Comparative Anatomy and Phylogeny of the Cloacae of Salamanders (Amphibia: Caudata). III. Amphiumidae

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ABSTRACT
Histological descriptions of female and male cloacae are presented for the three species of *Amphiuma*. Little interspecific variation occurs in either females or males. Females possess numerous spermathecae around the walls of the posterior cloacal tube and anterior cloacal chamber as well as rudimentary vent glands, of uncertain homology, in the caudal end of the cloacal chamber. Ventral glands such as those known from female salamanders in other families are absent, likely a secondary loss in the amphiumid clade. In a female *A. tridactylum* with large ovarian follicles (5- to 8-mm diameter), the lips of the cloacal orifice are highly cornified, and stored sperm in the spermathecae appear in organized bundles, either free in the lumen or with the sperm heads seemingly buried in the spermathecal epithelium. Males possess some gland clusters present in other members of the suborder Salamandroidea (dorsal pelvic glands, vent glands, Kingsbury’s glands, anterior ventral glands) but lack others (lateral pelvic glands, posterior ventral glands), resulting in the most plesiomorphic cloacal anatomy in the suborder. However, males possess an autapomorphic gland cluster, called the pit glands, in the posterior wall of the cloacal chamber. The pit glands consist of shallow infoldings of the cloacal epithelium unlike other cloacal glands, which are relatively tubular and elongate. The “pits” contain columnar eosinophilic cells that give positive staining reactions for acid mucopolysaccharides and proteins.

The salamander family Amphiumidae consists of three species of *Amphiuma* found in the southeastern United States. Amphiumas are elongate salamanders with reduced limbs, and adults retain one external gill slit, a paedomorphic character (Duellman and Trueb, '86). Individuals of two species, *Amphiuma means* and *A. tridactylum*, are large, often in excess of 70-cm snout-vent length (SVL), and are aquatic, while *A. pholeter* is a dwarf species, 20- to 25-cm SVL, that usually is found burrowing in mucky banks (Conant, '75).

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 ('42) provided a histological description of the cloaca of female *A. tridactylum*, but the only such account for male amphiumids is an abstract by Wilson ('41) on the same species. Sever ('91) 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. This paper presents histological descriptions of amphiumid cloacae in order to extend the observations of Wilson ('41) and Kreeger ('42) and to provide the anatomical basis for the character states used by Sever ('91).

MATERIALS AND METHODS

SVL refers to the distance from the tip of the snout to the posterior end of the vent. Specimens, as far as known, were initially preserved in 10% formalin, and some were stored in isopropanol or ethanol of varying concentrations before cloacae were excised for histological preparation. Specimens used are in the collections of the University of Michigan Museum of Zoology (UMMZ), Florida Museum of Natural History (FM), or in my possession (DMS). The following specimens were examined histologically: *Amphi-*
with diagnostic stains using procedures described by Humason ('79). Slides from female A. pholeter and one female A. tridactylum (UMMZ 187364) were stained with the ninhydrin-Schiff reaction/fast green (proteins), periodic acid-Schiff reagent/fast green FCF (PAS, neutral carbohydrates), and alcian blue/nuclear fast red at pH 2.5 (acid mucopolysaccharides). The cloacal sections of male A. pholeter were not subjected to diagnostic staining, but some from male A. means were stained with alcian blue. Sections from one male A. tridactylum (UMMZ 187363) were treated with PAS and alcian blue, and sections from another (DMS 4103) were treated with PAS, alcian blue, and ninhydrin Schiff. No tests for lipids were done since some reagents used for paraffin embedding remove lipids. Secretory products with a stringy appearance were considered "fibrous" while spheroidal secretory droplets were called "granular," if uniformly small, or "globular," if composed of coalesced granules.

Three-dimensional reconstructions of cloacae of female and male Amphiuma pholeter were done using PC3D software (Jandel Scientific, Corte Madera, California) and a Jandel digitizing tablet with a Zenith ZF-248 microcomputer. Every sixth (female) or tenth (male) transverse section was digitized for image reconstruction. Right lateral views were reconstructed by computer software, with the sections rotated 25° clockwise. The cloacal displays were stretched appropriately in the anteroposterior direction to help alleviate geometric distortions caused by skipping sections between those digitized. Each digitized section of cloacal glands was repeated once so that gaps between displayed sections were more nearly closed. Finally, alternate digitized sections of the cloacal walls were displayed to improve clarity.

Following Sever ('78), the cloaca is divided into the cloacal tube and the cloacal chamber. The cloacal tube is cylindrical and extends caudally to the cavity dorsal to the vent, the cloacal chamber. Relative length of the cloacal tube (cloacal tube length/total cloacal length = CTL/TCL elsewhere) may have phyletic significance (Sever, '91). To determine lengths, the number of transverse sections of the cloacal tube and cloacal chamber were counted. The most anterior section of the cloacal tube was considered the one immediately posterior to the junction of the Wolffian ducts with the hindgut.

RESULTS

Female cloacal anatomy

One female Amphiuma tridactylum (UMMZ 187364) has sperm in the spermathecae and enlarged ovarian follicles (5–8 mm in diameter). The other A. tridactylum and the A. means lack sperm in their spermathecae and possess small ovarian follicles (<1 mm in diameter). Spermathecae are well developed but devoid of sperm in the A. pholeter examined. Three-dimensional reconstructions through the cloaca of A. pholeter are shown in Figure 1, and transverse sections through cloacae of A. pholeter and A. tridactylum are illustrated in Figure 2.

Cloacal conformation and anatomy of the linings

The CTL/TCL quotient is 0.41 in A. means and 0.49 in A. pholeter. Some anterior and/or posterior sections are lacking in the Amphiuma tridactylum examined; thus the CTL/TCL could not be determined for that species.

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, while 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 (Figs. 1A, 2A–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. 2B,C). The epidermal lining gradually spreads dorsal, replacing the pseudostratified lining. In *Amphiuma phol-eter*, the entire caudal three-fourths of the cloacal chamber is lined with epidermis (Fig. 2D,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. The lining of the cloacal orifice (cloacal “lips”) in the *A. tridactylum* with enlarged ovarian follicles and spermatozoa in the spermathecae is especially thick and cornified (Fig. 2G).

**Cloacal glands**

Spermathecae and vent glands occur in all specimens examined. In the *Amphiuma tridactylum* with large ovarian follicles, spermatozoa occur in short, 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. Anteriorly, the first spermathecae appear dorsomedially, and more posteriorly they spread around the dorsal and lateral walls of the cloaca (Figs. 1B, 2A–D). In one *A. tridactylum* (DMS 16111) and in the *A. means* examined, some glands appear around the inferior border of the cloacal tube as well (the “ventral group” of spermathecae described by Kreeger, '42). The most posterior spermathecae are dorsomedial and, in *A. phol-eter*, pass into portions of the cloacal chamber where the lining has become epidermal (Fig. 2D).

Spermathecal cytoplasm is scant and the cells squamous in *Amphiuma means* and in the *A. tridactylum* lacking stored sperm. The spermathecal epithelium is eosinophilic and cuboidal in *A. phol-eter* and in the *A. tridactylum* containing sperm. In *A. phol-eter*, the spermathecae contain some small granules that are PAS+ and ninhydrin Schiff+. The apical cytoplasm of spermathecae is PAS+ in the individual of *A. tridactylum* with sperm. As illustrated by Kreeger ('42) and Sever ('87), 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 while others seem to have their heads embedded in the spermathecal cytoplasm. Light microscopy does not provide the necessary resolution to determine whether sperm–epithelial contacts are coincidental, or if embedded sperm are receiving nourishment or undergoing degradation.

A separate group of glands occurs at the posterior end of the cloaca in female *Amphi-uma* (Figs. 1B, 2E–F). These glands do not contain sperm in the *A. tridactylum* with sperm in the spermathecae and are cytologically different from spermathecae in all spec-
Fig. 2. Transverse sections through the cloaca of female *Amphiuma pholeter* (A–E) and *A. tridactylum* (F). A. Middle of 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 orifice. Bv, blood vessels; Ce, cornified epidermis of the cloacal lips; Ep, epidermal lining of the cloacal chamber; Ng, neural ganglion. Other labels as for Figure 1. Scale bar in lower right corner = 250 µm in A, B, C, D, 200 µm in E, and 500 µm in F.

imens. This posterior group of glands is similar in appearance and position to the vent glands found of male amphiumids (as described below) and males of many other members of the suborder Salamandroidea (Sever, '91). 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 (Fig. 1B). In *Amphiuma pholeter*, these short, tubular glands secrete only onto cornified epithelium (Fig. 2E), but in *A. means* and *A. tridactylum*, they secrete onto simple columnar epithelium as well as the cornified linings of the cloaca. In the *A. tridactylum* containing stored 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.
**Fig. 3.** Three-dimensional reconstruction of the cloaca of male *Amphiuma pholeter*. Lateral view, with the anterior end (toward the right) rotated clockwise 25°. A. Dorsal pelvic glands. B. Anterior ventral glands. C. Kingsbury's and vent glands. D. Pit glands. The distance between sections of the cloacal wall represents 200 μm. Av, anterior ventral glands; Dp, dorsal pelvic glands; Kg, Kingsbury's glands; Pi, pit glands. Other labels as for Figure 1.

**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. Three-dimensional reconstructions through the cloaca of male *A. pholeter* are shown in Figure 3, and transverse sections through the cloaca of this individual are illustrated in Figures 4 and 5. The cloacal glands in one individual of *A. tridactylum* (UMMZ 187366) possess scant cytoplasm, small lumina, and no apparent secretory activity, but those of the other specimens examined appear hypertrophied and contain secretory material.

Cloacal conformation and anatomy of the linings

The CTL/TCL quotient is 0.35 in *Amphiuma pholeter* and 0.16 in one *A. tridactylum* (UMMZ 187366). Sagittal sections were cut for *A. means,* and, thus, a CTL/TCL quotient could not be determined. 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 (Figs. 3, 4A–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 (Figs. 3, 4C–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.

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 bears unciliated papillae (Figs. 3, 4B–C). In the anterior end of the cloacal chamber, the dorsal portion narrows and a dorsal invagina-
tion occurs into the roof, resulting in the dorsal portion becoming a narrow slit lined with unciliated papillae (Figs. 3, 4D). 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 (Figs. 3D, 5A). 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. 5).

The most anterior pit glands are middorsal (Figs. 3D, 5A). Posteriorly, pit glands extend ventrally until they occupy the entire lining of the cloacal chamber except for the area just superior to the cloacal orifice (Figs. 3D, 5B,C). The caudal end of the cloacal chamber is abrupt (Fig. 3). Distal portions of pit glands extend beyond the posterior end of the cloacal orifice (Figs. 3D, 5D).

Cloacal glands

The pit glands are eosinophilic with columnar cells (Fig. 5). In the apical cytoplasm, an alcin blue+ fibrous material and numerous granules that are PAS+ and ninhydrin Schiff+ occur. The luminal secretion product appears as a mixture of alcin 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, '91) are not present.

Dorsal pelvic glands secrete onto the unciliated papillae of the roof of the posterior cloacal tube and anterior cloacal chamber (Figs. 3A, 4C,D, 5A). 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. 4A,B, 5B). The epithelium generally is cuboidal and, like that of pit glands, contains alcin blue+ fibrous material and granules that are PAS+.

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Fig. 4. Transverse sections through the cloaca of male *Amphiuma pholetar*. 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. Labels same as for Figures 1 and 3. Scale bar in lower right corner = 250 μm.
Fig. 5. Transverse sections through the cloaca of male Amphiuma, continued. A. Midpoint of the cloacal chamber. B. Posterior three fourths of the cloacal chamber. C. Posterior end of the cloacal chamber. D. Posterior to the cloacal orifice. Labels same as for Figures 1 and 3. Scale bar in lower right corner = 250 μm.

and ninhydrin Schiff+. The lumen contains a mixture of these granules in a matrix of the fibrous material, and the amount of secretory product in the lumen is greater 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 (Figs. 3C, 5C,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 (Figs. 3, 4A,B). Dorsal pelvic glands replace medial Kingsbury’s glands in the posterior end of the cloacal tube. Kingsbury’s glands have a fibrous secretion that is alcian blue+. This material is scant in 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 (Figs. 3B, 4, 5A,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. 5B,C). Distal ends of anterior ventral glands generally pass cranially (Fig. 3B). Histochemically, the secretion is like that of Kingsbury’s glands, a fibrous substance that is alcian blue+. Unlike Kings-
bury’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 +.

DISCUSSION

The ancestral condition in all salamanders except the Sirenidae (which lack cloacal glands) is the possession of at least one type of cloacal gland (Sever, ’91). In the clade leading to extant salamanders in the suborder Salamandroidea, parts of the ancestral cloacal gland mass are modified for production of spermatophores (males) or storage of sperm (females). Other regions of cloacal glands in both sexes may secrete mating pheromones, representing, perhaps, retention of the ancestral function for cloacal glands (Sever, ’91). Homologies of cloacal gland divisions in different groups of salamanders are determined by comparative morphology (e.g., position of glands in relation to regions of the cloacal cavities, cytology, staining reactions) and/or functions of glands in sperm storage, spermatophore formation, or pheromone production.

Although more work needs to be done on the functions of amphiumid cloacal glands, my results provide the morphological basis for recognition of homologous and autapomorphic cloacal structures in amphiumids. Previous studies, especially on males, lacked histological detail, failed to recognize the comparative value of some characters, or misinterpreted homologies.

Davison (1895) gave brief descriptions of the gross anatomy of the cloaca 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 (’37) 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, ’37, p. 208).

Kreeger (’42) 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. My results confirm Kreeger’s observations on spermathecae, and the glands she called “abdominal glands” are here referred to as female vent glands.

As regards 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 Rhacotritoninae of the Dicamptodontidae, 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, ’91).

Ventral glands in female salamanders usually are limited to the anterior end of the cloacal chamber, so the female vent glands of *Amphiuma* are not equivalent in position to ventral glands. Female amphiumid vent glands are not found in the area where “dorsal glands” have been described from female ambystomatid and plethodontid salamanders either. *Pleurodeles waltlii* and *Tylototriton verrucosus* of the Salamandridae possess some small, tubular glands in the posterior angle of the vent, although Sever (’91) could not ascertain whether these are homologous to vent glands of female amphiumids.

These divisions (ventral glands/vent glands) are derived from the same ancestral cloacal gland mass (Sever, ’91). Thus, the anterior ventral portion of the cloacal gland mass was lost in female amphiumids rather than the posterior ventral portion as in the ancestors to other salamander families in the Salamandroidea.

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, '88a). In some female salamanders, ventral glands have been implicated in mating pheromone production (Sever, '88b). 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 symplesiomorphic character for both sexes in a vestigial form.

As far as cloacal conformation and linings are concerned, female amphiumids show few specializations. Female amphiumids possess relatively long cloacal tubes (CTL/TL > 0.05) and cilia on the cloacal linings, both considered ancestral characters by Sever ('91). The extent of epidermis into the cloaca shows two states in female amphiumids. The ancestral condition is that a completely epidermal lining of the cloacal walls occurs only in the posterior half of the cloacal chamber (Amphiuma means, A. tridactylum), and in the derived condition, such a lining extends into the anterior half of the cloacal chamber (A. pholeter). Sever ('91) regarded the condition in female A. pholeter as an independently derived character within the Amphiumidae. This character also occurs in one genus in the Salamandridae, Euproctus, which has a specialized cloacal tube that again is most likely independently derived. The derived condition, however, is a synapomorphy for Rhacotritoninae-Plethodontidae-Proteidae (Sever, '91).

Male amphiumid cloacal anatomy previously had not been described in detail. Baker ('37), examining Amphiuma tridactylum, noted that males have cloacal glands in "oval, slightly rough patches having a white background with dotted lines of pigment" which are more numerous at the base and along the sides of the oval patches. Wilson ('41), 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 I could not locate his slides at Tulane University, where Wilson did the work. I assume that one of the groups of pelvic glands Wilson found are glands I recognize as Kingsbury's glands, and the glands he called "abdominal glands," I refer 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, '91), but the glands I believe Wilson was referring to as "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, '91). The place that the largest cluster of posterior ventral glands occurs in other salamanders is occupied largely by pit glands in amphiumas.

In their phylogeny incorporating data from comparisons of ribosomal RNA segments, Larson and Wilson ('89) found that the Plethodontidae is the sister group of all other salamander families and that among the taxa examined the tree topology is: Amphiuma (Andrias (Rhacotriton (Notophthalmus (Necturus (Ambystoma))))). Since among these genera, only Andrias possesses external fertilization (and reduction of cloacal glands to just the anterior ventral gland cluster), this tree implies that internal fertilization evolved at least twice, or a reversal to external fertilization occurred in Andrias. Morphological data do not support such a dichotomy between plethodontids and other salamanders nor the placement of Andrias (Sever, '91). It is interesting, however, that Larson and Wilson ('89) found Amphiuma the basal taxon in its clade, since the cloacal characters reveal that amphiumids have the most plesiomorphic cloacal anatomy of any of the Salamandroidea.

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**LITERATURE CITED**


