

Comparative Anatomy and Phylogeny of the Cloacae of Salamanders (Amphibia: Caudata). VI. Ambystomatidae and Dicamptodontidae

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ABSTRACT Histology of the cloacae of *Rhyacotriton olympicus* and representative species from the genera *Ambystoma* and *Dicamptodon* was examined by light microscopy. Females of *Ambystoma* possess sperm storage glands, the spermathecae, as well as ventral glands and dorsal glands, both of uncertain function. Females of *Ambystoma* examined from the subgenus *Linguaelapsus* differ from those in the subgenus *Ambystoma* by possessing more extensive ventral gland clusters and a shorter cloacal tube. Females of *Dicamptodon* possess spermathecae and ventral glands, but differ in cloacal conformation from females of *Ambystoma* and lack the dorsal glands. Females of *R. olympicus* possess more extensive epidermal lining in the cloaca than that found in females of *Ambystoma* and *Dicamptodon*, and the only glands present are spermathecae, which cluster around a tube in the dorsal roof. Males of *Ambystoma*, *Dicamptodon*, and *R. olympicus* possess five types of cloacal glands (dorsal pelvic glands, lateral pelvic glands, anterior ventral glands, posterior ventral glands, and Kingsbury's glands) that function in spermatophore formation, and vent glands that may produce a courtship pheromone. In *Ambystoma* and *Dicamptodon*, vent glands secrete along the medial borders of the cloacal orifice. Males of *A. opacum* and *A. talpoideum* differ from males of other species examined from the two genera by possessing more extensive vent glands. Males of *R. olympicus* possess unique vent glands in which tubules secrete onto the surface of vent lobes lateral to the posterior end of the cloacal orifice, and distal ends of the glands pass anteriorly, superficial to the fascia enclosing the other cloacal glands. The results from analysis of cloacal anatomy support other data indicating that *Ambystoma* and *Dicamptodon* are sister groups, and that *Rhyacotriton olympicus* is not closely related to either of the other two genera and merits placement in a separate family. © 1992 Wiley-Liss, Inc.

This paper reviews existing literature and presents additional descriptions of cloacal anatomy for the North American salamander families Ambystomatidae, containing the genera *Ambystoma* (27 species) and *Rhyacosiredon* (4 species), and Dicamptodontidae, consisting of *Dicamptodon* (4 species) and monotypic *Rhyacotriton* (Frost, '85). Descriptions exist for the male cloacal anatomy of *Dicamptodon tenebrosus* and *Rhyacotriton olympicus* (Sever, '88a) and for eight species of *Ambystoma* (Sever, '81, '87, '88a; Sever et al., '89; Licht and Sever, '91). Descriptions of female cloacal anatomy, however, are limited to brief reports on *A. tremblayi* and *R. olympicus* (Sever, '87) and more detailed studies on *A. maculatum* (Kingsbury, 1895) and *A. gracile* (Licht and Sever, '91). Duellman and

Trueb ('86) illustrate the external appearance of the cloacal region of male and female *A. jeffersonianum* in breeding condition, and the relationships of the cloacal cavities to urogenital and digestive structures of salamanders are shown by Sever ('92b). In the present paper, the female cloacal anatomy of three species of *Dicamptodon* and seven species of *Ambystoma* is described for the first time. This paper is the sixth in a series on the comparative anatomy and evolution of the cloacal region of salamanders (Sever, '91a,b; '92a–c).

MATERIALS AND METHODS

Snout-vent length (SVL) refers to the distance from the tip of the snout to the posterior edge of the vent. Cloacal anatomy was

examined following excision of the cloacal region and its histological preparation for light microscopy. Specimens examined (Appendix I) were initially preserved in 10% neutral-buffered formalin, and were stored in 60% isopropanol or 65% ethanol before removal of cloacal tissue.

Cloacal tissue was rinsed in water, dehydrated in ethanol, cleared in toluene or Histo-sol (National Diagnostics, Inc., Manville, New Jersey), and embedded in paraffin; 10- μ m sections were then cut with a rotary microtome. Some sections from each species were stained with hematoxylin-eosin (for general cytology), and others were stained or treated with Mallory's triple stain (connective tissue), ninhydrin Schiff/fast green (proteins), periodic acid-Schiff's (PAS) reagent/fast green FCF (neutral carbohydrates), and/or alcian blue/nuclear fast red at pH 2.5, or toluidine blue (acid mucopolysaccharides). Staining procedures followed Humason ('79).

Three-dimensional reconstructions were performed on transverse sections through the cloacae of a male *Ambystoma laterale*, and male and female *A. opacum*, *Dicamptodon copei*, and *Rhyacotriton olympicus*. These reconstructions were done using PC3D software (Jandel Scientific, Corte Madera, CA) and a Jandel digitizing tablet with a Zenith ZF-248 microcomputer. Every 4th to 15th section was digitized for image reconstruction following procedures outlined by Sever ('91a).

Sever ('91a) used a ratio of cloacal tube length divided by total cloacal length (CTL/TCL) to express the relative size of the cloacal tube. The cloacal tube is cylindrical and extends caudally to the cloacal chamber, a cavity dorsal to the vent. To determine lengths, the number of transverse sections of the cloacal tube and cloacal chamber was counted. The section immediately posterior to the junction of the Wolffian ducts with the hindgut was considered the most anterior section of the cloacal tube.

Dicamptodon copei normally is an obligate paedomorph, and *D. aterrimus* and *D. tenebrosus* are facultative paedomorphs (Nussbaum, '70, '83; Good, '89). *Dicamptodon aterrimus* and *D. tenebrosus* prior to 1989 were considered conspecific with *D. ensatus*, a species now restricted to parts of northern California (Good, '89) and not examined for the present study. Maturity of branchiate females was determined by large size of ovarian follicles, convolution of the oviducts,

and/or presence of sperm in the spermathecae. Stebbins ('54) reported that mature ova of *D. ensatus* are 5–6 mm in diameter, and Nussbaum ('85, '87) reported mean (oviposited) ova sizes of 5.5 mm for *D. copei* and 6.6 mm for *D. ensatus*.

In one *Dicamptodon copei* (UMMZ 134960.1, 101.5 mm SVL), ovarian follicles are only 0.75–1.25 mm in diameter, but oviducts are convoluted, and some sperm are present in the spermathecae; thus this specimen is mature. The other *D. copei* (UMMZ 134961) had the ovaries removed and lacks sperm in the spermathecae, but it is considered mature because the oviducts are convoluted and the specimen is even larger (110.0 mm SVL) than other mature specimens.

The only *Dicamptodon aterrimus* examined (UMMZ 134671, 135 mm SVL) is metamorphosed, has large ovarian follicles (4.5–5.25 mm in diameter), and is thus considered mature; however, it has not recently mated since the spermathecae lack sperm. Of the three *D. tenebrosus* used in the present study, only one is metamorphosed and definitely mature. This individual (UMMZ 134955, 279 mm SVL) has ovarian follicles about 5 mm in diameter and some sperm in the spermathecae. Neither branchiate *D. tenebrosus* examined possesses spermathecal sperm; both were collected September 13, 1975. One (UMMZ 137462.1, 130 mm SVL) has ovarian follicles 1.5–1.75 mm in diameter and is probably immature, while the other (UMMZ 137462.2, 141 mm SVL) has ovarian follicles 4.0–5.0 mm in diameter and may be mature.

RESULTS

Female cloacal anatomy

Ambystomatidae

Reconstructions based on serial sections through the cloaca of a female *Ambystoma opacum* are shown in Figure 1. Histological sections through cloacae are illustrated for *A. opacum*, *A. gracile*, *A. maculatum*, and *A. tremblayi* in Figure 2, and for *A. annulatum* and *A. barbouri* in Figure 3. The three types of glands Kingsbury (1895) reported for *A. maculatum* occur in all species. Interspecific variation occurs in cloacal conformation and anatomy of the ventral gland.

Cloacal conformation. The cloacal tube is present in all specimens examined except that of *Ambystoma annulatum*, in which the Wolffian ducts do not join until the anterior end of the cloacal tube, so that a definite

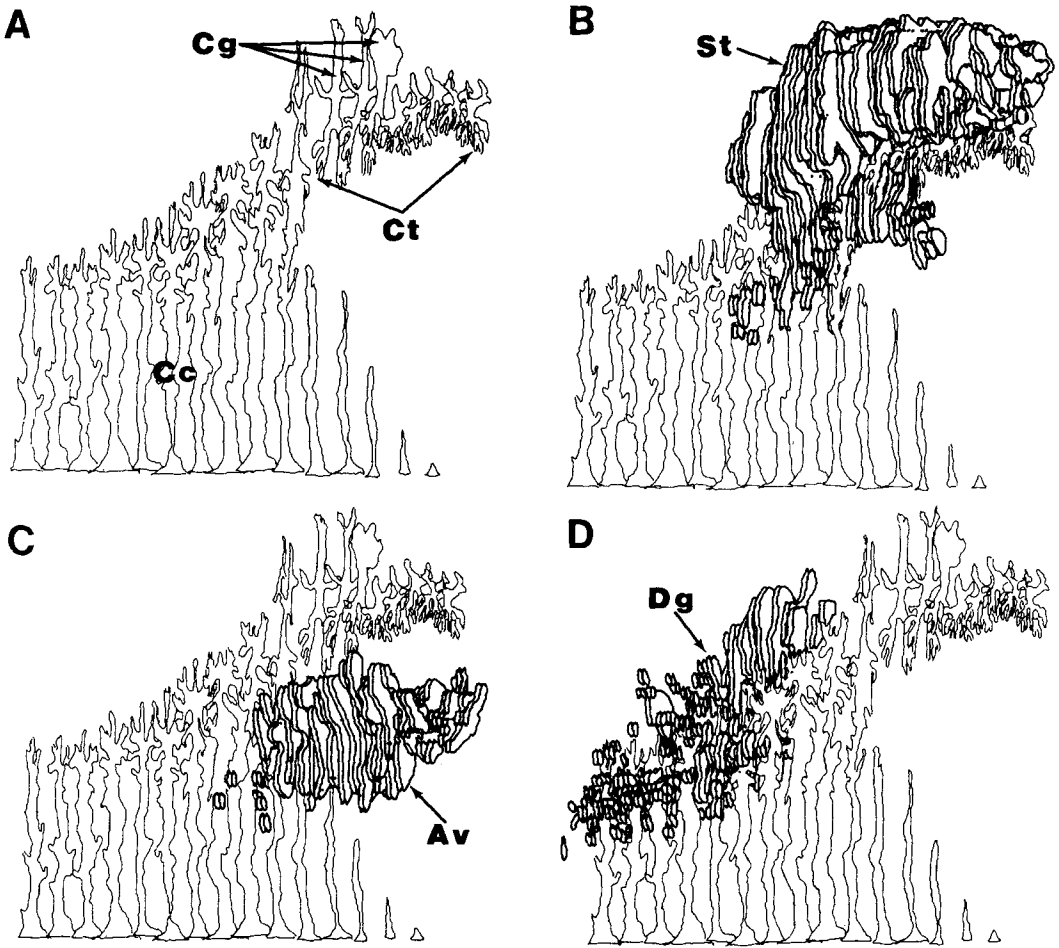


Fig. 1. Three-dimensional reconstructions of the cloaca of a female *Ambystoma opacum* (UMMZ 187373). Right lateral view with sections rotated 25° clockwise. **A:** Walls of the cloacal cavities. **B:** Spermathecae. **C:** Anterior ventral gland. **D:** Dorsal gland. The distance between

sections of the cloacal walls represents approximately 120 μm . Av, anterior ventral glands; Cc, cloacal chamber; Cg, central groove; Ct, cloacal tube; Dg, dorsal glands; St, spermathecae.

cloacal tube is not present (Fig. 3A). In the other forms, the CTL/TCL quotient ranges from 0.17 (*A. texanum*) to 0.38 (*A. laterale*).

In the species with a cloacal tube, the anterior half is narrowed dorsally and widened ventrally. The pseudostratified epithelium is formed into thick longitudinal folds or rugae (Fig. 2A) that are ciliated. At the midpoint of the cloacal tube, the dorsal half widens. In the posterior half of the cloacal tube, a pair of large folds appears in the lateral walls, forming a "central groove" between them (Figs. 1A, 2A). Cilia occur on the epithelium of the cloacal walls lateral to the folds but not on the lining of the folds or the central groove.

Posteriorly, the large dorsolateral folds join medially, obliterating the central groove. In *Ambystoma barbouri*, *A. jeffersonianum*, *A. laterale*, *A. opacum*, *A. platineum*, and *A. tremblayi*, obliteration of the central groove occurs at the anterior end of the cloacal chamber (Figs. 1A, 2B,C). In *A. gracile*, *A. maculatum*, *A. talpoideum*, and *A. tigrinum*, the central groove ends prior to the cloacal chamber, and the posterior fourth of the cloacal tube is a flattened slit (Fig. 2D). Since the cloacal tube is absent in *A. annulatum*, the central groove occurs in the anterior end of the cloacal chamber (Fig. 3A). The apex of the central groove is slanted posteriorly, so a

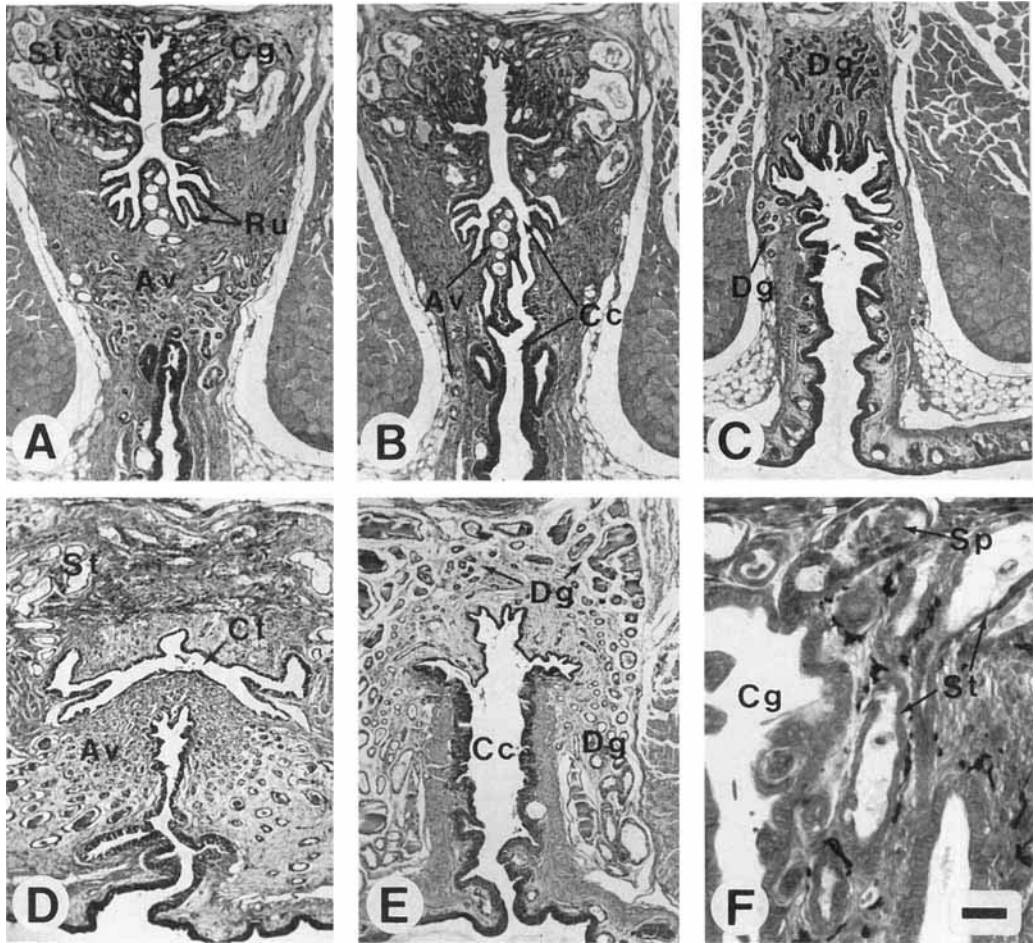


Fig. 2. Transverse sections through the cloacae of female *Ambystoma*. Sections stained with hematoxylin-eosin except F, stained with Mallory's triple stain. **A–C:** *A. opacum* (UMMZ 187373). **A:** Cloacal tube. **B:** Anterior cloacal chamber. **C:** Posterior cloacal chamber. **D:** *A. gracile* (UMMZ 133156), posterior cloacal tube. **E:** *A.*

maculatum (UMMZ 187377), posterior cloacal chamber. **F:** *A. tremblayi* (UMMZ 122783), spermathecae around dorsal end of the cloacal tube. Ru, rugae; Sp, sperm; other labels as for Figure 1. Scale bar = 275 μ m for A–C, E, 350 μ m for D, and 70 μ m for F.

small recess of the dorsal end still occurs in transverse sections after disappearance of the ventral end.

The cloacal chamber basically is a simple slit that gradually shortens posteriorly until it is even with the epidermis of the ventral tail wall. Posterior to the central groove, the dorsal portion of the cloacal chamber is widened, and the roof has short, blunt folds that lack cilia (Figs. 1A, 2C,E). Ciliated rugae from the lateral and ventral walls of the cloacal tube terminate in the anterior half of the cloacal chamber. At the anterior end of

the cloacal orifice, the lips of the cloacal orifice are lined with epidermis. As the chamber decreases in height posteriorly, the extent of the epidermal lining increases dorsally. The posterior third or fourth of the cloacal chamber is entirely lined with epidermis.

Cloacal glands. Cloacal glands are simple, tubular exocrine glands. The three types present are spermathecae, anterior ventral glands, and dorsal glands.

Spermathecae are glands for sperm storage. Sperm occur in the spermathecae of the

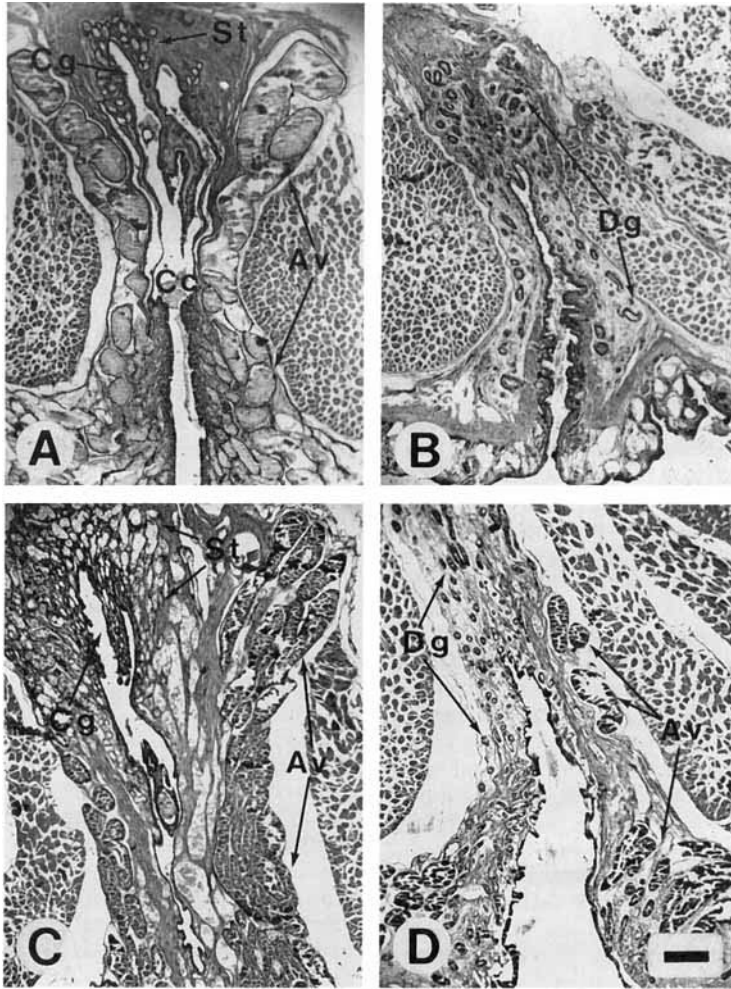


Fig. 3. Transverse sections through the cloacae of female *Ambystoma annulatum*, UMMZ 187374 (A,B) and *A. barbouri*, UMMZ 187376 (C,D). Sections stained with hematoxylin-eosin. A: Anterior cloacal chamber. B: Posterior cloacal chamber. C: Posterior cloacal tube. D: Posterior cloacal chamber. Labels as for Figure 1. Scale bar = 375 μ m for A–D.

specimens examined except for *Ambystoma laterale*, *A. talpoideum*, *A. platineum*, and some *A. tremblayi*. Whether spermatozoa occur or not, the spermathecal epithelium is alcian blue+, indicating presence of acid mucopolysaccharides. Spermathecae are short glands that are concentrated around the central groove, and most open into the central groove (Figs. 1B, 2A,B, 3A,B). However, other spermathecae open onto the walls of the cloaca anterior, lateral, and ventral to the central groove, and some spermathecae open upon the dorsal and lateral walls of the cloaca

posterior to the central groove (Figs. 1B, 2D). Melanophores are more abundant in the connective tissue around the spermathecae than other cloacal glands.

Ventral glands secrete onto the tips of the ciliated rugae lining the lateral walls of the anterior cloacal chamber (Figs. 1C, 2A,B,D). Ventral glands are long and convoluted. Their distal ends extend laterally and anteriorly. Anterior ends of some ventral glands are inferior to the posterior end of the intestine. Ventral gland lumina contain large globules, and granules occur in the cytoplasm. Glob-

ules and granules are PAS+, alcian blue-, and ninhydrin Schiff+, indicating a mucoprotein. Much variation occurs from specimen to specimen in the height of the ventral gland epithelium; the lumina of ventral glands in some specimens are obscured by the hypertrophy of the cells. Ventral gland hypertrophy probably is related to a particular stage of reproductive activity (Sever, '88b).

Both the *Ambystoma annulatum* and *A. texanum* examined have enlarged ovarian follicles, but no spermatozoa in the spermathecae, indicating their capture prior to breeding. In both species, the ventral glands are enlarged and convoluted. Anteriorly, distal ends of the ventral glands extend dorsally along the lateral borders of the cloacal cavities to the region just inferior to the posterior end of the kidneys (Fig. 3A,C).

Dorsal glands, in all species but *Ambystoma maculatum*, are similar in size to spermathecae. The glands appear most anteriorly in the roof of the cloacal chamber just posterior to the most caudad spermathecae (Figs. 1D, 2C, 3C,D). Further posteriorly, dorsal glands come to line the entire dorsal and dorsolateral walls of the cloacal chamber, persisting until the caudal angle of the vent, into which no cloacal glands secrete. Luminal contents are scant in most specimens, but the apical cytoplasm generally is PAS+, indicating presence of neutral carbohydrates.

In an *Ambystoma maculatum* collected just after oviposition, the dorsal glands are more elongate and hypertrophied, and luminal contents are copious (Fig. 2E). The contents are globular, PAS+, and ninhydrin Schiff+. Thus, the dorsal glands in *A. maculatum* are similar to the ventral glands, and the two clusters are difficult to distinguish except by position.

Cloacae were examined from specimens of *Ambystoma tremblayi* and *A. platineum* used by Uzzell ('64) to characterize these all-female forms of hybrid origin. Ovaries had been removed from these specimens, but the oviducts were convoluted. Spermatozoa occur in the spermathecae of two *A. tremblayi* collected in Delaware County, Indiana (Fig. 2F). None is apparent in the other specimens. A specimen (DMS 3439) of *A. (2) laterale-jeffersonianum* (using the nomenclature of Lowcock et al., '87) from Cass County, Michigan, has an egg in the cloaca, but no sperm are evident in the spermathecae (see Fig. 2D in Sever, '87).

Dicamptodontidae

No differences occur in cloacal conformation or the types of cloacal glands present in the female *Dicamptodon* examined. *Dicamptodon* and *Rhyacotriton*, however, differ greatly in female cloacal anatomy.

Cloacal conformation of Dicamptodon. The CTL/TCL quotient in *D. atterimus* is 0.21, in the 130-mm SVL *D. tenebrosus* 0.39, and in the 110.0-mm SVL *D. copei* 0.24. 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 (Figs. 4A, 5A–C). 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 (Figs. 4A, 5C–D). Thick dorsolateral folds with a central groove like that found in female ambystomatids are absent. Laterally, the walls of the cloacal tube are rugose, and the rugae persist into the anterior half of the cloacal chamber (Fig. 4A). Ventrally, a medial evagination of the floor of the cloacal tube occurs just anterior to its abrupt junction with the cloacal chamber (Figs. 4A, 5B). 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.

Cloacal glands of Dicamptodon. Spermathecae and ventral glands occur in female *Dicamptodon*. The spermathecae are short, tubular glands that occur around the cloacal tube and around the dorsal and dorsolateral borders of the anterior cloacal chamber (Figs. 4B, 5A–C,E,F). The apical cytoplasm of the spermathecae in the *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 (Figs. 4C, 5). Distal ends of the glands pass anteriorly, and those secreting into the dor-

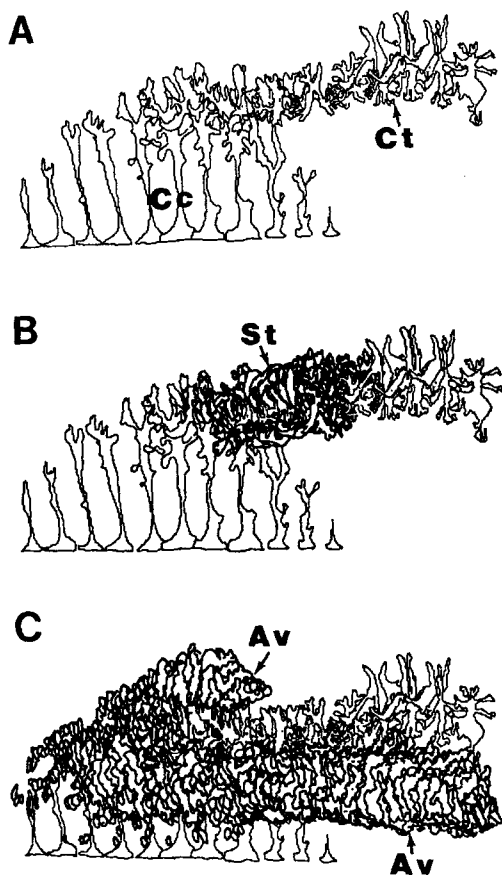


Fig. 4. Three-dimensional reconstructions of the cloaca of a female *Dicamptodon copei* (UMMZ 134981). Right lateral view with sections rotated 25° clockwise. A: Walls of the cloacal cavities. B: Spermathecae. C: Anterior ventral gland. The distance between sections of the cloacal walls represents approximately 260 μm . Labels as for Figure 1.

sal portion of the posterior cloacal chamber lie superior to the most caudad spermathecae (Fig. 5C). 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+.

In the 130-mm SVL, branchiate *Dicamptodon tenebrosus*, the spermathecae and ventral glands have narrow lumina and appear inactive or poorly developed, perhaps a sign of immaturity, since the glands in the 141-mm SVL specimen are hypertrophied.

Cloacal conformation of *Rhyacotriton olympicus*. One individual, 54.2 mm SVL,

has a CTL/TCL quotient of 0.125. The short cloacal tube is rugose and lined with pseudostriated epithelium. The rugae cease in the anterior cloacal chamber, which is a simple cavity almost completely lined with epidermis. Cilia are lacking in the cloaca. The most remarkable conformational feature is an abrupt invagination into the dorsal wall of the posterior cloacal tube and anterior cloacal chamber, forming a "dorsal tube" from which the spermathecae radiate (Figs. 6, 7A,C).

Cloacal glands of *Rhyacotriton olympicus*. The only glands are spermathecae. Individuals collected on May 29, 1983 (Multnomah County, Oregon) have large ovarian follicles (3.5–3.7 mm) and possess abundant spermatozoa in their spermathecae. Examined specimens from June 19, 1981 (Benton County, Oregon; Sever, '87) have small ovarian follicles and lack sperm in the spermathecae, which appear inactive. No diagnostic staining was done on female *R. olympicus*. The spermathecae are elongate, and radiate from sides and the apex of the dorsal tube (Figs. 6B, 7A–C). Sever ('87:111) noted a "... gland cluster of uncertain homology..." in the posterior cloacal region of female *Rhyacotriton olympicus*. At the caudal end of the cloaca, some short, tubular glands secrete onto the surface of the epidermis lateral and posterior to the cloacal orifice (Fig. 7D,E). These glands therefore are skin glands, but they are different from the mucous and granular glands found elsewhere in the skin but absent in this area.

Male cloacal anatomy

Ambystomatidae

Cloacal glands present include anterior and lateral pelvic glands, anterior and posterior ventral glands, Kingsbury's glands, and the vent gland (Licht and Sever, '91; Sever, '81, '88a, '91a; Sever et al., '89). In *Ambystoma opacum*, Noble and Brady ('33) reported that lateral to the pelvic gland is an eosinophilic "scaffolding" gland with a homogeneous secretion. This describes the lateral pelvic gland.

Male ambystomatids that have been examined are similar in cloacal anatomy. Sever ('88a), however, found the vent gland better developed in *Ambystoma opacum* and *A. talpoideum* than in the other species examined. Reconstructions through the cloacae of *Ambystoma opacum* and *A. laterale* are shown in Figure 8 and transverse sections through the

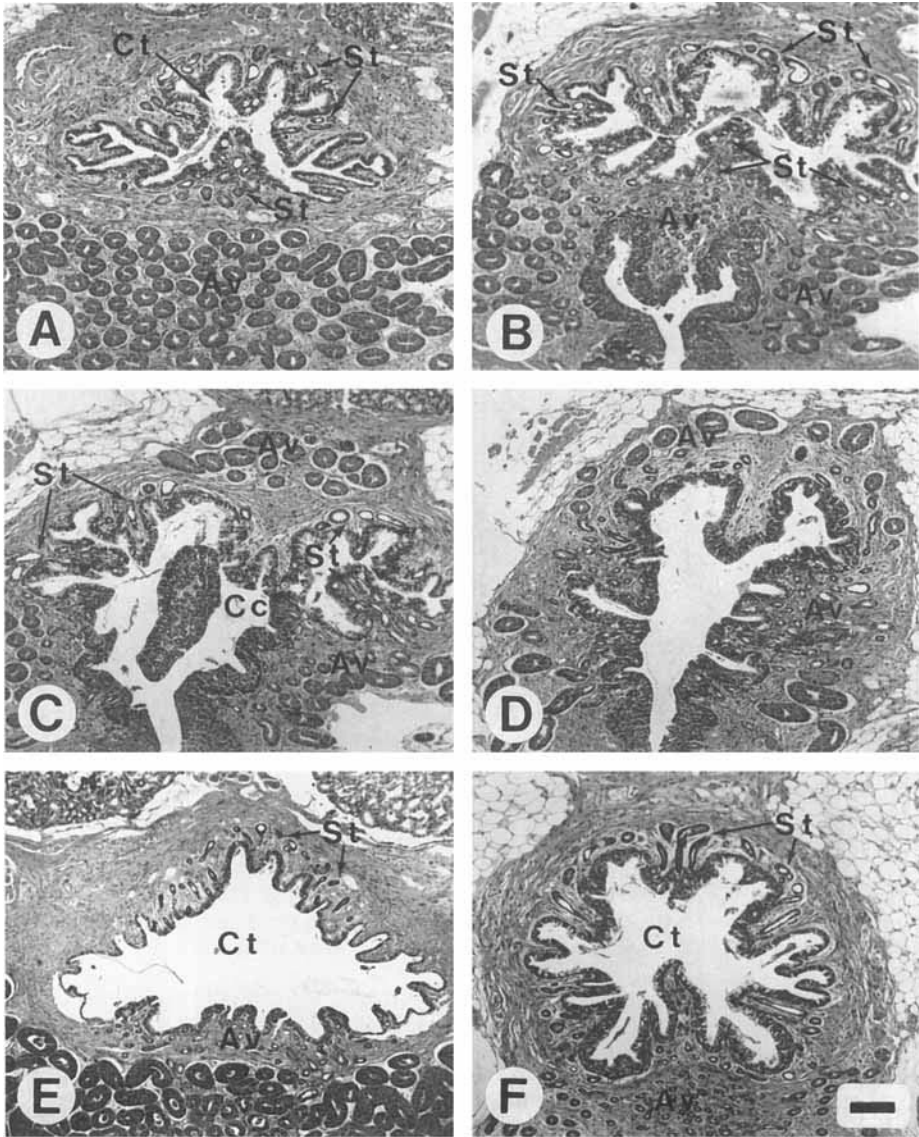


Fig. 5. Transverse sections through the cloacae of female *Dicamptodon*. Sections stained with hematoxylin-eosin. **A–D:** *D. copei* (UMMZ 134961). **A:** Anterior cloacal tube. **B:** Posterior cloacal tube. **C:** Anterior cloacal chamber. **D:** Posterior cloacal chamber. **E:** Metamor-

phosed *D. aterrimus* (UMMZ 134671), anterior cloacal tube. **F:** Paedomorphic *D. tenebrosus* (UMMZ 137462.1), anterior cloacal tube. Labels as for Figure 1. Scale bar = 250 μ m for A–D,F, and 320 μ m for E.

cloacae of these species are shown in Figure 9.

Cloacal conformation. The anterior end of the cloacal tube is narrow dorsally and wide ventrally (Fig. 8A,F), and is lined with unciliated, 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 (Figs. 8A, 9A–D). Concurrently, the dorsal portion of the cloacal tube widens, and two pairs of dorsolateral folds appear. These are the pri-

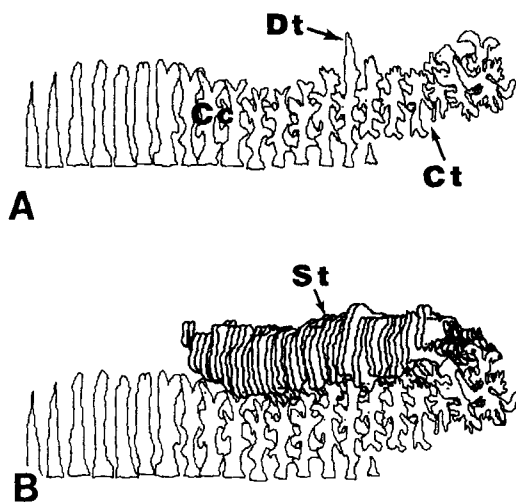


Fig. 6. Three-dimensional reconstructions of the cloaca of a female *Rhyacotriton olympicus* (UMMZ 135501.2). Right lateral view with sections rotated 25° clockwise. **A:** Walls of the cloacal cavities. **B:** Spermathecae. The distance between sections of the cloacal walls represents approximately 160 μm . Dt, dorsal tube; other labels as for Figure 1.

mary lateral folds (medially) and secondary lateral folds (Fig. 9A–D).

At the posterior end of the cloacal tube, a middorsal papilla appears with a medial groove (Fig. 9C,D). 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 end, and the lateral walls of the dorsal papilla merge with the walls of the dorsal cloacal chamber as that chamber shortens posteriorly (Figs. 8A,F, 9E,F). The cloacal chamber widens ventrally in the anterior end, but this area narrows posteriorly concordant with the absence of rugae. 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.

Cloacal glands. 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 and onto the tips of the primary lateral folds (Figs. 8D, 9A,B). 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 (Figs. 8C,D, 9). 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 and replace the anterior ventral glands caudad to the rugae. 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.

Additional eosinophilic glands with globular secretions are the dorsal pelvic, lateral pelvic, and vent glands. Dorsal pelvic glands and vent glands also 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 anterior cloacal chamber and are replaced caudally by posterior ventral glands. Posterior tubules possess more alcian blue+ granular secretion than anterior ones. Lateral pelvic glands occur between Kingsbury's glands and anterior ventral glands, and secrete onto the secondary lateral folds. Except in *Ambystoma opacum* and *A. talpoideum*, vent glands are few in number, and consist of thick, elongate tubules buried in the ventral gland masses (Figs. 8E–F, 9). In all species, vent glands secrete along the edges of the cloacal orifice and distal ends pass laterally.

In *Ambystoma opacum* and *A. talpoideum*, vent glands appear relatively more numerous and elongate. The vent glands of these species also extend laterally from the cloacal orifice, but the distal ends often curve anteriorly or posteriorly, resulting in transverse sections of vent glands appearing in some of the most cephalad as well as caudad sections of the cloaca (Figs. 8E, 9A,E). In *A. talpoideum*, some of the anteriorly passing vent

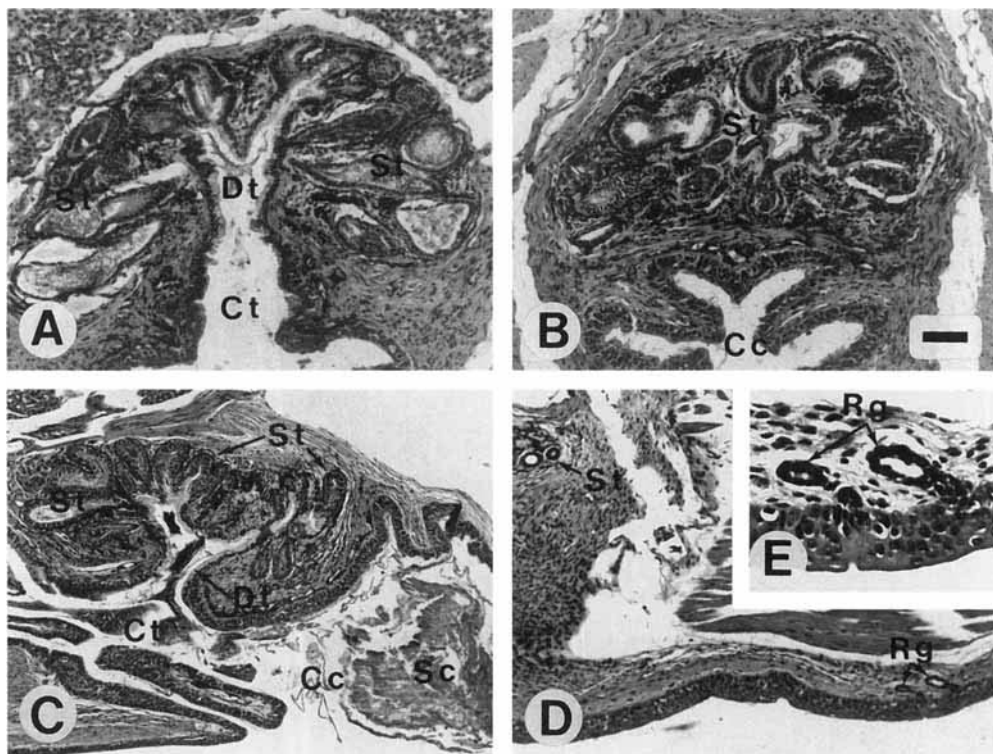


Fig. 7. Cloacae of female *Rhyacotriton olympicus*, showing transverse sections of UMMZ 135501.2 in A,B, and sagittal sections of UMMZ 135501.1 in C-E. Sections stained with hematoxylin-eosin. A: Posterior cloacal tube. B: Anterior cloacal chamber. C: Mid-sagittal section through the cloaca. D: Parasagittal section

through area posterior to the cloaca, showing rudimentary skin glands. E: Detail of skin glands shown in D. Rg, rudimentary glands; Sc, spermatophore cap; other labels same as for Figures 1 and 6. Scale bar = 150 μ m for A,B, 250 μ m for C, 200 μ m for D, and 70 μ m for E.

glands are so elongate that the distal ends curve dorsally to lie anterior to the pelvic gland cluster. In a 56.9-mm SVL *A. texanum* (UMMZ 184008) collected in western Louisiana and also examined by Sever ('88a), the vent gland cluster is like that of *A. opacum* rather than *A. texanum* from Pelee Island, Lake Erie, in which the vent gland cluster resembles *A. laterale* (Sever et al., '89).

Dicamptodontidae

Sever ('88a) described the cloacal anatomy of *Dicamptodon* and *Rhyacotriton olympicus*. However, three-dimensional reconstructions of the cloacae of male dicamptodontids have not been presented previously (Figs. 10, 11). Transverse sections in Figure 12 emphasize variation noted in vent gland anatomy. Histochemical reactions of the cloacal glands of dicamptodontids are similar to those of male ambystomatids (Sever, '88a).

As noted by Sever ('88a), *Dicamptodon* is similar to *Ambystoma* in cloacal anatomy (cf. Figs. 8 and 10). The vent gland of *Dicamptodon* is not like that of *A. opacum* and *A. talpoideum*, but is more like other *Ambystoma*. Thus, vent glands in *Dicamptodon* are limited to several layers of tubules inferior to the ventral glands (Figs. 10B, 12B-D). The area of secretion of vent glands is along the cloacal orifice, except for the most posterior end. Kingsbury's gland is a relatively smaller cluster of tubules in *Dicamptodon* than in *Ambystoma*.

Sever ('88a) noted that the posterior ventral glands are the only cloacal glands hypertrophied in a 122-mm SVL branchiate *D. tenebrosus* (UMMZ 137462.3; Fig. 12D), and a 106.0-mm SVL *D. copei* (UMMZ 134960). Meiotic divisions are found in the testes of these individuals, but no spermatozoa are present in the testes or vasa deferentia, as

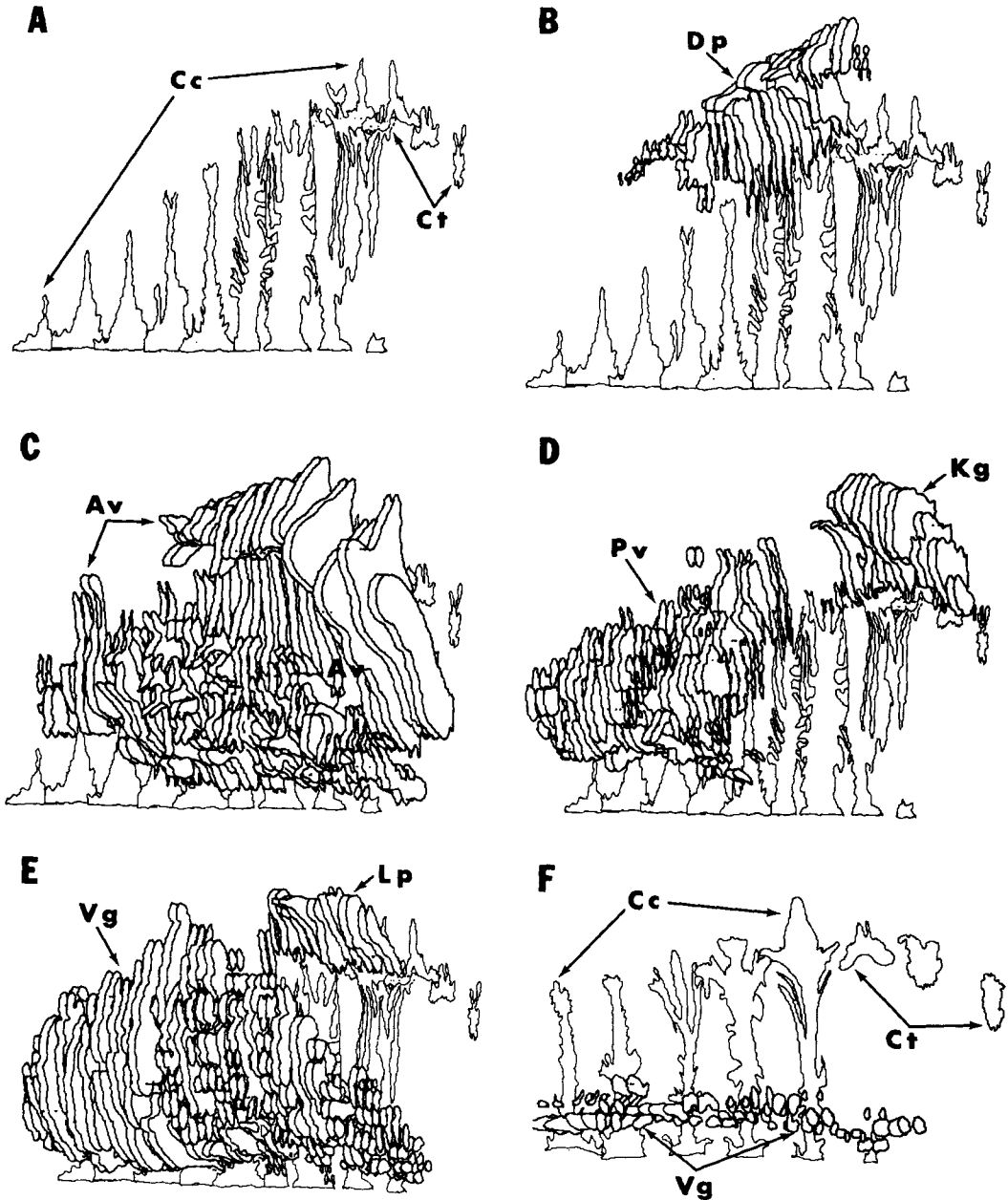


Fig. 8. Three-dimensional reconstructions through the cloacae of male *Ambystoma*. Right lateral view with sections rotated 25° clockwise. A–E: *A. opacum* (UMMZ 184009, 50.8 mm SVL, Fall 1983, Florida; specimen also used by Sever, '88a). A: Walls of the cloacal cavities. B: Dorsal pelvic glands. C: Anterior ventral glands. D: Kingsbury's glands and posterior ventral glands. E: Lateral pelvic glands and vent glands. F: *A. laterale* (DMS 6803)

showing cloacal walls and vent glands. Distance between sections of the cloacal walls represents approximately 200 μm for *A. opacum* (A–E) and 300 μm for *A. laterale* (F). Av, anterior ventral glands; Dg, dorsal glands; Dp, dorsal pelvic glands; Kg, Kingsbury's glands; Lp, lateral pelvic glands; Pv, posterior ventral glands; Vg, vent glands; other labels as for Figure 1.

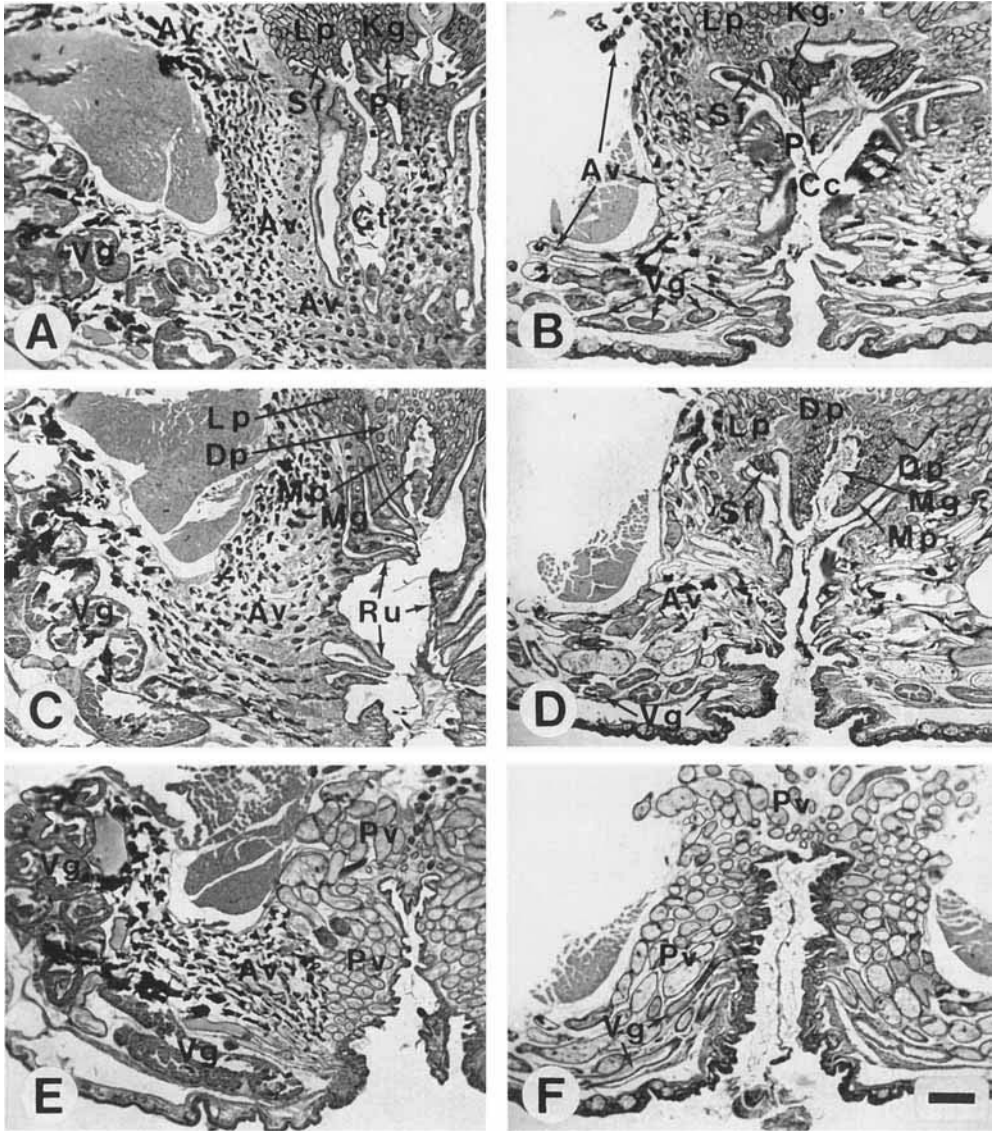


Fig. 9. Transverse sections through the cloacae of the male *Ambystoma opacum* (A,C,E) and *A. laterale* (B,D,F) used in Figure 8, showing sections through equivalent regions of the two species in horizontal rows. Sections stained with hematoxylin-eosin. A: Posterior end of the cloacal tube. B: Anterior end of the cloacal chamber.

C,D: Anterior third of the cloacal chamber. E,F: Posterior third of the cloacal chamber. Mg, middorsal groove; Mp, middorsal papilla; Pf, primary lateral folds; Sf, secondary lateral folds; other labels as for Figures 1, 2, 8. Scale bar = 325 μ m for A–F.

opposed to specimens in which all the cloacal glands are hypertrophied.

As in *Dicamptodon*, Kingsbury's gland in *Rhyacotriton olympicus* is a relatively small cluster. The primary and secondary folds of *R. olympicus* are not as well developed as in *Dicamptodon* and *Ambystoma*. Otherwise, the

cloacal conformation and the anatomy of the pelvic glands and ventral glands are similar to the ambystomatid pattern (Fig. 11).

The major difference in cloacal anatomy between *Rhyacotriton olympicus* and the other species is in the vent gland. *Rhyacotriton olympicus* possesses an autapomorphic

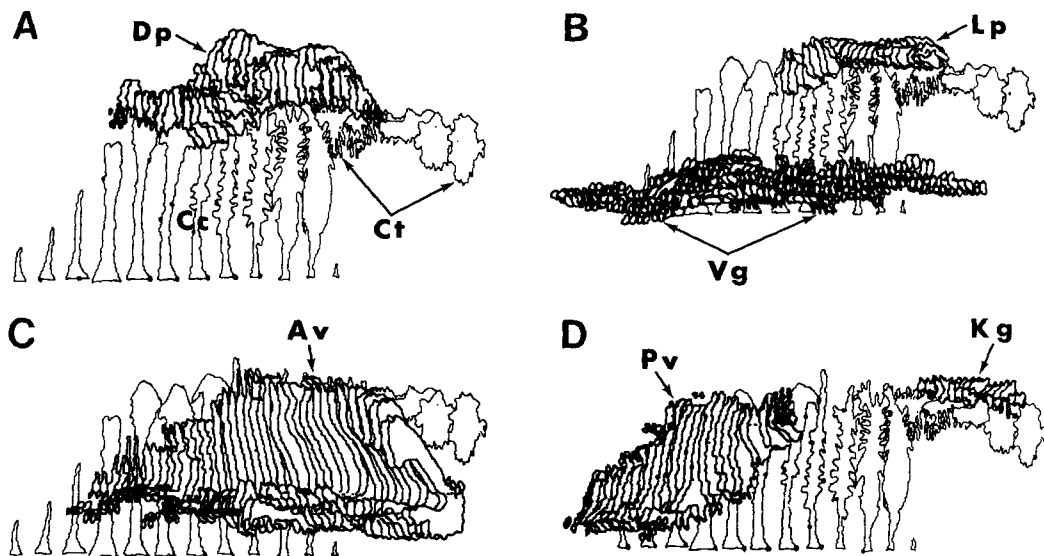


Fig. 10. Three-dimensional reconstructions through the cloaca of a male *Dicamptodon copei* (UMMZ 134960.3, 107.9 mm SVL, August 24, 1969, Skamania County, Washington; specimen also used by Sever, '88a). Right lateral view with sections rotated 25° clockwise. A: Dor-

sal pelvic glands. B: Lateral pelvic glands and vent glands. C: Anterior ventral glands. D: Kingsbury's glands and posterior ventral glands. Distance between sections of the cloacal walls represents approximately 260 μ m. Labels as for Figures 1 and 8.

vent gland structure in which the glands secrete at the posterior tips of squarish vent lobes dorsolateral to the cloacal orifice (Sever, '88a; Fig. 12A). The vent gland cluster of *R. olympicus* is relatively large in comparison to that of other species, even *Ambystoma opacum* and *A. talpoideum*. Vent glands pass from the area of secretion to the anterior end of the cloaca in large masses lateral to the musculature surrounding the cloacal cavities and other cloacal glands (Fig. 11F; Figs. 1 and 2 of Sever, '88a). Thus, in *R. olympicus*, the vent glands technically are not even cloacal glands, since they secrete onto the skin lateral to the cloaca, and vent gland tubules are superficial to the fascia enclosing glands that do secrete into the cloaca.

DISCUSSION

Male *Dicamptodon* are similar in cloacal anatomy to male *Ambystoma* except for *A. opacum* and *A. talpoideum*, which differ from *Dicamptodon* as well as other *Ambystoma* by possessing larger vent gland clusters. Females of the two genera, however, are easily distinguished by cloacal anatomy in that all female *Ambystoma* examined possess dorsal glands and the central groove, which are lacking in female *Dicamptodon*. The only other family of salamanders in which dorsal

glands are present is the Plethodontidae, and parsimony analyses of the evolution of cloacal characters support the hypothesis that possession of this character in the two families is due to convergence (Sever, '91a). Such analyses of cloacal characters show a sister group relationship between *Ambystoma* and *Dicamptodon*, a conclusion also reached from analysis of aligned nucleotide sequences of ribosomal RNA (Larson, '91). The sister group relationship revealed by analyses of cloacal characters, however, is due largely to symplesiomorphies (Sever, '91a). Therefore, the cloacal data provide equivocal additional evidence for the current view of separate familial status for *Ambystoma* and *Dicamptodon*.

The familial relationships of *Rhyacotriton olympicus* are another matter. Female *R. olympicus* differ significantly from *Ambystoma* and *Dicamptodon* in cloacal anatomy. Specifically, in *R. olympicus* the epidermal lining of the cloacal chamber is more extensive, the only cloacal glands present are the spermathecae, and the spermathecae are clustered around a "dorsal tube" that is similar to the common tube characteristic of the Plethodontidae. The dorsal tube in female *R. olympicus*, however, is not as narrow as the common tube of plethodontids. The sper-

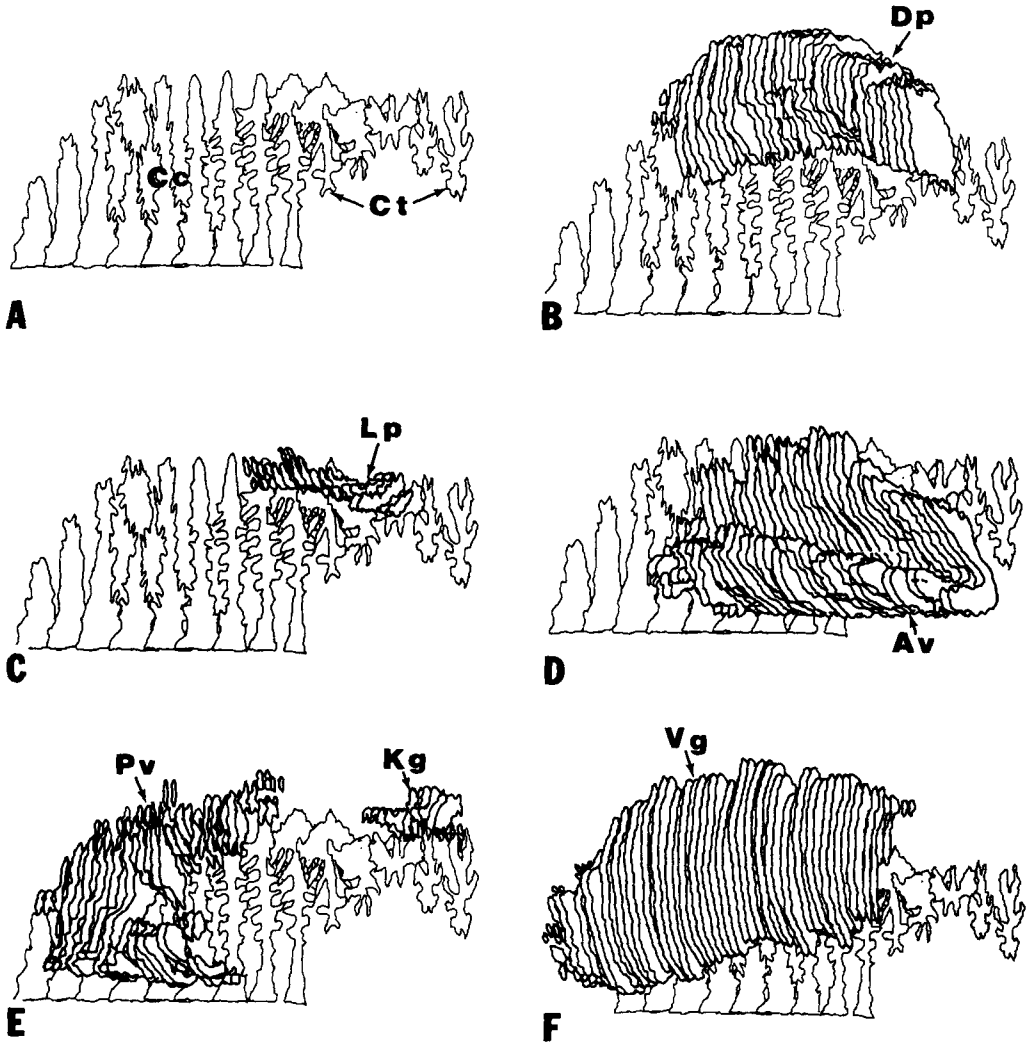


Fig. 11. Three-dimensional reconstructions through the cloaca of a male *Rhyacotriton olympicus* (UMMZ 135501.4, 52.5 mm SVL, May 29, 1983, Multnomah County, Oregon; specimen also used by Sever, '88a). Right lateral view with sections rotated 25° clockwise. A: Walls of the cloacal cavities. B: Dorsal pelvic glands. C:

Lateral pelvic glands. D: Anterior ventral glands. E: Kingsbury's glands and posterior ventral glands. F: Vent glands. Distance between sections of the cloacal walls represents approximately 160 μm . Labels as for Figures 1 and 8.

mathecae of *R. olympicus* evaginate from the entire upper half of the dorsal tube, whereas in plethodontids narrow neck tubules extend from the apex of the common tube and expand distally into spermathecal bulbs (Sever, '87).

Males of *Rhyacotriton olympicus* possess a unique vent gland (Sever, '88a). Males also possess primary and secondary lateral folds in the cloacal tube, characters otherwise

known only from male *Ambystoma* and *Dicamptodon* among salamanders. The phyletic relationships among *Ambystoma*, *Dicamptodon*, and *R. olympicus* found by parsimony analyses of cloacal characters differ by whether these conspicuous folds are symplesiomorphic for the genera or convergent in *Ambystoma* + *Dicamptodon* and in *R. olympicus*. The Dicamptodontidae, containing both *Dicamptodon* and *R. olympicus*, is

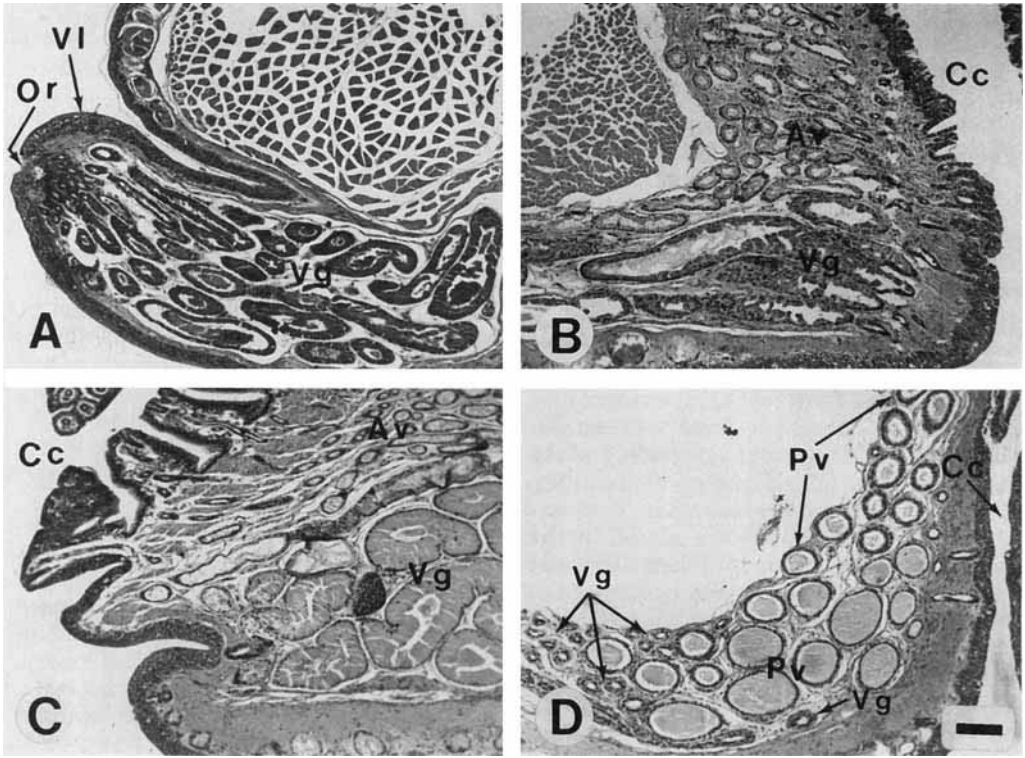


Fig. 12. Transverse sections through the cloacae of male *Dicamptodon* and *Rhyacotriton olympicus* showing sites of secretion and extent of the vent glands in the posterior cloacal chamber; specimens also used by Sever ('88a). Sections stained with hematoxylin-eosin. **A:** Posterior end of the cloacal chamber of an *R. olympicus* (UMMZ 13550.5, 50.8 mm SVL, May 29, 1983, Multnomah County, Oregon). **B:** Anterior end of the cloacal chamber

of a *D. copei* (same specimen used for Fig. 10). **C:** Anterior end of a metamorphosed *D. tenebrosus* (UMMZ 128006, 147 mm SVL, October 8, 1967, Benton County, Oregon). **D:** Posterior end of the cloacal chamber of a paedomorphic *D. tenebrosus* (UMMZ 137462.3, 122 mm SVL, September 13, 1975, Linn County, Oregon). Or, orifices; VI, lateral vent lobes. Other labels as for Figures 1 and 8. Scale bar = 250 μ m for A–D.

paraphyletic if the character is symplesiomorphic, and polyphyletic if the character is convergent (Sever, '91a). Neither choice supports the placement of *R. olympicus* within the Dicamptodontidae.

In the original description, *Rhyacotriton olympicus* was placed in the genus *Ranodon* and assigned to the Hynobiidae, although little justification for this action was given (Gaige, '17). Dunn ('20) erected the new genus *Rhyacotriton* and stated that it was most closely related to *Dicamptodon*. Based upon jaw anatomy, however, Eaton ('34) believed *R. olympicus* was derived from *Ambystoma* and not at all related to *Dicamptodon*. Tihen ('58) described many osteological differences between *Ambystoma*, *Dicamptodon*, and *R. olympicus* and placed the genera in different subfamilies in the Ambystomatidae. Regal ('66) subsequently placed both *Dicamptodon*

and *R. olympicus* in the same subfamily (Dicamptodontinae) based upon similarities in feeding specializations. Worthington and Wake ('71) found no derived characters common to *Dicamptodon* and *R. olympicus*, and preferred the classification of Tihen ('58).

Edwards ('76), in an analysis of spinal nerve patterns and osteological data, proposed the family Dicamptodontidae, with the extant subfamilies Dicamptodontinae and Rhyacotritoninae, and this is the classification most often followed currently (Estes, '81; Frost, '85; Duellman and Trueb, '87). Milner ('83), however, considered *Rhyacotriton olympicus* more closely related to plethodontids and amphiumids than to dicamptodontines, although he did not find this conclusion wholly satisfactory. Duellman and Trueb ('86) noted that the similarities between *R. olympicus* and plethodontids may be the result of conver-

gence of paedomorphic characters, but that synapomorphies linking *R. olympicus* and *Dicamptodon* are weak, and that Dicamptodontidae may be polyphyletic. Good et al. ('87) found that neither *Ambystoma* nor *Dicamptodon* is similar enough in its allozymes to make outgroup comparison with *R. olympicus* feasible. Beneski and Larsen ('89), however, felt that similarities in a stage of tooth development might indicate a close relationship between *Dicamptodon* and *R. olympicus*. Finally, Larson ('91), using an analysis of ribosomal RNA sequences, found that *R. olympicus* is not closely related to any other salamander genus. In summary, the evidence for monophyly at the family level between *Dicamptodon* and *Rhyacotriton* is poor, and the genera should be placed in separate families.

Two of the *Ambystoma* examined, *A. annulatum* and *A. texanum*, were placed in the subgenus *Linguaelapsus* by Tihen ('58), and *A. barbouri*, recently described by Kraus and Petranksa ('89), was considered a *linguaelapsid* by Shaffer et al. ('91). Other species of *Ambystoma* examined here are members of the subgenus *Ambystoma* (Tihen, '58). *Linguaelapsus* is the more highly derived group, and differs from the *Ambystoma* species in shape of the pterygoid and otoglossal of the hyoid, narrowness of the junction between the otoglossal and the second radials, and anatomy of the tongue plicae (Kraus, '88). Although morphological analyses provide unambiguous evidence for the monophyly of *Linguaelapsus*, analyses of allozyme data furnish equally compelling evidence that *Linguaelapsus* is polyphyletic (Shaffer et al., '91).

The female *linguaelapsids* examined here, *A. annulatum* and *A. barbouri*, share the same ventral gland morphology, in which these glands are more highly convoluted anteriorly and posteriorly than in other *Ambystoma*. Therefore this character may be a synapomorphy for female *Linguaelapsus*. However, the results from allozyme analyses (Shaffer et al., '91) hypothesize that *A. annulatum* and *A. barbouri* are in different clades, making the ventral gland morphology convergent in the two species. Variation in ventral gland morphology, especially in female *linguaelapsids* not examined here (*A. mabeei*, *A. cingulatum*, and *A. texanum*), merits further study to see if this character can help resolve conflicts in phylogenetic hypotheses concerning the status of *Linguaelapsus*.

The clash between results of phylogenetic analyses of morphological and molecular data also affects interpretation of vent gland evolution in male *Ambystoma opacum* and *A. talpoideum*, which have more extensive vent gland clusters than other *Ambystoma*. Based upon analyses of other morphological characters, Kraus ('84) found that *A. opacum* and *A. talpoideum* are sister-taxa, but results from analyses of allozyme data propose that *A. opacum* and *A. talpoideum* are in different clades (Shaffer et al., '91). Thus, when mapping the character on existing phylogenies, the vent gland morphology of *A. opacum* and *A. talpoideum* is either a synapomorphy (Kraus, '84) or convergent (Shaffer et al., '91). Cloacal characters may provide independent sources of data for constructing future phylogenetic hypotheses.

Few direct observational or experimental data are available on the function of female cloacal glands in *Ambystoma*, *Dicamptodon*, and *Rhyacotriton*. Spermathecae are used in sperm storage, but no detailed cytological study on this exists in these genera except for some observations reported in abstracts by Davitt and Larsen ('88a,b) on *Rhyacotriton olympicus*. Kingsbury (1895) reported that the secretion of the female ventral glands is used in adherence of the egg mass. No data exist to support or refute this idea. Sever ('88b) presented evidence that the ventral gland secretes a courtship pheromone in the females of the plethodontid *Eurycea cirrigera*. The function of the dorsal glands found in female *Ambystoma* is unknown. Dorsal glands are better developed in *Ambystoma* than in some genera in the Plethodontidae that also possess glands in this region.

Much literature exists on the overall appearance and histology of spermatophores of male *Ambystoma* (see Russell et al., '81, and Zalisko et al., '84, for reviews), but the relation between parts of the spermatophore and particular cloacal gland secretions has not been studied experimentally. In plethodontids, the pelvic, ventral, and Kingsbury's glands in the male cloaca are involved in spermatophore production (Sever and Houck, '85). Vent glands in male salamanders are assumed to produce a courtship pheromone (Sever, '91a); it is at least certain in *Rhyacotriton olympicus* that the vent glands, which secrete onto the lateral surface of the body, cannot be involved in spermatophore formation (Sever, '88a). As with females, experi-

mental data are needed for the roles of the various male cloacal glands in spermatophore formation and/or pheromone production.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation grants BSR-8715341 and BSR-9024918. For the loan or gift of specimens and/or aid in collecting, I thank A.L. Braswell, K. Mierzwa, R.A. Nussbaum, C.D. Sullivan, and T. Williams. I thank J.P. Bogart and L.E. Licht for identification of *Ambystoma* from Cass County, Michigan.

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Dicamptodontidae: *Dicamptodon aterrimus*—metamorph, UMMZ 134671, 135 mm SVL, May 10, 1967, Benewah County, Idaho. *D. copei*—UMMZ 134960.1, 101.5 mm SVL, and UMMZ 134961, 110.0 mm SVL, August 24, 1969, Skamania County, Washington; *D. tenebrosus*—metamorph, UMMZ 134955, 279 mm SVL, Linn County, Oregon; paedomorphs, UMMZ 137462.1, 130 mm SVL, and UMMZ 137462.2, 123 mm SVL, September 13, 1975, Linn County Oregon; *Rhyacotriton olympicus*—UMMZ 135501.1, 52.2 mm SVL, UMMZ 135501.2, 54.2 mm SVL, and UMMZ 135501.3, 50.4 mm SVL, May 29, 1983, Multnomah County, Oregon.

In addition, a male *A. annulatum* [UMMZ 187372, 91.4 mm SVL, August 1, 1978 (sacrificed July 25, 1979), Stone County, Missouri] and a male *A. laterale* in my possession (DMS 6803, 64.1 mm SVL, March 13, 1988, Cook County, Illinois) were examined.

APPENDIX I

Specimens examined include those listed in Sever ('81, '87, '88a), Sever et al. ('89), and Licht and Sever ('91) plus the following female specimens, all in the collections of the University of Michigan Museum of Zoology (UMMZ).

Ambystomatidae: *Ambystoma annulatum*—UMMZ 187374, 89.0 mm SVL, no collecting data; *A. barbouri*—UMMZ 187376, 83.5 mm SVL, February 22, 1970, Hamilton County, Ohio; *A. laterale*—UMMZ 187371, 57.1 mm SVL, September 13, 1975, Manitowoc County, Wisconsin; *A. maculatum*—UMMZ 187377, 89.5 mm SVL, April 11, 1980, Cass County, Michigan; *A. opacum*—UMMZ 187373, 68.3 mm SVL, Fall 1983, Missouri; *A. platineum*—UMMZ 122856, 78.4 mm SVL, UMMZ 122857, 79.0 mm SVL, UMMZ 122859, 85.0 mm SVL, and UMMZ 122860,