Cloacae, Cloacal Glands and Female Sperm Storage in Giant Salamanders

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Abstract

Most of the 620+ species of salamanders are relatively small animals, rarely exceeding 200 mm total length, but members of the families Cryptobranchidae (1800 mm), Sirenidae (978 mm), Amphiumidae (1162 mm), Proteidae (486 mm) and Dicamptodontidae (351 mm) can truly be considered “giant salamanders.” These families have few species (Proteidae with six species is the most speciose) and are aquatic paedomorphs except for Dicamptodontidae, in which the four species can metamorphose. All families except Cryptobranchidae, which has single species in China and Japan, are limited in distribution to North America. Cryptobranchidae and Sirenidae reproduce with external fertilization, whereas species in the other families have internal fertilization and females store sperm in cloacal spermathecae. Males of the internal fertilizers possess cloacal glands that make spermatophores. The only sister group relationship that has been proposed is between Sirenidae and Proteidae. This paper brings together and summarizes information from a number of separate studies on histology of the cloaca and cloacal glands and ultrastructure of sperm storage, when it occurs, in the giant salamanders. Proteids have cloacae similar to those of other internal fertilizing salamanders, and if the sister group relationship with sirenids is valid, this means that internal fertilization and associated cloacal structures were lost in sirenids or they evolved independently in proteids. The cloacal evidence, however, supports the proposal in one recent paper to return the *Dicamptodon*, the only extant genus in the Dicamptodontidae, to its traditional position in the Ambystomatidae.

Introduction

“Giant salamanders” do not form a clade or a taxonomic group, but the large size attained by many species in these five disparate families makes it interesting to consider their comparative anatomy to
determine if (1) large size leads to parallelisms in structure of organ systems, or (2) organ system
anatomy is independent of body size and instead related to phylogeny. In this chapter, I review
histological descriptions of cloacal anatomy and ultrastructure of female sperm storage in the giant
salamanders.

Sirenidae, usually considered the most basal salamander family (Frost et al., 2006), includes two
genera, *Siren* (two species) and *Pseudobranchus* (3 species). *Siren lacertina* can reach 978 mm total
length and *S. intermedia* 686 mm (Conant and Collins, 1998). *Siren lacertina* is limited to the coastal plain
of the southeastern United States from Maryland to Alabama. *Siren intermedia* overlaps the range of *S.
lacertina* and but continues westward to Texas and Mexico and up the Mississippi River valley and
tributaries to western Indiana; a disjunct population occurs in southwestern Michigan. *Pseudobranchus
striatus* and *P. axanthus* are smaller species with *P. striatus* reaching the largest size, 251 mm (Conant
and Collins, 1998). *Pseudobranchus* is largely limited to Florida, although *P. striatus* extends up the coast
to South Carolina. Sirenids are elongate aquatic paedomorphs with bushy external gills and an absence
of hind limbs. Usually they live in slow moving bodies of water or ponds. Fertilization is presumably
external (Sever et al., 1996c).

Cryptobranchidae includes *Cryptobranchus alleganiensis* found in the Appalachian and Ozark
regions of the eastern United States and *Andrias davidianus* from China and *A. japonicus* from Japan.
Chang (1936) reported a maximum size for *A. davdianus* of 1800 mm, certainly the largest salamander
ever reported! The largest *A. japonicus* on record is 1360 cm (Spareboom, 2010) and *C. alleganiensis*
reaches a maximum size of 740 mm (Conant and Collins, 1998). Cryptobranchids are aquatic
paedomorphs with robust bodies characterized by skin flaps along the lateral margins of the body. They
lack external gills and undergo external fertilization. Cryptobranchids live in fast flowing rivers and
streams.

Proteidae includes *Proteus anguinus* from northeastern Italy and parts of coastal Croatia, Slovenia,
Herzegovina and Bosnia, and five species of *Necturus* from eastern North America. The largest and most
widely ranging species is *N. maculosus*, which reaches a maximum length of 486 mm. Most of the other
species are considerably smaller, although *P. anguinus* can reach a length of 400 mm or more (Bulog
and Meijden, 2010). Proteids are aquatic paedomorphs that retain the bushy gills of larvae and laterally
flattened caudal fins throughout life. *Proteus anguinus* is found in caves and has degenerate eyes and generally lacks pigment. *Necturus* occurs in a variety of habitats from ponds to larger rivers. Frost et al. (2006) consider Sirenidae and Proteidae to be sister taxa, although proteids have internal fertilization and possess glands lacking in sirens that are involved in formation of spermatophores by males and storage of sperm by females.

Amphiumidae consists of three species found in the coastal plain and Mississippi River valley of southeastern United States. *Amphiuma means* with a maximum size of 1162 mm (Conant and Collins, 1998) occurs in the eastern portion of the range from Virginia to the Florida Parishes of Louisiana, and *A. tridactylum* with a total length of 1060 mm extends from Mississippi west to Texas and north to western Kentucky. A relatively small species, *A. pholeter*, has a maximum size of 330 cm and is limited to the panhandle of Florida and neighboring Alabama. These animals are elongate, robust aquatic paedomorphs that lack external gills, have reduced limbs, and prefer swamps, ditches and slower moving streams. They have internal fertilization, make spermatophores and store sperm.

*Dicamptodontdae* contains four species of *Dicamptodon* found in the Pacific northwest from northern California into British Columbia, where they inhabit the streams of mountainous coniferous forests. Adults of all species can exist as aquatic paedomorphs, although all can also metamorphose, but this is rare in *D. copei*. The largest species is *D. tenebrosus*, which can reach a length of 351 mm, but *D. ensatus* also can exceed 300 mm with a maximum known size of 305 mm (Petranka, 1998). Dicamptodontids are internal fertilizers with spermatophore producing glands in males and sperm storage glands in females. Dicamptodontids were placed in the family Ambystomatidae, a group of 30 species mostly found in eastern North America, until Edwards (1976) placed *Dicamptodon* in their own family based upon their hypothesized phylogenetic distance. Most ambystomatids are significantly shorter than dicamptodontids, although *Ambystoma tigrinum* may reach 350 mm (Petranka, 1998). Frost et al. (2006) rejected the hypothesis of Edwards (1976) and returned *Dicamptodon* to the Ambystomatidae, which reduced the redundancy of having two family group names each containing the sister genus of the other. Zhang and Wake (2008), however, returned Dicamptodontidae to full familial status due to their distinct morphology and fossil record.
Male and female cloacal anatomy and female sperm storage have been studied extensively in salamanders, and a relatively recent review was done by Sever (2003). These topics have each been analyzed phylogenetically (Sever, 1991a; Sever and Brizzi, 1996). In addition, cloacal characters have been combined with other morphological and molecular characters in phylogenetic analyses of salamander families (Larson and Dimmick, 1993; Larson et al. 2003; Frost et al. 2006). This chapter compiles observations on histology of cloacal anatomy and ultrastructural observations on sperm storage in the giant salamanders, and discusses aspects of the functional and evolutionary significance of these structures.

Cloacae and Cloacal Glands of the Sirenidae

Sirenids are unique among salamanders in that both sexes lack cloacal glands, absence of which Sever (1991a) considers ancestral character states for salamanders. Other ancestral states found in sirenids include the presence of a definitive cloacal tube (cloacal tube length/total cloacal length >0.05), absence of a cloacal recess, <10 pairs of rugae in the male cloaca, absence of primary and secondary folds, absence of a cloacal chamber depression, absence of dorsolateral recesses, and absence of a pseudopenis. Derived states for sirenids are the lack of cilia in the male and female cloacae, and the extension of epidermis into the anterior one-half of the cloacal chamber. The histological descriptions below are modified from Sever (1991b) who examined *P. striatus*, *S. intermedia*, and *S. lacertina*. Observations on *S. lacertina*, however, were limited to a single male.
Females.—In females, as the proximal ends of the oviducts and kidney collecting ducts descend toward the gut, the dorsal walls of the cavity evaginate, forming two dorsolateral recesses. The oviducts and collecting ducts pass down folds between the dorsolateral recesses to enter the hindgut. Ventrally, the floor of the cavity is lined with 3 (Pseudobranchus striatus) to 5 (S. intermedia) pairs of rugae continuous with those of the posterior large intestine.

Following the junction of the urogenital ducts with the hindgut, the walls of the dorsolateral recesses coalesce abruptly, leaving simply a small dorsomedial fold (Fig. 1A). In Pseudobranchus striatus, Sever (1991b) reported a cloacal tube length/total cloacal length (CTL/TCL) quotient of 0.62, indicating that the anterior tubular portion of the cloaca is longer than the chamber superior to the orifice. CTL/TCL quotients were not given for Siren, but Sever (1991b) indicated that the cloacal tube was distinct in S. intermedia and S. lacertina as well. The cloacal tube is composed of a dorsomedial fold and three pairs of rugae, and the tube decreases in height as it descends toward the cloacal orifice. The cloacal chamber consists of a cavity that steeply decreases in height from anterior to posterior end. The more ventral rugae terminate anterior to the more dorsal ones, so at the posterior end of the vent, the dorsal rugae project nearly vertically into the chamber just superior to the cloacal orifice.
The lining of the cloaca is non-ciliated. The columnar epithelium of the posterior intestine grades into an aglandular, pseudostratified lining in the cloacal tube and becomes stratified cuboidal by the anterior end of the cloacal chamber. Therefore, the lining of the cloacal chamber is similar to the epidermis. However, Leydig cells found in the epidermis adjacent to the cloaca are lacking in the lining of the cloacal chamber, and granular and mucous glands associated with the dermis do not extend into the cloaca.

**Males.**—As in females, the cloacal tube is distinct with CTL/TCL quotients of 0.40 in *Pseudobranchus striatus* and 0.33 in *Siren intermedia*. In *S. intermedia*, the kidney collecting ducts join the hindgut on small folds that exist in the apices of paired dorsolateral evaginations from the gut tube. In *P. striatus*, the hindgut seems to expand dorsally toward the collecting ducts, so that the dorsal end of the anterior cloacal tube is widened. The dorsolateral evaginations in *Siren intermedia* shorten, and the dorsal cavity in *Pseudobranchus striatus* narrows, so that the cloacal tube assumes a more circular appearance with rugose walls as it decreases in height toward the cloacal chamber. Like females, the cloacal chamber in males is a simple cavity (Fig. 1B) that abruptly shortens from the anterior to posterior end (especially in *S. intermedia*), with the most dorsal rugae being the last to terminate as the posterior angle of the cloacal orifice becomes even with the surrounding skin.

The cloaca in male sirens also lacks glands in the epithelium or subsurface connective tissue, and the epithelium is non-ciliated. In contrast to females, the epithelium of the cloacal chamber of males retains a pseudostratified appearance until the posterior angle of the cloaca.

Sever (1991b) only examined one male specimen of *S. lacertina* (35.4 cm snout-vent length) and transverse sections were made through five different regions of the cloaca. The cloaca is similar to that of other sirens except for greater vascularity in the connective tissue of the cloacal sheath. Capillary networks and other blood vessels are frequent around the cloaca in all sirens, but they are especially numerous in *S. lacertina*.

**Cloacae and Cloacal Glands of the Cryptobranchidae**

Sever (1991b) examined male and female cloacae of the North American species, *Cryptobranchus alleganiensis*, but not the Asian forms *Andrias davidianus* and *A. japonicus*, whose cloacal anatomy remains undescribed. The only derived cloacal characters in female and male *Cryptobranchus*...
alleganiensis are possession of anterior ventral glands (Sever 1991a,b; Fig. 2). Sever (1991a) considered male and female anterior ventral glands homologous. In both sexes of C. alleganiensis the glands are basophilic, positive with periodic acid and Schiff reagent (PAS+, indicating neutral carbohydrates), alcian blue+ at pH 2.5 (mostly carboxylated glycosaminoglycans), and female ventral glands are ninhydrin-Schiff+ (proteins) while those of the male are ninhydrin-Schiff-. Transverse sections through the cloacal chamber of a female and male C. alleganiensis and detail of their ventral glands is illustrated in Figure 2.

Female cloacal anatomy.—The CTL/TCL quotient is 0.40, so the ancestral condition of a definitive cloacal tube is present. The pseudostratified cloacal epithelium is ciliated. Rugae are limited to the inferior wall. The lining of the medial borders of the cloacal orifice is continuous with the epidermis of the skin. The skin contains vacuolated Leydig cells and numerous capillaries that abut on the squamous layer of the epidermis; these features extend into the lining of the cloacal orifice. Groove-like invaginations of the skin lateral to the anterior half of the cloacal orifice result in obvious folds, the cloacal lips. Posteriorly in the cloacal chamber, the dorsal portion narrows and shortens, and the extent of the epidermal lining increases. Cilia are present dorsally on the simple columnar epithelium to the start of the posterior two-fifths of the cloacal chamber, where the entire cavity is lined with epidermis. At the posterior angle of the vent, granular and mucous glands, which are abundant in the dermis lateral to the cloacal orifice, are present over the dorsal apex of the cloacal chamber.

Ventral glands secrete into the cloacal orifice from the anterior end to the posterior angle where mucous and granular glands surround the orifice. Distal ends of ventral glands pass anteriorly. In the anterior half of the cloacal chamber, ventral glands secrete onto the epidermal surface of the cloacal lips, including the borders of the invaginated grooves, but not into the cloaca. Mucous and granular glands do not secrete onto the surface of the cloacal lips. In the caudal half of the cloacal chamber (posterior to the cloacal lips) ventral glands are present around the chamber and secrete onto its dorsal and lateral walls instead of the skin lateral to the orifice, where mucous and granular glands are abundant.

Luminal contents of the ventral glands include fibrous material as well as granules. The apical cytoplasm and the luminal fibrous material are strongly alcian blue+. This reaction with alcian blue
is more intense (darker) than that of mucous glands in the skin. The luminal granules are PAS+, and some PAS+ sites are scattered in the apical cytoplasm of the ventral glands. Weak ninhydrin-Schiff+ reactions occur in the cytoplasm and lumen.

Fig. 2. Transverse sections through the cloacal chamber (Cc) of Cryptobranchus alleganiensis, stained with hematoxylin and eosin. Scale bar in lower right corner = 350 µm for A,B and 150 µm for C,D. A. Female. B. Male. C. Detail of female anterior ventral glands (Av) and skin glands. D. Detail of male anterior ventral glands and skin. Gg, granular glands; Mg, mucous glands; Ss, stratum spongiosum. Original.
Male cloacal anatomy.—The CTL/TCL quotient is 0.38 in the specimen examined by Sever (1991b). As in the female, the pseudostratified cloacal epithelium is ciliated. However, the cloacal tube of the male is rugose, bearing prominent middorsal and ventral folds and four pairs of lateral folds. Also, blood vessels are more abundant around the cloacal cavity than in the female. The skin and the lining of the medial border of the cloacal orifice is the same as in the female. The external cloacal lips lateral to the cloacal orifice are more prominent in the male. Cloacal lips occur anterior to the vent (ventral to approximately the posterior sixth of the cloacal tube) and continue along the lateral borders of the anterior three-fifths of the cloacal orifice.

Posterior to the junction of the cloacal tube and cloacal chamber, the chamber gradually shortens in height, with the most ventral rugae ending first. Rugae are absent and the ciliated columnar epithelium completely replaced by epidermis in the posterior half of the cloacal chamber, nearly concomitant with the end of the cloacal lips. The cloacal chamber becomes a narrow slit with smooth walls that simply reduces in height to the level of the skin. Like the epidermis of the skin lateral to the cloaca, the epidermal lining of the cloaca contains numerous capillaries and vacuolated Leydig cells.

The ventral glands of the male are more numerous and convoluted than those of the female (Fig. 2). The secretion again is basophilic and alcian blue+, although the alcian blue reaction is lighter than that of the female and similar to that of mucous glands in the skin. The secretory product in the cytoplasm is diffuse and abundant, but luminal materials are scant. The luminal substance is fibrous, although some granular material occurs. The weak ninhydrin-Schiff+ reaction noted in female ventral glands is not detectable in those of the male. The ventral glands secrete onto the surface of cloacal lips and the groove lateral to the cloacal lips, and not into the cloacal chamber itself (Fig. 2B). Ventral glands do not secrete posterior to the end of the cloacal lips, in contrast to the female. Like females, however, mucous and granular glands do not occur on the cloacal lips. The distal ends of the ventral glands radiate from the cloacal lips, with some extending anteriorly as far as the posterior end of the large intestine and others extending medial and lateral to the posterior end of the cloaca. The glands are branched, and a transverse section may contain more than one section of a given gland. In the caudal two-fifths of the
cloacal chamber (posterior to the cloacal lips) mucous and granular glands secrete onto the surface of the epidermal lining.

Cloacae and Cloacal Glands of the Proteidae

Kingsbury (1895) provided a histological description of the cloaca of female *Necturus maculosus*, and Dawson (1922) described the male cloaca of that species. Sever (1992b) examined a female *Proteus anguinus* and males and females of all *Necturus* except *N. alabamensis*, i.e., *N. beryeri*, *N. lewisi*, *N. maculosus*, and *N. punctatus*. Some of his observations on *N. maculosus* differed from those of Kingsbury (1895) and Dawson (1922).

Duellman and Trueb (1986) placed proteids are placed in the suborder Salamandroidea, seven families characterized by the presence of sperm storage glands (spermathecae) in females and cloacal glands that make spermatophores in males, resulting in internal fertilization (Sever, 1991a). Monophyly of this suborder has been questioned (Hillis, 1991; Larson and Dimmick, 1993; Frost et al. 2006), and the occurrence of these reproductive structures, which Sever (1991a) considers a synapomorphy for the suborder, is the most important suite of characters uniting these families in one clade. Of the 25 cloacal characters recognized by Sever (1991a), proteids possess three derived states for the cloacal linings and eight derived characters for cloacal glands.

**Female cloacal anatomy.**—Transverse sections through the cloacae of *Necturus lewisi* are illustrated in Figure 3 and *Proteus anguinus* in Figure 4. The cloacal tube is well-developed. The CTL/TCL quotient is 0.38 in *Necturus beryeri*, 0.29 in *N. lewisi*, 0.41 in *N. punctatus*, and 0.28 in *Proteus anguinus*. Prior to the junction with the oviducts and Wolffian ducts, the posterior intestine is circular, with rugose walls of glandular, simple columnar epithelium. The oviducts and Wolffian ducts descend toward the gut through folds that evaginate from the dorsolateral walls of the cavity (Fig. 3A). In this region, the ventral walls consist of longitudinal folds. The pseudostratified epithelium contains a superficial columnar layer and an interrupted basal layer of squamous cells. The apical cytoplasm of the columnar cells is alcian blue+, indicating presence of glycosaminoglycans, in specimens in which cloacal glands are active (contain secretory substances). Cilia are absent on the cloacal epithelium.
Fig. 3. Transverse sections through the cloaca of female Necturus lewisi. Sections A,B,D,E stained with hematoxylin-eosin, C with ninhydrin-Schiff, and F with PAS and alcian blue at pH 2.5. Scale bar in lower right corner of C = 350 µm for A,B,D,F; 80 µm for C, and 100 µm for F. A. Posterior end of the intestine. B. Anterior end of the cloacal tube. C. Detail of sperm from a sperm cap entering a spermathecal tubule. D. Anterior end of the cloacal chamber. E. Posterior end of the cloacal chamber. F. Skin glands posterior to the cloaca. Av, anterior ventral glands; Cc, cloacal chamber; Cs, spermatophore cap; Ct, cloacal tube; Df, deep fibrous layer of the dermis; Di, intermediate layer of the dermis; Ep, epidermis; Gg, granular glands; Le, Leydig cells; Mg, mucous gland; Od, oviduct; Pi, posterior intestine; Sp, sperm; St, spermathecae. Modified from Sever, D. M. 1992b. Herpetologica 48:320, Fig. 2.

Just posterior to the junction of the Wolffian ducts with the gut, the dorsolateral folds cease, and the dorsal portion of the cloacal tube appears as a widened cavity. This area quickly narrows, and the dorsal walls also become rugose and lined with pseudostratified epithelium (Fig. 3B). The cloacal tube opens into the cloacal chamber (Fig. 3D), and a gradual dorsoventral shortening of the cavity occurs caudally.
The cloacal orifice is a narrow slit lined with 3-4 layers of highly vacuolated, cuboidal epithelial cells like those in the adjacent epidermis of the body wall. The epidermal lining of the cloaca, however, lacks the Leydig cells found in the skin (Fig. 3F). At the anterior end of the cloacal orifice, the epidermal lining is restricted to just the cloacal orifice, but more posteriorly, the extent of epidermis into the cloacal chamber increases dorsally so that the entire cavity is lined with epidermis by the start of the posterior one-half (Fig. 3E). Rugae from the cloacal tube continue until the midpoint of the cloacal chamber, after which the walls of the cavity are smooth.

No interspecific variation in these structures occurs among female *Necturus*. *Proteus anguinus* possesses some differences in cloacal conformation. In *P. anguinus*, the oviducts (Fig. 4A) and Wolffian ducts descend toward the gut through a single broad evagination from the dorsal wall. Caudal to the junction of the Wolffian ducts with the cavity, the evagination from the roof ends, creating a wide cavity dorsal to a narrow vertical slit that represents the ventral portion of the cloacal tube (Fig. 4B). Ventral to the cloacal tube is a horizontally flattened cavity that represents a recess from the anterior end of the cloacal chamber (Fig. 4A,B). The ventral portion of the cloacal tube joins this recess just anterior to the cloacal orifice. At the anterior end of the cloacal orifice, extensions from the flattened portion pass dorsally and pinch off tissue representing a posterior projection from the cloacal tube (Fig. 4C). This projection ends quickly, and the anterior end of the cloacal chamber is therefore widened dorsally (Fig. 4D). In the anterior one-fourth of the cloacal chamber, the cavity narrows into a slit with smooth walls (Fig. 4E), and the chamber rapidly decreases in height to the posterior end.

Simple spermathecae and ventral glands occur in all species. In *Necturus beyeri*, *N. maculosus*, and *Proteus anguinus* examined by Sever (1992b), the spermathecae did not contain spermatozoa, and all glands appear inactive (devoid of a secretory product and reduced in size). In these specimens, ovarian follicles are small, and the oviducts are thin and straight. In one *N. lewisi*, however, a spermatophore cap occurs in the cloaca. In this species and in *N. punctatus*, the spermathecae are filled with spermatozoa, and the ventral glands are hypertrophied and contain copious secretions. Ovarian follicles are large, and the oviducts are thick and convoluted. The active spermathecae of *Necturus lewisi* and *N. punctatus* are simple tubular glands with basophilic, columnar epithelia, wide lumina, and narrow pores that open into the cloaca (Fig. 3C). Spermathecae have a fibrous secretion in the apical cytoplasm and in the lumen that
is alcian blue+ (indicating glycosaminoglycans). In *N. lewisi*, granules that are PAS+ (neutral carbohydrates) also occur among the luminal secretory products. Active ventral glands are eosinophilic with columnar cells, and elongate, thick, convoluted tubules. Ventral glands contain a globular luminal substance and a granular product in the apical cytoplasm, and these substances are ninhydrin Schiff + (indicative of proteins) and PAS+ but are alcian blue-.

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Fig. 4. Transverse sections through the cloaca of female *Proteus anguinus* (UMMZ 187489). Sections stained with hematoxylin and eosin. Scale bar in lower right corner of F = 250 µm for A—F and 80 µm for G. A. Posterior end of the intestine. B. Anterior end of the cloacal tube. C. Anterior end of the cloacal orifice. D. Anterior one-fourth of the cloacal chamber. E. Mid-cloacal chamber. F. Glands secreting into the epidermis posterior to the cloacal orifice. G. Detail of glands shown in F. Av, anterior ventral glands; Cc, cloacal chamber; Ct, cloacal tube; Ep, epidermis; Gx, unnamed glands; Od, oviducts; Pc, pleuroperitoneal coelom; Pi, posterior intestine; St, spermathecae. Modified from Sever, D. M. 1992b. Herpetologica 48: 321, Fig. 3.
In *Necturus*, spermathecae are present at the anterior end of the cloacal tube, just posterior to the union of the Wolffian ducts with the tube (Fig. 3B). Spermathecae open into the dorsal and lateral walls of the entire cloacal tube and the anterior one-half of the cloacal chamber (Fig. 3B-D). Distal ends of the glands pass dorsally with the tips curving posteriorly, so tubules still appear in transverse sections dorsal to the caudal one-half of the cloacal chamber (Fig. 3E). In *Proteus anguinus*, spermathecal tubules do not appear until the anterior end of the cloacal chamber but otherwise are similar to those of *Necturus* (Fig. 4D,E).

As noted above, a spermatophore cap is lodged in the anterior cloacal chamber (Fig. 3C,D) of a *N. lewisi* examined by Sever (1992b). Most sperm are associated with the periphery of the cap, which consists of alcian blue+ fibrous material. Towards the center of the cap are globules that are PAS+ and ninhydrin Schiff+. Streams of sperm extending from the apex of the cap appear in proximal ends of spermathecae (Fig. 3C). These sperm are in the lumina of the spermathecae and are not in contact with the spermathecal walls.

The ventral glands of *Necturus* secrete onto the rugae in the ventral portion of the posterior one-half of the cloacal tube and anterior one-third of the cloacal chamber. Distal ends of the ventral glands radiate from the areas of secretion. Anteriorly, tubules extend inferior to the urogenital region to lie ventral to the posterior intestine (Fig. 3A). Posteriorly, the distal ends of tubules extend to the posterior end of the cloaca. In *Proteus anguinus*, ventral glands secrete into the ventral floor of the anterior recess of the cloacal chamber and onto the walls lining the anterior one-half of the vent (Fig. 4B-E). As in *Necturus*, no cloacal glands secrete into the posterior end of the cloacal chamber.

*Necturus* also possess granular glands deep to the inner-most fibrous layer (stratum compactum) of the dermis and lateral to the posterior end of the cloaca (Fig. 3F). The ducts pass through the dermis and epidermis to open upon the surface of the skin. In *Proteus anguinus*, numerous short tubular glands of unknown homology occur deep to the skin caudad of the cloaca (Fig. 4F,G). As with the spermathecae and ventral glands of this species, these glands also appear inactive.

In female *Necturus maculosus*, Kingsbury (1895) found spermathecae, ventral glands, and a third gland cluster, which he called the "convoluted tubules", that occurs on the antero-dorsal side of the
cloaca. Sever (1992b) proposed that these convoluted tubules also are spermathecae. Otherwise, the findings of Kingsbury (1895) and those of Sever (1992b) agree. Female proteids are similar in cloacal anatomy to species found in other families in the Salamandroidea, in which possession of spermathecae and ventral glands is synapomorphic (Sever, 1991a). Additional types of cloacal glands are known in females of some families. However, the glands that secrete onto the surface of the epidermis posterior to the cloacal orifice in female *Proteus anguinus* may be an autapomorphy for the species. Small tubular glands called “female vent glands” by Sever (1991a) secrete into the posterior angle of the vent in females of *Amphiuma* (Amphiumidae) and in *Pleurodeles* and *Tylototriton* (Salamandridae), but the glands in *P. anguinus* secrete onto the surface of the skin and not into the cloaca and therefore are not homologous.

**Male cloacal anatomy.**—Transverse sections through the cloacae of a male *Necturus lewisi* are illustrated in Figures 5—7. Both the cloacal tube and cloacal chamber are lined with pseudostratified epithelium, except for the edges of the cloacal orifice and the most posterior angle of the cloacal chamber, which are lined with epidermis. The cloacal tube is relatively long. The CTL/TCL quotient is 0.64 in *Necturus beyeri*, 0.68 in *N. lewisi*, and 0.69 in *N. punctatus*. The anterior end of the cloacal tube is rugose and similar to the posterior intestine, except the ventral floor is widened laterally (Fig.5A).
Fig. 5. Transverse sections through the cloaca of male *Necturus lewisi*, stained with hematoxylin and eosin. Scale bar in lower right corner = 300 µm. A. anterior end of the cloacal tube. B. Anterior one-third of the cloacal tube. Av, anterior ventral glands; Ct, cloacal tube; Dp, dorsal pelvic glands; Kg, Kingsbury's glands; Lp, lateral pelvic glands; Ru, rugae. Modified from Sever, D. M. 1992b. Herpetologica 48: 324, Fig. 5.

In the anterior one-fifth of the cloacal tube, the dorsal one-half flattens horizontally forming the “lateral furrows” noted by Dawson (1922) and is lined with papillae. Concurrently, the ventral floor becomes ciliated and invaginates ventrally, with the walls formed into a dozen pairs of long, narrow, dorsomedially directed rugae (Fig. 5B). At the beginning of the posterior two-thirds of the cloacal tube, a dorsal invagination occurs, creating a groove lined with non-ciliated papillae (Fig. 6A; “the median dorsal groove” of Dawson, 1922). In the posterior one-third of the cloacal tube, the entire cavity widens, the lateral furrows end, and a posteriorly projecting, medial evagination (Me) of the floor occurs (Fig. 6B).

At the anterior end of the cloacal chamber, the floor opens into the vent by a narrow slit, thereby eliminating the medial evagination of the floor, and the rugae terminate and are replaced by non-ciliated papillae (Fig. 7A). In the posterior one-half of the cloacal chamber, the widened areas narrow, papillae are absent, and the chamber gradually decreases in height until it is even with the surrounding skin (Fig. 7B).
Fig. 6. Transverse sections through the cloaca of male *Necturus lewisi*, continued from Fig. 5. Scale bar in lower right corner = 300 µm. A. Mid-cloacal tube. B. Posterior one-third of the cloacal tube. Av, anterior ventral glands; Cc, cloacal chamber; Ct, cloacal tube; Dg, dorsal median groove; Dp, dorsal pelvic glands; Kg, Kingsbury’s glands; Lf, lateral furrows; Lp, lateral pelvic glands; Me, medial evagination of the floor of the posterior cloacal tube; Pv, posterior ventral glands; Ru, rugae; Se, secretion. Modified from Sever, D. M. 1992b. Herpetologica 48: 325, Fig. 6.

Ventral glands and Kingsbury’s glands are basophilic, with a fibrous secretion that is alcian blue+ and ninhydrin Schiff -. Kingsbury’s glands are short tubules which occur around the dorsal portion of the anterior cloacal tube (Fig. 5A,B). As the “median dorsal groove” appears in the cloacal tube, Kingsbury’s glands become restricted to the widened middle area, and they are replaced by posterior ventral glands in the caudal one-half of the cloacal tube (Fig. 6A,B).

Anterior ventral glands are the largest mass of glands and surround the anterior and lateral borders of the cloacal orifice; they are absent only around the posterior end of the orifice (Figs. 5-7). Anterior ventral glands are largely responsible for the swollen cloacal area conspicuous externally in sexually active males. These glands are thick, elongate glands that radiate outward from their secretory areas.
Anterior ventral glands secrete onto the rugae of the cloacal tube and on the papilla lining the ventral portion of the cloacal chamber.

Posterior ventral glands appear in the caudal one-half of the cloacal tube in the middle, laterally widened area, and spread dorsally around the cavity until they surround the dorsal portion of the posterior cloacal tube (Fig. 6). These glands stain more intensely with alcian blue than the anterior ventral glands, and their tubules are posteriorly directed and appear shorter than those of anterior ventral glands. Posterior ventral glands no longer secrete into the cloaca at the anterior end of the cloacal chamber, although distal ends of some caudally directed tubules occur in transverse sections in the anterior one-fourth of the cloacal chamber (Fig. 7A).

Pelvic glands are eosinophilic glands that can be divided into two groups based on location and some differences in nature of the secretion. Dorsal pelvic glands are elongate glands that secrete into the median dorsal groove of the cloacal tube until these glands are replaced by posterior ventral glands in the caudal end of the cloacal tube (Figs. 5, 6). The distal ends of dorsal pelvic glands pass anteriorly and

Fig. 7. Transverse sections through the cloaca of male *Necturus lewisi*, continued from Fig. 6. Scale bar in lower right corner = 300 µm. A. Anterior end of the cloacal chamber. B. Posterior one-third of the cloacal chamber. Av, anterior ventral glands; Vg, vent glands. Modified from Sever, D.M. 1992b. Herpetologica 48:326, Fig. 7.
laterally as well as posteriorly. The anteriorly and laterally passing glands have a secretion consisting of globules that are PAS+ and ninhydrin Schiff+ as well as some fibrous material that is alcian blue+. In the posteriorly passing dorsal pelvic glands, which are more medial, the alcian blue+ and ninhydrin Schiff+ substances are not as abundant, and globules that fill the lumina are intensely PAS+. Distal ends of the medial dorsal pelvic glands extend superior to the posterior ventral glands in the caudal end of the cloacal tube and the anterior cloacal chamber (Figs. 6B, 7A).

Lateral pelvic glands secrete into the widened area of the cloacal tube between Kingsbury’s glands and anterior ventral glands (Figs. 5A,B, 6A). The posterior extent of the lateral pelvic glands coincides with the anterior appearance of the posterior ventral glands (Fig. 6B). Distal ends of lateral pelvic glands pass anterolaterally. These glands have a fibrous secretion that fills the lumen and is PAS+, alcian blue-, and ninhydrin Schiff-.

Vent glands are relatively thick, elongate, and convoluted glands found in dorsal and ventral groups in the cloacal chamber. The most cranial vent glands of the ventral group are inferior to the anterior ventral glands in the posterior end of the cloacal tube. Distal ends of these glands extend laterally around the inferior border of the anterior ventral gland. The vent glands secrete onto the most inferior papillae of the posterior cloacal tube and cloacal chamber until the papillae end in the posterior cloacal chamber (Fig. 7A). At this point, no more anterior ventral glands are found, and the ventral group of vent glands merges with the dorsal group (Fig. 7B).

The most cranial vent glands of the dorsal group occur in the anterior cloacal chamber between the most caudal posterior ventral glands and anterior ventral glands (Fig. 7A). The proximal ends pass posterolaterally to secretory regions in the posterior end of the cloaca, where vent glands of the dorsal and ventral groups come into contact. Vent glands are the only glands in the posterior end of the cloacal chamber. Here the vent glands secrete onto papillae or smooth epidermal surfaces, including those of paired skin folds prominent at the caudal end of the cloacal orifice and called the "external papillae" by Dawson (1922). Vent glands usually have a mixture of fibrous and granular luminal products that are eosinophilic, although the proximal ends of some glands, especially in the ventral group, contain only basophilic, fibrous secretions. The secretions are PAS+, alcian blue-, and ninhydrin Schiff-. As in
females, skin glands occur deep to the stratum compactum at the posterior end of the cloaca. Both mucous and granular glands occur deep to the stratum compactum in the area lateral and posterior to the external papillae.

Dawson (1922) described the cloaca and cloacal glands of male *Necturus maculosus*. Dawson’s detailed descriptions of cloacal conformation and linings are consistent with the present findings. Dawson recognized three types of cloacal glands: pelvic glands, "cloacal" (ventral) glands, and "abdominal" (vent) glands. Dawson, however, also realized the distinctiveness of the lateral pelvic gland and of Kingsbury’s gland (which he called “median cephalic” pelvic gland). Dawson’s findings on the histology of these glands agree with those reported by Sever (1992b).

The major difference between the results of Sever (1992b) and those of Dawson (1922) is the extent of the vent gland. Dawson found the vent gland "almost vestigial" and located only in the caudal end of the cloacal orifice. In specimens examined by Sever (1992b), the vent gland was extensive and well-developed. Distal ends of the dorsal group of vent glands extend into the anterior cloacal chamber, and tubules of the ventral group secrete along the entire cloacal orifice, not just the most posterior end.

The skin of *Necturus* is a mosaic of metamorphosed and larval characters, in which Leydig cells, typical of amphibian larvae, are abundant in the epidermis, whereas granular and mucous glands, absent in typical larvae, occur in the dermis (Dawson, 1920). The occurrence of mucous and granular glands deep to the dermis has not been reported previously from amphibians and is of unknown significance. Hedonic (courtship) glands, however, are known from deep to the dermis in some paedomorphic plethodontids (Sever, 1985).

**Cloacae and Cloacal Glands of the Amphiumidae**

The salamander family Amphiumidae consists of three paedomorphic species of *Amphiuma* found in the southeastern United States. Amphiumas are elongate salamanders with reduced limbs, and adults retain one external gill slit. Two species, *Amphiuma means* and *A. tridactylum*, are large, with record lengths in excess of 1000 mm total length and are aquatic, whereas *A. pholeter* is a smaller species, maximum length of 330 mm (Conant and Collins, 1998), that usually is found burrowing in mucky banks (Petranka, 1998).
Davison (1895) described the gross anatomy of the cloacae of male and female *Amphiuma means* and was the first to provide anatomical evidence for internal fertilization in the family. Kreeger (1942) provided a histological description of the cloaca of female *A. tridactylum*, but the only such account for male amphiumids, prior to Sever (1991a, 1992a) was an abstract by Wilson (1941) on the same species. Sever (1991a) listed presence/absence data for 23 cloacal characters in amphiumids and other salamanders, and his cladistic analysis of these characters found that amphiumids possess the most plesiomorphic cloacal anatomy in the suborder Salamandroidea. Amphiumids possess no derived characters of the cloacal linings and seven derived cloacal gland characters. Sever (1992a) extended the observations of Wilson (1941) and Kreeger (1942) by providing histological descriptions of the cloacae of all species of *Amphiuma*.

**Female cloacal anatomy.**—Sever (1992a) reported a CTL/TCL quotient of 0.41 in *Amphiuma means* and 0.49 in *A. pholeter*, so a distinct cloacal tube exists. Posterior to the junction of the urogenital ducts, the cloacal tube has rugose walls lined with glandular, alcian blue+, pseudostratified epithelium. Some epithelial cells in the cloacal tube possess cilia. Cilia do not occur dorsally beyond the midpoint of the cloacal tube, whereas cilia remain ventrally until the anterior end of the cloacal chamber. A dorsomedial fold is prominent in the cloacal tube, but at the anterior end of the cloacal chamber, the dorsal apex narrows, and the dorsolateral wall becomes less rugose (Fig. 8A-C). Ventrally, three pairs of rugae continue from the cloacal tube into the anterior end of the cloacal chamber. The infolding of epithelium at the anterior end of the cloacal orifice is epidermal, consisting of stratified cuboidal cells with a superficial cornified layer (Fig. 8B,C). The epidermal lining gradually spreads dorsally, replacing the pseudostratified lining. In *Amphiuma pholeter*, the entire caudal three-fourths of the cloacal chamber is lined with epidermis (Fig. 8D,E), but in *A. means* and *A. tridactylum*, the dorsal end of the cloacal chamber retains the pseudostratified lining until the caudal angle of the vent. Sever (1992a) noted that the lining of the cloacal orifice (cloacal “lips”) in an *A. tridactylum* with enlarged ovarian follicles and spermatozoa in the spermathecae is especially thick and cornified (Fig. 8F).

Cloacal glands are simple spermathecae and vent glands. In an *Amphiuma tridactylum* with large ovarian follicles, spermatozoa occur in short, simple tubular glands in the posterior half of the cloacal tube and the anterior two-fifths of the cloacal chamber, defining these glands as spermathecae. Glands similar
in appearance and location, but devoid of sperm, occur in these regions in the other female amphiumids examined by Sever (1992a). Anteriorly, the first spermathecae appear dorsomedially, and more posteriorly they spread around the dorsal and lateral walls of the cloaca (Fig. 8A-D). In several *A. tridactylum* and *A. means*, some glands appear around the inferior border of the cloacal tube as well (the “ventral group” of spermathecae described by Kreeger, 1942). The most posterior spermathecae are dorsomedial and, in *A. pholeter*, pass into portions of the cloacal chamber where the lining has become epidermal (Fig. 8D).

Fig. 8. Transverse sections through the cloaca of female *Amphiuma pholeter* (A—E) and *A.tridactylum* (F), stained with hematoxylin and eosin. Scale bar in lower right corner = 250 µm in A,B,C,D, 200 µm in E, and 500 µm in F. A. Middle of the cloacal tube. B. Posterior end of the cloacal tube. C. Anterior end of the cloacal chamber. D. Start of the posterior three-fourths of the cloacal chamber. E. Posterior fourth of the cloacal chamber. F. Highly cornified epidermis around the cloacal chamber. Bv, blood vessels; C,
cloacal chamber; Ce, cornified epithelium of the cloacal lips; Ct, cloacal tube; Ep, epidermal lining of the cloacal chamber; Ng, neural ganglion; St, spermathecae; Vg, vent glands. Modified from Sever, D. M. 1992a. Journal of Morphology 211:66, Fig. 2.

Spermathecal cytoplasm is scant and the cells are squamous in *Amphiuma means* and in *A. tridactylum* that lack stored sperm. The spermathecal epithelium is eosinophilic and cuboidal in *A. pholeter* and in a *A. tridactylum* containing sperm. In *A. pholeter*, the spermathecae contain some small granules that are PAS+ and ninhydrin Schiff-. The apical cytoplasm of spermathecae is PAS+ in an individual of *A. tridactylum* with sperm. The sperm in the spermathecae are organized into bundles with sperm heads usually oriented in the same direction. Some groups of sperm appear free in the lumen of these glands whereas others seem to have their heads embedded in the spermathecal cytoplasm.

A separate group of glands occurs at the posterior end of the cloaca in female *Amphiuma* (Fig. 8E,F). These glands do not contain sperm in an *A. tridactylum* with sperm in the spermathecae and are cytologically different from spermathecae in all specimens. This posterior group of glands is similar in appearance and position to the vent glands found in male amphiumids (as described below) and males of many other members in the suborder Salamandroidea (Sever, 1991a). Hence, these are considered female vent glands.

Vent glands occur in the caudal half of the cloacal chamber, and some secrete onto the skin posterior to the cloacal orifice. In *Amphiuma pholeter*, these short, tubular glands secrete only onto cornified epithelium (Fig. 8E), but in *A. means* and *A. tridactylum*, they secrete onto simple columnar epithelium as well as the cornified linings of the cloaca. In an *A. tridactylum* storing sperm, the cells of vent glands are columnar and basophilic, and the apical cytoplasm is strongly PAS+, although the lumina are devoid of secretory product. In the other specimens, vent gland cytoplasm is scant, and the glands seem inactive. In *A. pholeter*, however, small PAS+ granules occur in the vent gland cytoplasm.

**Male cloacal anatomy.**—The male cloacal anatomy of all three species of *Amphiuma* is similar. Sever (1992a) reported a CTL/TCL quotient of 0.35 in *Amphiuma pholeter* and 0.16 in *A. tridactylum*, so a cloacal tube is distinct. The epithelium is pseudostratified throughout the cloaca except on the medial edges of the cloacal orifice where the lining is epidermal and continuous with that of the skin. Anteriorly,
the cloacal tube is laterally narrowed dorsally and widened ventrally. In the anterior cloacal tube, a middorsal fold is prominent and 4-5 pairs of rugae occur ventrally (Fig. 9A,B). Posteriorly in the cloacal tube, the floor narrows and invaginates toward the cloacal orifice. Rugae continue into the anterior half of the cloacal chamber (Fig. 9C,D). The rugae are ciliated in individuals with active cloacal glands, project medially into the cloacal cavity, and are obliquely slanted with the anterior end superior to the posterior end.

Fig. 9. Transverse sections through the cloaca of male *Amphiuma pholeter*, stained with hematoxylin and eosin. Scale bar in lower right corner = 250 µm. A. Anterior end of the cloacal tube. B. Posterior end of the cloacal tube. C. Anterior end of the cloacal chamber. D. Anterior fourth of the cloacal chamber. Av, anterior ventral glands; Ct, cloacal tube; Dp, dorsal pelvic glands; Kg, Kingsbury’s glands. Modified from Sever, D. M. 1992a. Journal of Morphology 211:68, Fig. 4.

At the midpoint of the cloacal tube, the dorsal portion of the cloacal tube widens, and the roof in the posterior half of the cloacal tube and anterior end of the cloacal chamber (Fig. 9C) bears non-ciliated papillae. In the anterior end of the cloacal chamber, the dorsal portion narrows and a dorsal invagination occurs into the roof, resulting in the dorsal portion becoming a narrow slit lined with non-ciliated papillae.
(Fig. 9D). Thus, the anterior half of the cloacal chamber consists of a narrow cavity with papillae dorsally and rugae ventrally.

In the posterior half of the cloacal chamber, the ventral portion becomes less rugose, and the lining of the dorsal portion becomes glandular and infolded, forming the pit glands (Fig. 10A,B). All cloacal glands are exocrine, apparently formed by invaginations of the epithelial surface of the cloacal lining, but cloacal glands other than the pit glands are relatively elongate, tubular structures with orifices smaller than the diameter of the tubules. The pit glands are simply shallow infoldings of the surface, without definitive tubular structure or rounded orifices (Fig. 10).

The most anterior pit glands are middorsal (Fig. 10A). Posteriorly, pit glands extend ventrally until they occupy the entire lining of the cloaca chamber except for the area just superior to the cloacal orifice (Figs. 10B,C). The caudal end of the cloacal chamber is abrupt. Distal portions of pit glands extend beyond the posterior end of the cloacal orifice (Fig. 10D).

The pit glands are eosinophilic with columnar cells. In the apical cytoplasm, an alcian blue+ fibrous material and numerous granules that are PAS+ and ninhydrin Schiff+ occur. The luminal secretory product appears as a mixture of alcian blue+ fibrous and PAS+ globular material. The other eosinophilic glands are the dorsal pelvic glands and the vent glands.

Lateral pelvic glands such as those described from males of some other species in the Salamandroidea (Sever, 1991a) are not present. Dorsal pelvic glands secrete onto the non-ciliated papillae of the roof of the posterior cloacal tube and anterior cloacal chamber (Figs. 9C,D; 10A). Distal ends of glands radiate from the cloaca, so that sections through tubules occur anterior as well as posterior to the area of secretion (Figs. 9A,B; 10B). The epithelium generally is cuboidal and, like that of pit glands, contains alcian blue+ fibrous material and granules that are PAS+ mixed together in the lumen, but in greater quantity than in pit glands.
The vent glands are short and tubular with distal ends that pass posteriorly. Vent glands secrete into the caudal end of the cloacal orifice and onto the skin surrounding the posterior end of the vent (Fig. 10C,D). Vent glands replace in position the most caudal anterior ventral glands. Vent glands are not numerous, but are convoluted so that two sections of the same gland may appear in the same transverse section. The epithelium is columnar, although somewhat festooned, and contains granules that are PAS+ and ninhydrin Schiff+. Luminal material, however, generally is fibrous and alcian blue+. Like that of pit glands, luminal product is less evident than in dorsal pelvic glands.

Basophilic glands are Kingsbury’s glands and the anterior ventral glands. Kingsbury’s glands are short tubules that secrete along the borders of the dorsomedial fold in the anterior end of the cloacal tube and, after the roof flattens and widens, some Kingsbury’s glands secrete onto the roof of the cloacal tube.
medial to dorsal pelvic glands (Fig. 9A,B). Dorsal pelvic glands replace medial Kingsbury’s glands in the posterior end of the cloacal tube, and no Kingsbury’s glands are found at the anterior end of the cloacal chamber (Fig. 9C). Kingsbury’s glands have a fibrous secretion that is alcian blue+. This material is scant in the lumina but abundant in apices of the columnar/cuboidal epithelial cells.

Anterior ventral glands secrete onto the surfaces of the rugae that line the lateral walls of the posterior cloacal tube and anterior half of the cloacal chamber (Fig. 9A,B). After the inferior lining of the cloacal chamber becomes smooth midway through the cloacal chamber, some anterior ventral glands secrete into this area, but ventral glands are replaced more posteriorly by vent glands (Fig. 10B,C). Distal ends of anterior ventral glands generally pass cranially. Histochemically, the secretion is like that of Kingsbury’s glands, a fibrous substance that is alcian blue+. Unlike Kingsbury’s glands, the lumina of anterior ventral glands contain copious amounts of secretory material. Epithelial cells generally are squamous, and the luminal borders also are alcian blue+. Amphiumids lack posterior ventral glands, which are known from all other families within the Salamandroidea.

Davison (1895) gave brief descriptions of the gross anatomy of the cloacae of male and female Amphiuma means and, after finding sperm in a “viscid substance” exuding from the vent, concluded that fertilization was internal, by cloacal apposition. His other findings were somewhat fanciful, as he described the cloacal walls as lined with “membranous laminae” (= rugae?) that connect to a series of “capillary tubes” around the cloacal orifice that “induce” spermatozoa into the female cloaca following apposition (Davison, 1895).

In A. tridactylum, Baker (1937) found that sex easily could be determined by examining the cloaca. The inside of the female’s cloaca is darkly pigmented with soft, somewhat folded walls, while males possess a white cloaca with a “small rather deep pocket in the dorsal roof. . . .” (Baker, 1937, p. 208).

Kreeger (1942) described the cloacal histology of female Amphiuma tridactylum. She found spermathecae around the dorsal and ventral portions of the cloacal tube and anterior cloacal chamber. Kreeger reported a small group of glands at the posterior end of the cloaca, which she thought were nonfunctional and possibly rudimentary “abdominal” glands. Sever’s (1992a) results confirm Kreeger’s observations on spermathecae, and the glands she called “abdominal glands” were recognized by Sever
(1992a) as female vent glands. As regards to the spermathecae, those of female amphiumids are similar in appearance and distribution to those found in most other members of the Salamandroidea with the notable exception of the Plethodontidae, in which all spermathecae unite with the cloacal wall by means of a common tube, an autapomorphic trait.

Female amphiumids are nearly unique, however, in the absence of anterior ventral glands and the presence of vent glands. The only groups of salamanders in which ventral glands are absent in females are the Sirenidae (in which all cloacal glands are absent), the Rhyacotritonidae, and the Amphiumidae. In addition, females of some species in each of the subfamilies of the Plethodontidae lack ventral glands. The absence of ventral glands is considered the ancestral condition for Sirenidae and a secondary loss when it occurs in other species (Sever, 1991a).

Female vent glands are similar in position and some aspects of cytology to the male vent glands in amphiumids and in other males of the Salamandroidea. The male vent glands show considerable variation among the many species in the suborder that have been examined, and a role for the glands in mating pheromone production has been postulated (Sever, 1988a, 2003). In some female salamanders, ventral glands have been implicated in mating pheromone production (Sever, 1988b). Since female amphiumids lack ventral glands, perhaps their vent glands assume the role of pheromone production, which is, as noted above, the hypothesized function for vent glands in males. The vent glands in female amphiumids, however, may be vestigial structures with no function in mating. The function of vent glands in female amphiumids awaits further study, but their occurrence could represent retention of a sympleiomorphic character for both sexes in a vestigial form.

Wilson (1941), in an intriguing abstract four sentences long, stated that he found five types of glands in male *A.tridactylum*, "anterior pelvic, middle pelvic, cloacal, abdominal and pit glands." He reported that he had studied both the development and seasonal variation of these glands. Unfortunately, Wilson never published his completed study, and Sever (1992a) could not locate his slides at Tulane University, where Wilson did the work. Sever (1992a) assumed that one of the groups of pelvic glands Wilson found are glands he recognized as Kingsbury’s glands, and the glands he called “abdominal glands,” Sever (1992b) referred to as vent glands. The homologies of Kingsbury’s glands and vent glands with glands of the same name in other salamanders are clear (Sever, 1991a), but the glands Wilson (1941) and Sever
(1992b) called “pit glands” are unique in amphiumids. Studies correlating gland secretion with spermatophore structure in amphiumids are necessary to see if the pit glands are involved in spermatophore formation or if they have another function, such as pheromone production.

The autapomorphic pit glands do not elucidate phyletic relations of amphiumids with other salamander families. The basal position of the Amphiumidae in their suborder resulting from analysis of cloacal characters is due to the lack of a definitive posterior ventral gland cluster like that known from other male salamanders in the Salamandroidea (Sever, 1991a). The place that the largest cluster of posterior ventral glands occurs in other salamanders is occupied largely by pit glands in amphiumas.

**Cloaca and Cloacal Glands of the Dicamptodontidae**

The Dicamptodontidae consists of four species of salamanders, *Dicamptodon aterimus*, *D. copei*, *D. ensatus*, and *D. tenebrosus* that occur along the Pacific coast of western North America. Paedomorphosis is common, especially in *D. copei*, which rarely undergoes full metamorphosis. Sever (1988a, 1992c) described cloacal anatomy in all species except *D. ensatus*. The cloacal anatomy is similar to that of the Ambystomatidae, and some workers consider *Dicamptodon* as a genus in that family (Frost et al. 2006).

Dicamptodontids have only one derived character in anatomy of the cloacal linings, the presence of primary and secondary folds in the male cloacal tube, a trait that is shared with male *Ambystoma* and *Rhyacotriton* (Sever, 1991a). Females possess simple spermathecae and ventral glands and lack other cloacal glands, all ancestral states for the Salamandroidea. Likewise, male cloacal glands present represent the ancestral state for the suborder and include dorsal and lateral pelvic glands, anterior and posterior ventral glands, Kingsbury’s glands, and the vent gland.

**Female cloacal anatomy.**—The CTL/TCL quotient is 0.21 in *Dicamptodon atterimus*, 0.39 in *D. tenebrosus*, and 0.24 in *D. copei*. The cloacal conformation of dicamptodontids is simple. Anteriorly, a dorsomedial fold appears subsequent to the junction of the urogenital ducts with the cloaca, and this fold continues until a widening of the dorsal portion in the posterior half of the cloacal tube (Fig. 11A,B,F). Additional folds appear in the dorsal roof of the anterior end of the cloacal chamber, and merge into a second dorsomedial fold that persists into the posterior end of the chamber (Fig. 11C). Thick dorsolateral
folds with a central groove like that found in females in the Ambystomatidae (the sister group of Dicamptodontidae) is absent. Laterally, the walls of the cloacal tube are rugose, and the rugae persist into the anterior half of the cloacal chamber (Fig. 11C). Ventrally, a medial evagination of the floor of the cloacal tube occurs just anterior to an abrupt junction with the cloacal chamber.

The epithelium is pseudostratified in the cloacal tube and is lightly ciliated. Except for the most anterior end, cilia are absent in the cloacal chamber. Posteriorly, the cloacal chamber simply decreases in height concordant with an increase in the extent of epidermis into the cloaca. The walls of the entire posterior fourth of the cloacal chamber are smooth and lined completely with epidermis.

Spermathecae and ventral glands occur in female *Dicamptodon*. The spermathecae are short, simple tubular glands that occur around the cloacal tube and around the dorsal and dorsolateral borders of the anterior cloacal chamber (Fig. 11A-C,E,F). The apical cytoplasm of the spermathecae of a *D. copei* containing spermatozoa is PAS+ and alcian blue+.

Ventral glands are numerous, elongate, thick, and convoluted. These glands secrete into the lateral borders of the cloacal chamber and, posterior to the spermathecae, into the dorsal portion of the cloacal chamber (Fig. 11C,D). Distal ends of the glands pass anteriorly, and those secreting into the dorsal portion of the posterior cloacal chamber lie superior to the most caudad spermathecae (Fig. 11C). The ventral glands have granules in the cytoplasm, and the luminal product usually appears as a large globule. The secretory products are PAS+ and ninhydrin Schiff+. 
Male cloacal anatomy.— Male *Dicamptodon* that have been examined are similar in cloacal anatomy to male *Ambystoma*. The anterior end of the cloacal tube is narrow dorsally and wide ventrally (Fig. 12A), and is lined with non-ciliated, pseudostratified epithelium. A ventral, ciliated evagination occurs
in the anterior fourth of the cloacal tube. Posteriorly, the floor of the cavity invaginates toward the cloacal orifice, with the walls composed of ciliated rugae that extend into the anterior third of the cloacal chamber (Fig. 12C,D). Concurrently, the dorsal portion of the cloacal tube widens, and two pairs of dorsolateral folds appear. These are the primary lateral folds (medially) and secondary lateral folds (Fig. 12B,C). At the posterior end of the cloacal tube, a middorsal papilla appears with a medial groove (Fig. 12C). At this point, the entire cavity is ciliated except for the medial groove, which is ciliated only at the inferior end, and the lateral borders of the secondary folds.

In the anterior third of the cloacal chamber, the primary and secondary lateral folds terminate, and the lateral walls of the dorsal papilla merge with the walls of the dorsal cloacal chamber as that chamber shortens posteriorly (Fig. 12D). The cloacal chamber widens ventrally in the anterior end, but this area narrows posteriorly concordant with the absence of rugae (Fig. 12E). In the posterior two-thirds of the cloacal chamber, cilia are lacking. The walls of the middle third of the cloacal chamber are papillose. The walls of the most posterior angle of the vent are smooth and lined with epidermis (Fig. 12F).

The basophilic glands present are Kingsbury's glands, anterior ventral glands, and posterior ventral glands. These glands contain alcian blue+ granular secretions. Kingsbury's glands secrete into the dorsal, narrowed portion of the cloacal tube (Fig. 12A) and onto the tips of the primary lateral folds. Kingsbury's gland is a relatively smaller cluster of tubules in *Dicamptodon* than in *Ambystoma*.

Ventral glands are responsible for the swelling of the cloacal region apparent in sexually active males. Anterior ventral glands form the large mass of glands surrounding the cloacal orifice, except for the posterolateral portions where posterior ventral glands occur (Fig. 12A-F). Anterior ventral glands secrete onto the tips of the rugae forming the lateral walls of the posterior cloacal tube and anterior cloacal chamber. Posterior ventral glands secrete into the dorsal end of the cloaca caudal to the medial papilla (Fig. 12D,E) and replace the anterior ventral glands caudad to the rugae (Fig. 12F). No glands secrete into the most posterior angle of the vent. Posterior ventral glands are larger in diameter and stain less intensely than anterior ventral glands. In addition to the basophilic fibrous secretion, posterior ventral glands possess eosinophilic globules in their lumina, especially in the more anterior and dorsal tubules.
Fig. 12. Transverse sections through the cloaca of a male *Dicamptodon copei*, stained with hematoxylin and eosin. Scale bar in lower right corner = 300 µm. A. Anterior end of the cloacal tube. B. Depression of the cloacal tube roof. C. Anterior end of the cloacal chamber. D. Anterior one-third of the cloacal chamber. E. Anterior two-thirds of the cloacal chamber. F. Posterior end of the cloacal chamber. Av, anterior ventral glands; Cc, cloacal chamber; Ct, cloacal tube; Dp, dorsal pelvic glands; PF, primary folds; Pv, posterior ventral glands; SF, secondary folds; Vg, vent glands. Modified from Sever, D. M. 1988a. Herpetologica 44:278, Fig. 3.
Eosinophilic glands with globular secretions are the dorsal pelvic, lateral pelvic, and vent glands. Dorsal pelvic glands and vent glands often contain some granular, basophilic secretion similar in appearance and staining to that of the ventral glands. The globular secretions of the eosinophilic glands are PAS+ and ninhydrin Schiff+, while granular material in the lumina and cytoplasm of dorsal pelvic glands and vent glands is alcian blue+. Dorsal pelvic glands secrete along the medial groove and superior portions of the cloacal chamber until they are replaced by posterior ventral glands in the caudal end. Posterior tubules possess more alcian blue+ granular secretion than anterior ones. Lateral pelvic glands occur between Kingsbury’s glands and anterior ventral glands (Fig. 12 C), secrete onto the secondary lateral folds, and are a relatively small cluster in comparison to many other salamandroids.

Vent glands are limited to several layers of thick, elongate tubules inferior to the ventral glands (Fig. 12A-F). Vent glands secrete along the edges of the cloacal orifice and distal ends pass laterally. Thus, the area of secretion of vent glands is along the cloacal orifice, except for the most posterior end.

**Sperm Storage in Female *Necturus beyeri***

Sever and Bart (1996) studied the ultrastructure of sperm storage in 17 specimens of *Necturus beyeri* collected 13 December 1993 to 8 January 1995 in southeastern Louisiana. Collections, however, were absent from the warm water months of June—November, when *N. beyeri* adults appear to leave smaller streams that were sampled. Nesting and oviposition occur in May. As reported by Sever (1992a) and reviewed earlier in the chapter, the spermathecae of female *N. beyeri* are simple tubular exocrine glands that opened onto the roof of the cloaca. Sperm occurred in the spermathecae of all specimens examined by Sever and Bart (1996). Sperm were not evenly distributed among the glands, however, and some glands or at least portions of some glands were devoid of sperm in each specimen.

When present in a gland, sperm are randomly oriented and clustered in the center of the lumen (Fig. 13). Sperm seem most numerous in those specimens collected in March (Fig. 13C). Many sperm still remain after oviposition, but the glands appear relatively reduced in diameter, especially those glands that lack sperm (Fig. 13D). In the latter glands, "light cells," whose ultrastructure is described below, are particularly conspicuous (Fig. 13D).
Fig. 13. Sections through the spermathecae of *Necturus beyeri*, stained with hematoxylin and eosin. A. TU 23402, a 110 mm SVL gravid specimen collected 13 Dec. B. TU 23407, a 145 mm SVL gravid specimen collected 2 Jan. C. TU 23412, a 127 mm SVL gravid specimen collected 13 March. D. TU 23417, a 117 mm SVL spent specimen collected 22 May. Ep, epithelial spermathecal cells; Lc, light cells; Sp, sperm in the lumen of spermathecae; Tp, tunica propria. Modified from Sever, D. M. and Bart, H. L. 1996. Copeia 1996:929, Fig. 1.
In paraffin sections, apical portions of the spermathecal epithelium are mainly PAS+, although some alcian blue+ areas occur as well. PAS+ substances also occur around clusters of sperm in the lumen. In plastic sections, the secretory material stains darkly with toluidine blue but is not metachromatic. The staining reactions are most intense in those specimens sacrificed prior to oviposition.

Transmission electron microscopy revealed two types of epithelial cells that are distinguished based upon the size of their secretory vacuoles and the electron density of their cytoplasm (Fig. 14A,B). In “dark cells,” the secretory vacuoles are generally 1-2 µm dia and consist of a central dense area (0.5-0.7 µm dia) surrounded by an outer, more lucent, flocculent material (Fig. 14B). Secretory vacuoles also are present in light cells, at least prior to oviposition, but these vacuoles are 0.5-1.0 µm dia, with a central dense material that usually is 0.3-0.5 µm dia (Fig. 14A). Light cells are interspersed among dark cells, and apical ends of both cell types abut on the luminal border. The intercellular canaliculi between light cells and dark cells are narrow and interdigitating, and desmosomes between the cell types are numerous (Fig. 14C).

The secretory vacuoles in the light and dark cells probably are responsible for the PAS+ reaction in the cytoplasm because the PAS+ reaction is most intense around the luminal border where the secretory vacuoles are most numerous. Therefore, release of the product likely is responsible for the PAS+ reaction around sperm in the lumen. Golgi bodies and rough endoplasmic reticulum (Rer) are associated with the vacuoles in the cytoplasm (Fig. 14C). A PAS+ reaction combined with these synthetic organelles indicates the product contains glycoproteins (Kiernan, 1990).
Fig. 14. Transmission electron micrographs through the spermathecae of TU 23403, a 106 mm SVL *Necturus beyeri* collected gravid on 13 Dec. A. Overview of a light cell and adjacent dark cell. B. A dark cell. C. Detail of secretory vacuoles and organelles involved in synthesis of product in a dark cell. Cv, condensing vacuole; Dc, dark cell; De, desmosome; Dm, dense material; Fm, flocculent material; Go, Golgi complexes; Ic, intercellular canalculi; Lc, light cell; Mf, microfilaments; Nu, nuclei; Rer, rough endoplasmic reticulum; Sv, secretory vacuoles. Modified from Sever, D. M. and Bart, H. L. 1996. Copeia 1996:930—931, Figs. 3A, 2B, 2C.
In specimens collected in May, shortly after oviposition, sperm still are numerous in some spermathecae (Fig. 13D) although other tubules appear empty (Fig. 15A). The luminal sperm appear normal in cytology. Instead of a wide supranuclear area filled with secretory vacuoles, the apical cytoplasm of specimens collected after oviposition contains relatively few vacuoles (Fig. 15B,C). Also, the secretory vacuoles appear more uniform in density (Fig. 15C) than those observed in specimens sacrificed prior to oviposition. Microvilli seemed relatively more elongate after oviposition (Fig. 15C).
Fig. 15. Light microscopy (A) and transmission electron micrographs (B—D) through the spermathecae of TU 23416, a 138 mm SVL *Necturus beyeri* collected spent on 22 Dec. A. Plastic semithin section stained with toluidine blue. B. Light cells. C. Luminal border of a light cell. D. Cytoplasm of a dark cell. Modified from Sever, D. M. and Bart, H. L. 1996. *Copeia* 1996:933, 935, Figs. 6A, 6B, 4C, 4D.

No evidence of spermiophagy by the spermathecal epithelium was noted, but structures consisting of concentric membranes are common especially basally in the cytoplasm of specimens sacrificed after oviposition (Fig. 15D). These concentric membrane structures often are associated with Golgi bodies (Fig. 15D). Intercellular canaliculi are wider basally following oviposition, and desmosomes occur frequently at the basal borders of adjacent cells. Basal globules that appear to be lipid droplets are sporadically found in individuals sacrificed after oviposition.

Light cells seem especially depleted after oviposition. Secretory vacuoles and organelles involved in synthetic activity are not noticeable in these light cells (Fig. 15B). The most conspicuous structures are small dense bodies (about 0.3 µm) that occasionally are found in the cytoplasm.

Secretory vacuoles in the plethodontid *Eurycea cirrigera* (Sever, 1991c, 1992d) and in the salamandrid *Notophthalmus viridescens* (Sever et al., 1996b) are of uniform density throughout the period of production. Secretory vacuoles consisting of a central dense area and a surrounding flocculent material have been noted in the salamandrid *Salamandrina terdigitata* (Brizzi et al. 1989, 1995) and in the ambystomatids *Ambystoma opacum* (Sever and Kloepfer, 1993) and *A. tigrinum* (Sever, 1995). In *S. terdigitata* and *A. opacum*, both the dense material and the flocculent material are exported into the lumen to bathe the sperm during storage (Brizzi et al., 1995; Sever and Kloepfer, 1993). In *A. tigrinum*, however, Sever (1995) believed that the flocculent material results from dissociation of the compacted granules, and the vacuole is then released into the lumen via exocytosis.

The process of release of the secretory material was not determined in *N. beyeri*, but the quantity of secretory vacuoles along the apical border decreased during sperm storage, and the release of the product probably is merocrine as reported in other salamanders (Brizzi et al., 1995; Sever, 1991a, 1995). The secretion bathed the sperm during storage in *N. beyeri*, as reported in salamandrids (Brizzi et al., 1995, Sever et al., 1996a) and in *A. opacum* (Sever and Kloepfer, 1993). Since sperm are inactive during
storage (Hardy and Dent, 1986), Sever and Kloepfer (1993) proposed that this type of secretion helps maintain the environment for sperm viability and quiescence. In A. tigrinum, however, the secretions are not released until oviposition and may serve to flush the sperm from the spermathecae (Sever, 1995).

*Necturus beyeri* can store sperm for at least six months prior to oviposition (Dec—May), and the secretions apparently are released slowly throughout the period of storage, since some secretory vacuoles still remained (at least in the dark cells) after oviposition. Thus, Sever and Bart (1996) hypothesized that the secretions provide the environment for keeping the sperm viable but inactive during long-term storage in *N. beyeri*.

Two distinct types of spermathecal epithelial cells, dark cells and light cells, occur in *Necturus beyeri*. Only one other study reported more than one type of epithelial cell in spermathecae of salamanders. Davitt and Larsen (1990), in an abstract, stated that an unspecified "plethodontid salamander" has five types of cells in its spermathecae. Their cell type II consists of an electron dense core surrounded by more lucent areas, much like the secretory vacuoles we found in *N. beyeri*. The other cell types found by Davitt and Larsen (1990) contain vacuoles that differ in size and shape as well as the density of the product, which is uniform in each cell type. Davitt and Larson (1990) proposed that some of the secretions are involved in sperm activation whereas others, including those in cell type II, help modulate continued alterations in spermathecal function during storage. In *N. beyeri*, both spermathecal cell types release their products into the lumen during sperm storage, and the main difference between the cell types is the size of the secretory vacuoles. Also, the light cells apparently are depleted before the dark cells.

The presence of sperm in all 17 females of *N. beyeri* examined by Sever and Bart (1996) raises the question regarding whether sperm storage can continue from one breeding season to the next, as suggested for some other salamanders (Baylis, 1939; Massey, 1990) but never substantiated (Sever, 2003). Shoop (1965) found that male *N. beyeri* collected during December and January from this population contain motile sperm in the Wolffian ducts, and several females collected in January have the remains of spermatophores in their cloacae. Shoop's observations provide additional evidence that females may store sperm up to 5–6 months prior to fertilization and oviposition.
Because of the absence of samples from June—November, the fate of sperm left in the spermathecae of *N. beyeri* in these months following oviposition is unknown. However, the spermathecae showed depletion of secretory product after oviposition. If the secretions do indeed help maintain the environment for sperm viability, the depletion of secretory product could limit the storage of remaining sperm during the summer and fall months.

In many species of salamanders that have been studied, remnant sperm are phagocytized, and often spermiophagy by the spermathecal epithelium occurs even prior to oviposition (Davitt and Larson, 1988; Sever, 1992c; Sever and Kloepfer, 1993). No evidence of spermiophagy was found in *N. beyeri*, a situation also noted in *Ambystoma tigrinum* (Sever, 1995) and *Amphiuma tridactylum*. Perhaps the sperm remaining in the spermathecae simply "leak out" gradually or are expelled by myoepithelial contractions, as reported for sperm remaining in the vas deferens of *Ambystoma macrodactylum* (Zalisko and Larsen, 1989).

**Sperm Storage in Female *Amphiuma tridactylum***

As noted earlier in this chapter, a number of reports exist on the reproductive biology of *Amphiuma*, but most of these are anecdotal and some are contradictory (see Table 1 in Sever et al., 1996a). The majority of the literature concerns *A. tridactylum*. Sperm in the female cloaca of this species were first observed by Davison (1895). The only report of mating behavior is an observation of copulation by cloacal apposition (Baker et al., 1947). Sperm storage glands, spermathecae, in the female cloaca were first described by Kreeger (1942), and Sever (1992a) extended her observations on spermathecae of *A. tridactylum* and described these glands in *A. means* and *A. phloleter*.

Kreeger (1942) stated that female *A. tridactylum* could store sperm in their spermathecae for 7 or 8 months, and Baker (1962) found that isolated females could retain living sperm for 15 months. In females of other salamanders, the longest period that Sever (2003) felt was reliable for duration of sperm storage was 6 months. Sever et al. (1996a) cytologically examined the spermathecae of ten female *A. tridactylum* sacrificed during the mating and ovipository period from March--August, and presented their findings on the ultrastructure of sperm storage in this species.
Sever et al. (1996a) also examined testes and ducts from males sacrificed in March, May, and October. Sperm occur in the vasa deferentia of all of these males, and sperm are relatively most numerous in vasa deferentia of specimens sacrificed in March. The testes of the specimen examined from October contain lobules full with spermatids and spermatozoa, whereas testes of the specimens from March contain few sperm and those of the specimen sacrificed in May are totally evacuated of sperm.

Fig. 16. Female *Amphiuma tridactylum*, 40.4 cm SVL, collected 10 April and sacrificed 24 April with 93 yolky ovarian oocytes 4.0 mm mean diameter. A. Light micrograph of a plastic semithin section stained
with toluidine blue showing overview of spermathecal tubules containing sperm and the surrounding connective tissue. B. Electron micrograph showing supranuclear cytoplasm with numerous secretory vacuoles. C. Electron micrograph showing detail of cytoplasm containing synthetic organelles and the relative density of secretory vacuoles. Cr, cristae; Cv, condensing vacuoles; Dm, dense material; Fm, flocculent material; Ic, intercellular canaliculi; Lu, lumen; Mf, microfilaments; Mi, mitochondria; Nu, epithelial cell nucleus; Po, polyribosomes; Se, spermathecal epithelium; Spl, sperm in the lumen; Sv, secretory vacuoles; Tu, tunica propria, Ve, vesicles. Modified from Sever, D. M. et al., 1996a. Journal of Morphology 230:84, Fig. 2.

Females containing vitellogenic follicles possess sperm in their spermathecae, and oocytes in specimens sacrificed March-May are smaller (3.7—4.7 mm) than in those sacrificed in August (5.8—6.0). Four of the 10 mature females examined by Sever (1996b) lack vitellogenic follicles. Two of these females, however, one sacrificed 14 March and the other sacrificed 22 August, contain sperm in their spermathecae.

As noted by Sever (1992a) and reviewed earlier in this chapter, the spermathecae are numerous, simple, tubular exocrine glands opening into the roof of the cloaca. Sperm are not present in all spermathecae observed in a given section and seem most abundant in the widened lumina of the alveolar end of the glands (Fig. 16A). The epithelial cells are cuboidal to columnar, and the largely euchromatic nuclei are basal and oriented to the long axes of the cells. Round to oval secretory vacuoles (0.3-0.7 km) are abundant in the apical cytoplasm, and intercellular canaliculi are narrow (Fig. 16B). Abundant vesicles, ribosomal complexes, microfilaments, and elongate mitochondria with tubular cristae are associated with secretory vacuoles in the perinuclear cytoplasm (Fig. 16C). Since alpha and beta particles of glycogen are absent, the secretory vacuoles are responsible for the PAS+ reaction in paraffin sections. The combination of ribosomal organelles responsible for peptide synthesis and a PAS+ reaction indicates that the product contains glycoproteins (Kiernan, 1990). No evidence of release of the secretory product occurs in specimens sacrificed March—May.

Inactive spermathecae are found in two apparently mature females (53.0 and 56.2 cm SVL) sacrificed 21 April and 5 May, which lack both large yolky ovarian oocytes and sperm in the
spermathecae (Fig. 17A). Spermathecae of these specimens possess heterochromatic nuclei and scant cytoplasm (Fig. 17B), and electron-dense secretory vacuoles are absent from the apical cytoplasm. The most abundant organelles in the cytoplasm are mitochondria, although scattered vesicles and areas rich in microfilaments also are numerous (Fig. 17C).

Fig. 17. Female *Amphiuma tridactylum*, 53.0 cm SVL, collected 10 April and sacrificed 21 April lacking enlarged yolky ovarian oocytes and possessing inactive spermathecae lacking sperm. A. Light micrograph of a plastic semithin section stained in toluidine blue showing overview of spermathecal tubules and surrounding connective tissue. B. Electron micrograph showing overview of perinuclear cytoplasm of a spermathecal epithelial cell. Cr, cristae; Cv, condensing vacuole; Ic, intercellular canaliculi;
As mentioned previously, two non-gravid females were found to contain stored sperm. The cytology of their spermathecal epithelium is the same as that of gravid females sacrificed in the same periods. Release of secretory product into the lumen is apparent in a nongravid female sacrificed 22 August (Fig. 18A). The release of secretory material involves the loss of apical cytoplasm, making the mode apocrine. Clusters of secretory granules, either free in the lumen or still surrounded by cell membranes, occur in lumina (Fig. 18A) The flocculent portion of the secretory vacuoles dissociates from the more electron-dense portion during apocrine release of the material. Apical areas of the spermathecal cytoplasm containing dissociated secretory vacuoles are separated from the nuclear regions of the cells by intracellular canaliculi that served as cleavage lines for the apocrine release of these portions of the cytoplasm.

When present, sperm occur in the lumen in small clusters in which sperm have similar orientations (Fig. 18B,C). These small clusters are often adjacent to other groups of sperm with different orientations. Portions of sperm cells are sometimes found associated with the apical epithelium or actually embedded in the apical cytoplasm (Fig. 19A,B). These sperm, however, usually appear normal in cytology.

In the nongravid specimen containing sperm that was sacrificed 22 August, however, degradation of luminal sperm is evidenced by distortion of the axial fiber and loss of electron density (Fig. 19C). In several instances, sperm are embedded in desquamated epithelial cells, and these cells, as indicated by vacuolated spaces as well as the distorted sperm contained within the epithelium, appear to be degenerating (Fig. 19C).
Fig. 18. Transmission electron micrographs. A. Female *Amphiuma tridactylum*, 51.0 cm SVL, collected between 9—13 May and sacrificed 22 August lacking enlarged oocytes. Apical cytoplasm and adjacent lumen showing apocrine mode of release of secretory vacuoles and secretory material. B.—C. Specimen 40.4 cm SVL collected 10 April and sacrificed 24 April with 93 yolky ovarian oocytes 4.0 mm mean diameter, showing portions of sperm cells in the lumen of a spermathecal tubule. Clusters of sperm cells have similar orientations. B. Mostly middle pieces of the tail. C. Primarily principal pieces of the tail. Acl, apical cytoplasm free in the lumen and containing secretory vacuoles; Dml, dense material free in the lumen; Eac, evagination of apical cytoplasm; Fml, flocculent material in the lumen; Lu, lumen; mpt, middle...
Fig. 19. Transmission electron micrographs. A—B. Female *Amphiuma tridactylum*, 59.6 cm SVL, collected 6 March and sacrificed 14 March, lacking enlarged yolk oocytes. A. Overview of spermathecal epithelium and adjacent luminal areas. B. Supranuclear cytoplasm of the spermathecal epithelium. C. Female *A. tridactylum*, 51.0 cm SVL, collected between 9—13 May and sacrificed 22 August lacking...
enlarged yolky oocytes. Degradation of sperm in spermathecal epithelium adjacent to the lumen. Aaf, abnormal axial fiber; Dmi, disrupted mitochondria from axial fiber; Ev endocytic vacuole; Lu, lumen; Mpt, middle piece of the tail; Mv, microvilli; Nu, nucleus of an epithelial cell; Ppt, principal piece of the tail; Se, spermathecal epithelium; Sn, sperm nuclei; Sv, secretory vacuoles, Va, vacuolated spaces. Modified from Sever, D. M. et al., 1996a. Journal of Morphology 230:88—89, Figs. 5C,D, 6C.

Due to the long period of vitellogenesis (September—May) and nesting period (June—November), *Amphiuma tridactylum* probably is unable to develop the follicles necessary for annual reproduction in Louisiana (Fontenot, 1990). Only 35-48% of adult females examined by various authors are gravid in any given year, leading to predictions of biennial (Wilson, 1942; Cagle, 1948) or even triennial (Fontenot, 1990) reproduction in females. Four of the 10 females examined by Sever et al. (1996a) were not gravid, lending support to the notion of a biennial or triennial female reproductive cycle.

Males, however, breed every year (Wilson, 1942). For *A. means* from Florida and *A. tridactylum* from Louisiana, Baker (1962) noted that mature sperm can be obtained from the testes October—April and from the sperm ducts November—May. In the study of Sever et al. (1996a), male *A. tridactylum* were sacrificed from October—May and sperm occur in the vasa deferentia of males in each sample. The testis are filled with mature and maturing sperm in October and are largely evacuated by March.

Kreeger (1942) found that female *Amphiuma tridactylum* collected and isolated in March and April still possess active sperm in their cloacae in April and December, respectively. Baker (1962) observed sperm in a female’s cloaca 15 months after complete isolation from males. Such females never lay eggs in captivity, and, if eggs are present, they are probably resorbed (Baker, 1962). Pituitary injections generally have been ineffective in inducing oviposition in *Amphiuma* (Baker, 1937; Kammeraad, 1942), although one female *A. tridactylum* was induced to lay 49 eggs following intramuscular injection of bovine pituitary (Kammeraad, 1942). Baker (1945) stated that 31% of those eggs were fertile, but this information is not in the original paper (Kammeraad, 1942). Finally, we should note Dundee and Rossman’s (1989) report that Percy Viosca, an accurate chronicler of natural history in Louisiana, wrote in his field notes that a female *A. tridactylum* collected April-May 1937 contained “two quarts of eggs with active embryos,” which implies ovoviviparity.
The number of large, yolky oocytes in gravid females of *Amphiuma tridactylum* examined by Sever et al. (1996a) was 93-269, with the smallest oocytes in a female sacrificed 3 March (3.7 mm mean diameter) and the largest in females collected in spring and kept in the cattle tanks and sacrificed on 22 August (5.8-6.0 mm mean diameter). All of the gravid females possess sperm in their spermathecae. These results are consistent with reports of Wilson (1940) and Rose (1967) that vitellogenesis begins in late fall and winter and with findings by Wilson (1940, 1941), Cagle (1948), and Rose (1967) that mating occurs winter and spring. Despite the apparent seasonality of mating activity, the capability for mating exists over a longer period, because, as noted above, males have sperm in their vasa deferentia at least from October—May.

Two of the nongravid females contain sperm in their spermathecae. Either these individuals stored sperm from a previous breeding season when they were gravid, or mating had occurred in the current breeding season even though the females were not vitellogenic. These alternatives cannot be resolved based upon available data. However, no differences occur in the cytology of the spermathecae and stored sperm between gravid and nongravid individuals. It is interesting to note that Sever et al. (1996b) observed hypertrophy of the spermathecal epithelium in a nonvitellogenic female *Notophthalmus viridescens* during the breeding season similar to the extent found in gravid females. The coupling among vitellogenesis, hypertrophy of secondary sexual characters (including the spermathecae), and mating behavior in salamanders needs further study.

At least some of the sperm in the gravid and nongravid females sacrificed 22 August were degenerating, and further production of secretory vacuoles was not occurring. Sever et al. (1996a) proposed that the spermathecal secretions of *Amphiuma tridactylum* function in sperm degeneration, because release was only observed in August and was often associated with distorted sperm. Sperm storage between breeding seasons has not been found in any salamander in which the annual cycle of sperm storage has been studied (Sever, 2003). The total length of time over which *Amphiuma tridactylum* can store viable sperm was not determined by Sever et al. (1996a) or in any previous study. The data indicate, however, that the potential for long-term sperm storage is present, perhaps as long or longer than the 15 months suggested by Baker (1962).

**Discussion**
Male Cloacal Glands

The absence of male cloacal glands in sirens indicates that they are unable to form spermatophores characteristic of the families in the Salamandroidea. If the sister taxon relationship proposed by Frost et al. (2006) between Sirenidae and Proteidae is accepted, this means that either cloacal glands were lost in sirens or that proteids independently evolved cloacal glands similar in overall histology and organization to other spermatophore producing families. Neither of these two scenarios are parsimonious to a hypothesis of a single origin of spermatophore producing glands in the ancestor of a Salamandroidea in which sirens are excluded and proteids are included with other families that produce spermatophores. If we accept the traditional view that sirens represent the most basal family of salamanders (Zhang and Wake, 2009), then the ancestral state for the Urodela is the absence of cloacal glands (Sever, 1991a).

Cryptobranchidae is considered by many workers, including Frost et al. (2006) and Zhang and Wake, 2009), to be the sister taxon of Hynobiidae. Males of neither cryptobranchids nor hynoiids produce spermatophores, and possess only one type of cloacal gland, the anterior ventral gland, with the exception of the hynobiid *Onychodactylus japonicus*, which possesses an autapomorphic variety of glands (Sever, 1991b). The ventral glands of male *Cryptobranchus*, however, do not secrete into the cloaca, but onto the surface of the cloacal lips and into the groove lateral to the cloacal lips along the anterior three-fifths of the cloaca. In many hynobiids examined by Sever (1991b), ventral glands secrete not only onto epidermal areas along the cloacal orifice but also onto the dorsal and lateral walls of the cloaca. Sever (1988b) hypothesized that the ancestral function of the anterior ventral gland is secretion of a pheromone.

The ancestor of the cryptobranchids and hynobiids is the sister taxon of the ancestor of the Salamandroidea, the spermatophore producing families (Zhang and Wake, 2009). Possession of anterior ventral glands is a synapomorphy for the common ancestor of these two clades (Sever, 1991a).

The spermatophore producing glands in the seven spermatophore producing families are easily recognized, although some variation exists. For example, amphiumids lack lateral pelvic glands and posterior ventral glands present in all other salamandroid families. The area is occupied by posterior ventral glands in other families, however, is filled in amphiumids by autapomorphic pit glands. Males in
the family Plethodontidae possess a easily distinguished caudal pelvic gland cluster that is absent in other families. Perhaps the most variation occurs in dorsal and vent glands, glands that secrete into the posterior angle of the cloaca. In the Salamandridae, these glands are highly elaborated and often extend from their posterior secretory pores dorsal to other cloacal glands, even into the posterior trunk, hence the name dorsal glands is used in this family. In other salamandroid families, including those of the giant salamanders examined herein, the glands are more reduced and limited to epidermal areas adjacent to the posterior end of the vent, so they are called vent glands.

Variation also occurs in cloacal conformation and histology of the linings, as noted in this chapter for the Proteidae, Amphiumidae, and Dicamptodontidae. Of special note is the observation that dicamptodontids have primary lateral folds, on which Kingsbury’s glands secrete, and secondary lateral folds, on which lateral pelvic glands secrete. This character is shared with males in the Ambystomatidae and Rhyacotritonidae (Sever, 1992c). Rhyacotritonids, however, have other characters in both males and females that readily distinguish them from dicamptodontids and ambystomatids. For example, male rhyacotritonids have relatively large vent gland clusters that secrete into pores that open on the epidermis of lobes lateral to the cloaca (Sever, 1988a, 1992c). Sever (1992c) concluded that analysis of cloacal anatomy supports other data indicating Ambystoma and Dicamptodon are sister groups and that Rhyacotriton is not closely related to either of the other two genera.

Frost et al. (2006) and Zhang and Wake (2009) both recognized that Ambystoma and Dicamptodon are sister taxa. Frost et al. (2006) proposed that they be placed in a single family, Ambystomatidae, to remove the redundancy of having two family-group names, each containing a single genus. Zhang and Wake (2009), however, proposed retention of the two family names because Dicamptodon has a long fossil history, differs from Ambystoma in easily visible features of morphology, and has perennial stream-adapted larvae rather than the short-lived pond larvae characteristic of non-neotenic Ambystoma.

Female Cloacal Anatomy

Females in the Sirenidae, like males, lack cloacal glands and thus do not possess sperm storage glands found in the Salamandroidea. Sever et al. (1996c) believed that sirens undergo external fertilization and that is the ancestral state for salamanders. Females of the Cryptobranchidae and Hynobiidae, like males in these two families, have anterior ventral glands, making possession of such
glands the ancestral condition for females in ancestor of their clade and the Salamandroidea. The ventral glands in female *Cryptobranchus alleganiensis* are not as numerous or hypertrophied as in males, but they are more extensive in females, secreting along the entire border of the cloacal orifice rather than just the anterior three-fifths as in males.

Females of all seven families in the Salamandroidea, including those giant salamanders in the Proteidae, Amphiumidae and Dicamptodontidae, possess sperm storage glands called spermathecae in the roof of the cloaca. In the giant salamanders these sperm storage glands are similar, being composed of simple tubular glands. As far as other glands are concerned, female amphiumids differ from females in other families by lacking ventral glands (a secondary loss) and possession of glands that resemble male vent glands, although the homology is unsure (Sever, 1992a). Similar vestigial vent glands are known in several species in the Salamandridae, an co-occurrence that remains unresolved (Sever, 1991a) but is likely convergent. Other differences among females in these three families involve what appear to be minor species specific differences in cloacal conformation and histology of linings.

**Ultrastructure of Female Sperm Storage**

Although ultrastructural studies exist on sperm storage in female *Necturus beyeri* (Proteidae; Sever and Bart, 1996) and *Amphiuma tridactylum* (Amphiumidae; Sever et al., 1996a), none have been done on a representative of the Dicamptodontidae. Studies exist, however, on females of *Ambystoma opacum* (Sever and Kloepfer, 1993; Sever et al., 1995) and *A. tigrinum* (Sever, 1995) of the sister taxon, Ambystomatidae. It will be interesting to see how closely *Dicamptodon* resembles *Ambystoma* in spermathecal cytology, considering how similar they are at the light microscopy level.

Sever and Brizzi (1998) found that *Necturus beyeri* and *Amphiuma tridactylum* share 15 character states for spermathecal characters. Differences include three autapomorphies for *A. tridactylum*: secretion during luminal degeneration of sperm, apocrine mode of secretion, and desquamation of the apical cytoplasm (as a consequence of apocrine secretion). Females of *N. beyeri* differ from those of *A. tridactylum* by having six months of effective sperm storage rather than 4-5 months observed in *A. tridactylum* and by an autapomorphy for *N. beyeri*, the possession of two types of epithelial epithelial cells.

**Conclusion**
I shall end with a quote from George Gaylord Simpson (1969): “In spite of omitting so much, this paper has grown unduly long. Brevity here suggests a single conclusion.” Differences and similarities in cloacal anatomy and ultrastructure of sperm storage are related to phylogeny and not the relatively large body size obtained by a group of divergent salamanders.

**Literature Cited**


the spermathecal epithelium of the female salamander *Rhyacotriton olympicus* Amer Zool 28:30A.


Rose FL. 1967. Seasonal changes in lipid levels of the salamander *Amphiuma means*. Copeia


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