitthöller (1913) mapped nervous system of Anodonta cygnea (as Anodonta cellensis) and characterized gross morphological features of cerebral ganglia, pedal ganglia, visceral ganglia and statocysts. Motley (1943) stimulated the heart with electrical impulses and discerned that cardiac tissue is responsive to such stimulation; stimulation of cerebral and visceral ganglia did not depress heart rate, but instead caused contractions in the pedal musculature. Kraemer (1967) observed mantle flapping of Lampsis cardium (as Lampsis ventricosa) and explained how flapping was positively associated with light intensity. Additionally, he noted the presence of a ganglion near the mantle flap and a richer supply of axons in this area as compared to the siphon. Kraemer (1978) described the cellular structure of statocysts of Lampsis cardium (as Lampsis ventricosa). Yokley (1968) described the cerebral, pedal, and visceral ganglia from Pleurobema cordatum. Gross anatomy of visceral ganglion was described by Margaritifera margaritifera, Margaritifera monodonta (as Cumberlandia monodonta) (Smith, 1980) and Margaritifera hembeli (Smith, 1988).

Freshwater mussels have a decentralized nervous system consisting of a pair of anterior cerebral ganglia (Figs. 117, 248, 377), a bilobed pedal ganglion (Figs. 112, 243, 372) and visceral ganglion located posteriorly (Figs. 118, 250, 379). Each ganglion has a distinct cortex and medulla. Herein, we show that the cellular composition of each ganglion is similar. Furthermore, we observed subtle interspecific variation in the histological characteristics of the pedal ganglion of F. cerina as compared...
to *V. nebulosa* and *S. connasaugaensis*. The pedal ganglion of *F. cerina* had thickened axons and connective tissue surrounding each hemisphere (Figs. 244, 246; Table 11). It is unclear how the function of the pedal ganglion of *F. cerina* may differ from that of *V. nebulosa*, and *S. connasaugaensis* considering the later two species lacked thickened axons and connective tissue fibers (Figs. 112, 114, 372, 373). Each ganglion is associated with local tissue function and movement of musculature. Based upon experiments in which bodily regions are separated from the cerebral ganglion, it appears that the cerebral ganglion is required for complex motor functions. Moreover, ganglia are linked to each other and can work together to regulate tissue function. For example, the visceral ganglion enables shell closure, but opening requires signals from cerebral ganglia (Tauc, 1966). Prior work on the cellular anatomy of the bivalve nervous system has indicated that unipolar neurons are typically located in the cortex while bipolar and multipolar neurons tend to be located within the medulla (Tauc, 1966). However, cell bodies of the medulla in *V. nebulosa*, *F. cerina* and *S. connasaugaensis* were minute, making it difficult to determine whether some are neurons or glial cells.

The spherical statocysts located laterally to the pedal ganglion are associated with equilibrium. Statocysts are fluid-filled capsules containing a ciliated epithelium (Figs. 123, 254, 383). Sensation of equilibrium may be derived from neural impulses created following contact between the statolith and ciliated cells. The statolith had a dense consistency similar to the crystalline style since the statolith tended to wrinkle during microtomy. We speculate that the statolith is gelatinous and becomes rigid following tissue function. For example, the visceral ganglion enables shell closure, but opening requires signals from cerebral ganglia (Tauc, 1966). Evidence of statocyst function is derived from experimental investigations in which impaired righting response was observed following mechanical damage to the statocyst or its associated nerve (Cragg & Nott, 1977). Our observations suggest that statocysts of unionids are histologically conserved.

**Reproductive System**

Reproductive tissues in freshwater mussels comprise ovarian and testicular acini, ciliated ducts that transfer ova and sperm dorsally to gonopores (Henley et al., 2007). Histology has been commonly used to determine spawning period for freshwater mussels (Van der Schalie & Van der Schalie, 1963; Yokley, 1972; Smith, 1978; Zale & Neves, 1982; Smith, 1988; Gordon & Smith, 1990; Jirka & Neves, 1992; Grande et al., 2001; Smith et al., 2003; Henley et al., 2007), and numerous authors have demonstrated seasonality of gametogenesis (Van der Schalie & Van der Schalie, 1963; Yokley, 1972; Zale & Neves, 1982; Smith, 1988; Gordon & Smith, 1990; Jirka & Neves, 1992; Grande et al., 2001; Smith et al., 2003; Henley et al., 2007).

Regarding the male anatomy, spermatocytes, spermatids and spermatooza are minute such that only cellular shape and staining characteristics can be observed with light microscopy. Furthermore, differentiating early and late staged spermatocytes or spermatids and spermatooza is difficult. Ovarian acini contain larger oocytes by comparison, with a distinct vitelline mass, and centrally located nucleus is typically seen in published micrographs. Spermatogenesis and morphology of nuclei and organelles of spermatocytes, spermatids and spermatooza have been rendered from transmission electron microscopy studies (Heard, 1975; Healy, 1989; Peredo et al., 1990; Rocha & Azevedo, 1990). Seasonality of male gametogenesis is characterized by typical and atypical spermatogenesis. Typical spermatogenesis occurs during the summer when males are ready to spawn. Spermatooza differentiate from spherical spermatocytes, and spermatooza have chromosomes or granular chromatin. Atypical spermatogenesis occurs between the fall and spring and is distinguished by the presence of spermatocyte clusters called sperm morulae given their resemblance to morula-stage embryos. Spermatooza of sperm morulae have a minimal amount of cytoplasm surrounding a nucleus with condensed chromatin (Heard, 1975). Male mussels disperse spermatooza by packaging them into spherical masses resembling *Volvox* called spermatoozegmatas. Spermatoozegmatas were described from *Anodontoides ferussacianus* using light microscopy. Spermatooza are spherical such that the acrosome was facing the median and the tail extended distally, allowing the entire mass to be propelled and steered by different groups of cells (Edgar, 1965). Waller & Lasee’s (1997) SEM observations of spermatooza and spermatoozegmatas of *Truncilla truncata* revealed that a single spermatoozegmata had 8,000–9,000 spermatooza (see also Edgar, 1965).
Regarding the female anatomy, Van der Schalie & Locke (1941) presented a detailed description of ova and was one of the first publications to specifically describe formation of sperm and ova with photomicrographs. Ova may have an irregular shape initially and maintain a connection to the acinus wall by means of a pellicle. During oogenesis, ova are surrounded by nurse cells, which may secrete a nutritive substance to become incorporated into a developing ovum. When ova reach maturity they are appreciably enlarged and contain a double nucleus (Beams & Sekhon, 1966). Ovarian acini contain degenerative or atretic cells between the fall and spring (Yokley, 1972).

Herein we observed interspecific variation in vitelline cells between the fall and spring (Yokley, 1972). Ovarian acini contain degenerative or atretic cells between the fall and spring (Yokley, 1972). Ovarian acini contain degenerative or atretic cells between the fall and spring (Yokley, 1972).

We observed seasonality of gametogenesis in V. nebulosa, F. cerina and S. connasaugensis. Specifically, the peak spawning period for each species was different. The peak spawning period of V. nebulosa in Terrapin Creek was between late July and early August similar to the spawning time reported from Big Moccasin Creek, Virginia (Zale & Neves, 1982). Similarly, peak gametogenesis occurs in S. connasaugensis between late July and early August. The spawning period of F. cerina in Cahaba River however occurs during May, similar to the spawning period of Fusconaia cuneolus in Clinch River, Virginia (Bruenderman & Neves, 1993). Acini expand to accommodate a small number of enlarged, mature oocytes in females (Figs. 126, 257, 386) and a large volume of spermatozoa in males (Figs. 129, 260, 389). Testicular (Figs. 128, 259, 388), and especially ovarian acini (Figs. 125, 256, 385) contained a preponderance of eosinophilic granules when gametogenesis was depressed. The small granules likely constitute fluid, however the larger granules in ovarian acini possibly represent a combination of polar bodies and degenerative oocytes considering the abundance of pycnotic nuclei within the lumen. Testicular acini also contained pycnotic nuclei to a lesser extent when gametogenesis was limited. Seemingly, gametogenesis occurs outside of the spawning season and that oocytes and spermatocytes breakdown prematurely. Specific seasonal cellular changes to acini were previously described from Pleurobema cordatum (Yokley, 1972).

Interspecific variation in the histological appearance of mature ova was pronounced owing to their great size (Table 11). However, it is difficult to determine the polarity of vitelline structure based on the available literature. For example, some comparative studies are inconsistent with the portrayal of each structure because photographs were taken at different orientations (Jirka & Neves, 1992; Henley et al., 2007). The vitelline membrane was the most distinctively different feature of oocytes (Figs. 126, 257, 386). The structure of oocytes of V. nebulosa correspond to the appearance of V. nebulosa oocytes that appear in Zale & Neves (1982), while the oocytes of F. cerina appear to be similar to oocytes of Fusconaia flava (Van
nder Schalie, 1970). Based upon histological sections of filled marsupia of *F. cerina*, it seems that the structure of vitelline membranes may be important for embryo packing. The vitelline membranes were irregular, filamentous extensions of ova, and ova appeared to be held together within a conglutinate by vitelline membranes (Figs. 160, 162). Therefore, the vitelline membranes of *F. cerina* may be adhesive and provide strength to a conglutinate. Histological sections of filled marsupia of *V. nebulosa* and *S. connasaugaensis* were derived from animals collected months after the spawning period, making it difficult to link structural characteristics of vitelline membranes to embryo packing (Figs. 30, 294). Differences in the thickness or composition of the vitelline membrane of bivalve ova are typically matched by corresponding adaptations of the spermatozoa. For example, the thickened ova of *Mytilus edulis*, may be fertilized by a spermatozoa that possess a large acrosome, which would be required to penetrate a thickened, gelatinous membrane (Morse & Zardus, 1997).

There were no obvious differences in the structure of spermatocytes or spermatozoa of each unionid species considering their minute size (Figs. 129, 260, 389). Spermatocytes and sperm morula of stage 1 testicular acini were loosely arranged, making it difficult to describe specific details of cellular progression from a spherical, single-celled spermatocyte to a spermatid and spermatozoa. Spermatogenesis in stage 1 and stage 3 acini was different. Stage 1 acini were characterized by "atypical" spermatogenesis consisting of copious sperm morula (Figs. 128, 259, 388) while stage 3 acini were characterized as exhibiting "typical" spermatogenesis (Figs. 129, 260, 389; Van der Schalie & Locke, 1941; Heard, 1975). Atypical and typical spermatogenesis may produce sperm cells that are identical based on light microscopy, but it is unclear whether there is a difference at the ultrastructure level. Considering the interspecific variation in oocyte structure, we speculate that spermatozoa of *V. nebulosa*, *F. cerina* and *S. connasaugaensis* would be structurally distinct from each other. Spermatozoa of marine bivalves can be highly species specific at the ultrastructure level and variation is associated with fertilization strategies. Differences are associated with external fertilization, fertilization in mantle cavity, and whether sperm are released freely or packaged into spermatophores (Morse & Zardus, 1997). Regarding reproductive biology of freshwater mussels, unionoids are well known for their widely different dispersal strategies (Barnhart et al., 2008). Mussels are either short-term brooders (tachytic), exhibiting gametogenesis during the early spring followed by the release of their glochidia shortly thereafter, or long-term brooders (bradytic) spawning later in the summer and holding the glochidia within marsupia for weeks or months (Cummings & Graf, 2010). Potentially, variation in oocyte and spermatozoa morphology may correspond to dispersal strategies of *V. nebulosa*, *F. cerina* and *S. connasaugaensis*, or to differences in brooding.

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CONRAD, T. A., 1834, New freshwater shells of the United States, with coloured illustrations: and a monograph of the genus Anculus of Say; also a synopsis of the American naiades. J. Dobson, Philadelphia, Pennsylvania, 76 pp., 8 pls.


