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REPORT OF THE DIRECTOR OF THE REELFOOT LAKE BIOLOGICAL STATION

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The thirty-third season of this research station of the Tennessee Academy of Science, supported by an appropriation from the State of Tennessee, was restricted to research relating to Reelfoot Lake and its environs.

Dr. Robert J. Schoffman of Griffin High School, Springfield, Illinois, spent his twenty-sixth consecutive summer at the station specializing in studies on the growth rate of fishes. He is now able to compare their present growth rate with that for the past two decades and he finds considerable increase in the number of white crappie in the lake with a correspondingly larger proportion of younger crappie.

Dr. Robert B. Short and Sherman S. Hendrix of Florida State University, Tallahassee, Florida, spent a brief period at the station investigating Aspidogastrid trematodes in fresh water mussels and found an abundant supply.

Other investigators and students utilized our facilities for short terms observing and collecting biological material for further use and study.

While the environment is not comparable to a health resort, the station and vicinity offer a wealth of limnological, herpetological, parasitological and ornithological material for teaching purposes or advanced research. Our facilities are limited but there is available board and room and collecting equipment for brief periods for competent investigators at no charge. These facilities are available during June, July and August to biologists.

THE MALE UROGENITAL SYSTEM OF THE SALAMANDRIDAE[1]

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The urogenital system of the five species of *Ambystoma* of the family Ambystomidae has been described (Baker and Taylor, 1964) along with a rather complete bibliographical account of the present status of this system in vertebrate literature. It will therefore suffice here to merely point out that individuals of the Ambystomidae are completely transformed, particularly in regard to the urogenital system, indicating a degree of development considerably above that of the Proteidae and Cryptobranchidae in terms of the number of vasa efferentia from the testes to the longitudinal canal, the loss of a clear alignment of the epididymal nephrons, and the emancipation of the Wolffian duct from urine transport thereby establishing it as a true vas deferens or sperm duct.

This system in three genera of Salamandridae, *Diemictylus (Triturus) viridescens viridescens,* the eastern red-spotted newt, *Taricha granulosa,* the western rough-skinned newt, and *Cynops pyrrhogaster,* the Japanese red-bellied newt, is, in most respects, similar to that of the ambystomids and offers evidence for concluding that these two families of Urodela are perhaps closely related or at least represent similar degrees of development.

The urogenital systems of the Sirenidae (Willett, 1965) and the Cryptobranchidae (Ratcliff, 1965) offer further evidence of phyletic affinity in that the Sirenidae are more primitive than any of the Urodela and should perhaps be separated from this group, while the Cryptobranchidae are considerably more advanced than is assumed by Cochran (1961) and Jollie (1962).

This system in *Taricha torosa* (McCurdy, 1931) has four to five pairs of testes, those on each side connected by cords with a germ cell cord at each end. Each testicular lobe has two parts; the anterior is ivory and the posterior is biege in color. There is a longitudinal furrow on the dorsal side. A longitudinal canal arises in the most anterior renal corpuscle of the sexual or epididymal portion and gives off branches to each nephron. The first three or four urinary ducts of the definitive kidney connect directly with the Wolffian duct while 18-20 posterior ones pass to the cloacal region where they unite in a short ureter which joins the Wolffian duct as it enters the urogenital papilla.

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Adams (1940) finds this system in *Diemictylus viridescens viridescens* essentially similar to that of *Taricha torosa, Triton cristatus,* and *Ambystoma tigrinum.*

Portions of this system in *Cynops pyrrhogaster* have been described and indicate a progressive dissociation of the true ureters from the Wolffian duct during the course of evolution (Yamagiva, 1924).

Salamandra salamandra (Francis, 1934) is somewhat typical of other salamandrids in having bi-partite multiple testes, 4-5 vasa efferentia, a longitudinal canal (Bidder's canal), and a linear arrangement of nephrons in the sexual kidney each connecting to the Wolffian duct by a transverse tubule. The sexual kidney is presumed to secrete urine, thus making the Wolffian duct a true urogenital duct. The Wolffian duct is not joined by the purely urinary ducts from the definitive kidney until quite close to their common origin into the cloaca through a short common duct or ureter.

A comparative study of this system in these three genera of Salamandridae will clarify our concept of some of the finer structures such as the number of afferent epididymal ducts passing from the longitudinal canal to the epididymis, whether each nephron has such a connecting duct or a glomerulus, and the number of efferent epididymal ducts from the epididymis to the Wolffian duct in relation to the number of nephrons. Evidence will be presented to show that the Wolffian duct is definitely a true vas deferens and does not transport urine from the definitive kidney, thus it represents an advanced stage of development for this family.

Material and Methods

Diemictylus viridescens viridescens were obtained in quantities near Murphy, N. C., *Taricha granulosa* were collected near Gladstone, Oregon, while the *Cynops pyrrhogaster* were purchased from local tropical fish dealers who import them from Japan. Ten or more males of each genera were dissected and the urogenital system was studied in situ, sketched, removed carefully in toto and fixed. Several of each were serially sectioned 30 micra thick. Visual reconstructions were used for the details of the fine structures. A single anterior nephron was reconstructed from scale drawings from projection sections that were transferred to wax, cut out, and stacked.

Observations

The urogenital systems of these three salamandrids are quite similar in general appearance, and it is almost possible to consider any of them as a "typical" system. The testes are paired, bilobed and multiple, and interconnected on each side by slender constricted testicular regions. The bilobed condition is due to the degree of development of the reproductive cells, the anterior portion having spermatids or earlier stages while the posterior part contains mature sperm, especially during the fall and winter seasons. Four to five vasa efferentia pass from the testes to the longitudinal canal. Efferent epididymal ducts are 4-5 in *Diemictylus* and *Cynops* and 5-9 in *Taricha.* The epididymides are quite uniform in the three with a single row of nephrons, almost every one of which branches into an efferent epididymal duct that passes across to the Wolffian duct. A majority of the nephrons have glomeruli, and an absence of an afferent epididymal duct usually indicates the absence of a glomerulus. The Wolffian duct is coiled and may be filled with sperm previous to the breeding season. A rudimentary Mullerian duct is present in each; it arises near the pectoral girdle and enters the Wolffian duct tissue on its lateral side as it arches from the first nephron. This Mullerian duct cannot be observed externally after it enters the tissue of the Wolffian duct.

The most posterior efferent epididymal duct opens into the Wolffian duct lateral to the definitive kidney and may easily appear to be a urinary tubule. Serial sections show this duct clearly draining the posterior nephron of the epididymis. Urinary tubules are 11-20, being more numerous in *Taricha.* Each originates on the dorso-lateral border of the definitive kidney, enlarges as it curves ventrally and all of them become confluent with a short single ureter. The openings into the cloaca may be through a urogenital sinus in *Taricha* and *Cynops* or through separate openings in *Diemictylus.*

Diemictylus viridescens viridescens (Fig. 1) may have either one or two pair of testes with each being bilobed or single depending on the sexual activity. The usual number of vasa efferentia (va. eff.) is four and these enter the longitudinal canal (lo. c.). The afferent epididymal ducts (af. ep. d.), connecting the longitudinal canal to the epididymis (epi.), vary from four to six while there may be ten or more nephrons in the epididymis; therefore some nephrons do not connect to the testicular network. Additional nephrons have connections to the Wolffian duct (W. d.) and there are from seven to eleven efferent epididymal ducts (ef. ep. d.). A glomerulus is present in each nephron. The Wolffian duct receives the most posterior efferent epididymal duct alongside the definitive kidney, giving this duct the appearance of a urinary tubule. There are 12-16 definite urinary tubules; they all converge into a short ureter that has a separate opening into the cloaca through the urinary papilla (u. pap.) adjacent

Abbreviations Used in Figures

adrenal gland	adr.
afferent epididymal duct	af. ep. d.
definitive kidney	d. ki.
efferent epididymal duct	ef. ep. d.
epididymis	epi.
genital papilla	g. pap.
longitudinal canal	lo. c.
postcaval vein	p. c. v.
testis	tes.
rudimentary Mullerian duct	r. M. d.
urinary papilla	u. pap.
urogenital sinus	u-g. si.
urinary tubules	ur. tu.
vasa efferentia	va. eff.
ventral cloacal gland	v. c. g.
Wolffian duct	W. d.

to the opening from the Wolffian duct, the genital papilla (g. pap.). Adrenal glands are distributed along the medial ventral edge of both the epididymis and definitive kidney as small, lightly pigmented masses. A typical rudimentary Mullerian duct enters the arch of the Wolffian duct and extends its entire length buried in its tissue (r. M. d.).

Taricha granulosa (Fig. 2, 4) testes are definitely

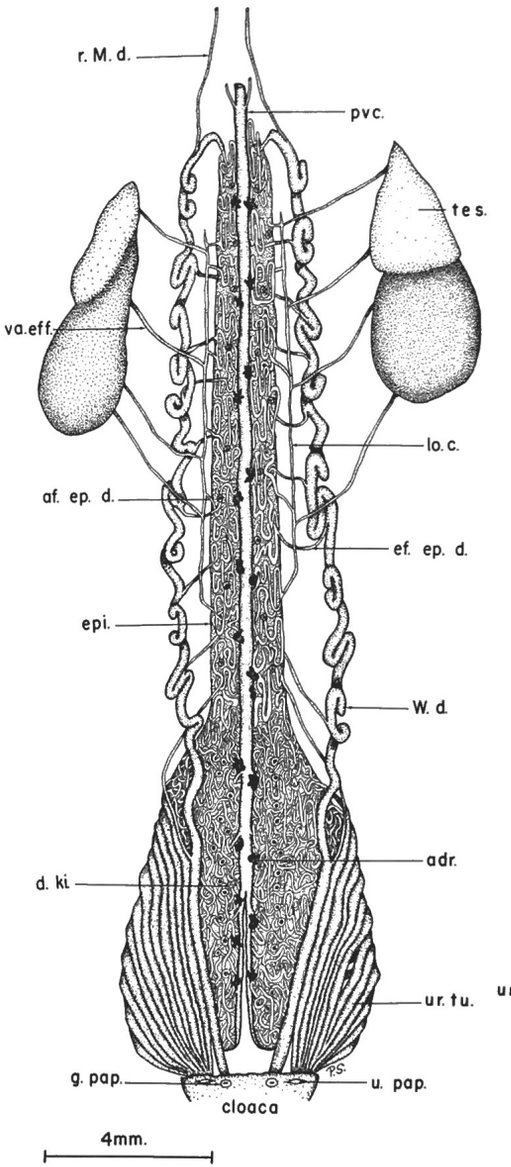


Fig. 1. Urogenital system of **Diemictylus viridescens viridescens.** The testes are extended laterally to show the underlying structures.

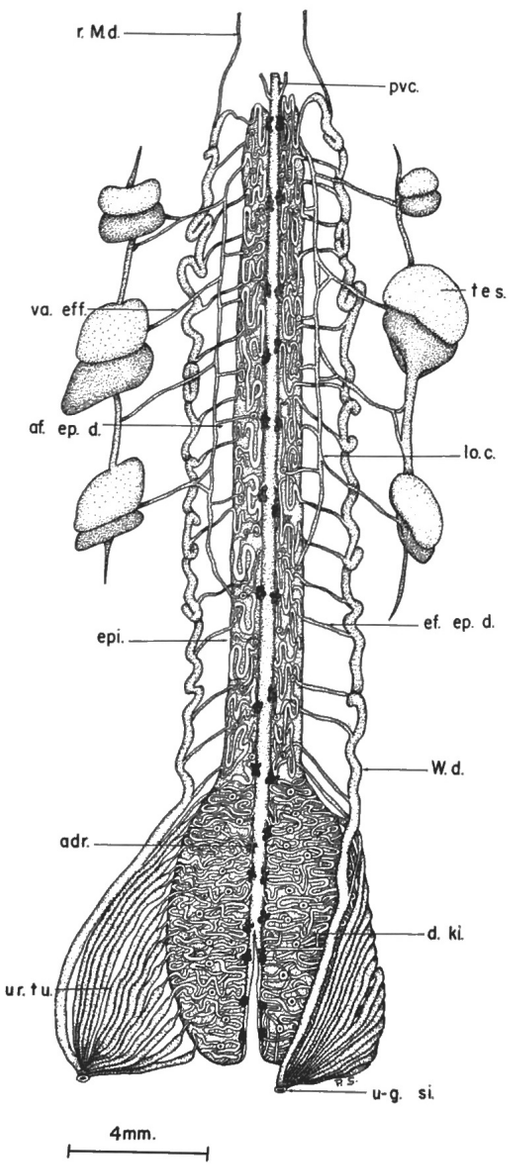


Fig. 2. Urogenital system of **Taricha granulosa.** The testes are extended laterally to show the underlying structures, and the urinary tubules and Wolffian duct on the right side are displaced so the underlying definitive kidney can be seen.

bilobed, and the number of pairs varies in adults from two to five. Seven of fourteen animals examined had three pair. In some the posterior pair may be quite small, and in one case there were four on the right side and five on the left side. The vasa efferentia (va. eff.) vary from four to seven and some may arise from the constricted testicular stalk. Two vasa efferentia may sometimes unite to form a single one. Both ends of the longitudinal canal (lo. c.) seem to merge into the epididymis. The afferent epididymal ducts (af. ep. d.) are 5-9 with the larger number in the animal with five pair of testes. The efferent epididymal ducts (ef. ep. d.) are 14-19 and seem to be the same as the number of nephrons in the epididymis.

The urinary tubules are 17-20 in number and enter a short ureter into which the Wolffian duct opens (Fig. 4,D) just before this urogenital sinus opens into the cloaca. Adrenal glands are distributed along the entire length of the opisthonephros. The rudimentary Mullerian duct enters the arch of the Wolffian duct and extends in its tissue for almost its entire length.

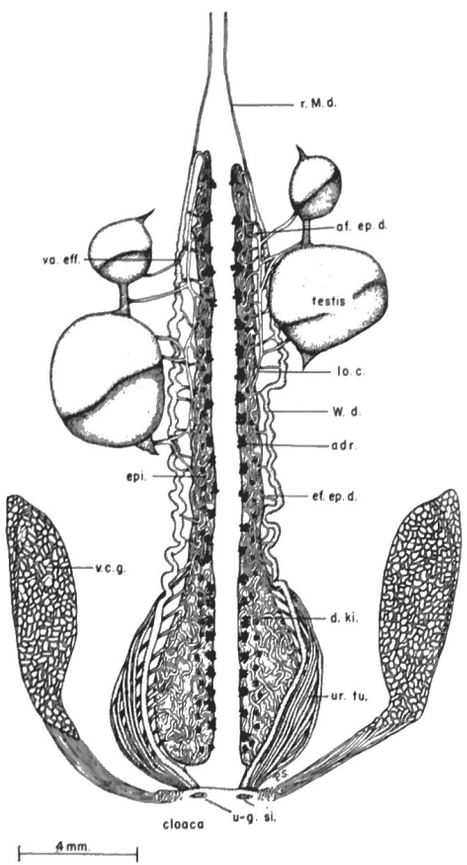


Fig. 3. The urogenital system of **Cynops pyrrhogaster.** The testes are extended laterally to show the underlying testicular network.

Cynops pyrrhogaster (Fig. 3) also has the typical bilobed, multiple testes varying from a single pair to four pair. These may all be the same size or the anterior or posterior pair may be quite small. Vasa efferentia are 4-5, a longitudinal canal connects them to the afferent epididymal ducts which may be 5-6. Approximately 16 nephrons are aligned in a single row in the epididymis and each has an efferent epididymal duct connecting to the Wolffian duct. The most posterior efferent epididymal duct enters the Wolffian duct alongside the definitive kidney. There may be 11-14 urinary tubules that arise on the lateral margin of the definitive kidney, arch ventrally to merge into a short ureter into which the Wolffian duct opens, forming a short urogenital papilla that opens into the cloaca. A typical rudimentary Mullerian duct enters the tissue of the Wolffian duct and passes almost its entire length.

A distinctive feature of *Cynops* is a very prominent ventral cloacal gland (v. c. g.) that opens into the cloaca by a number of tubules.

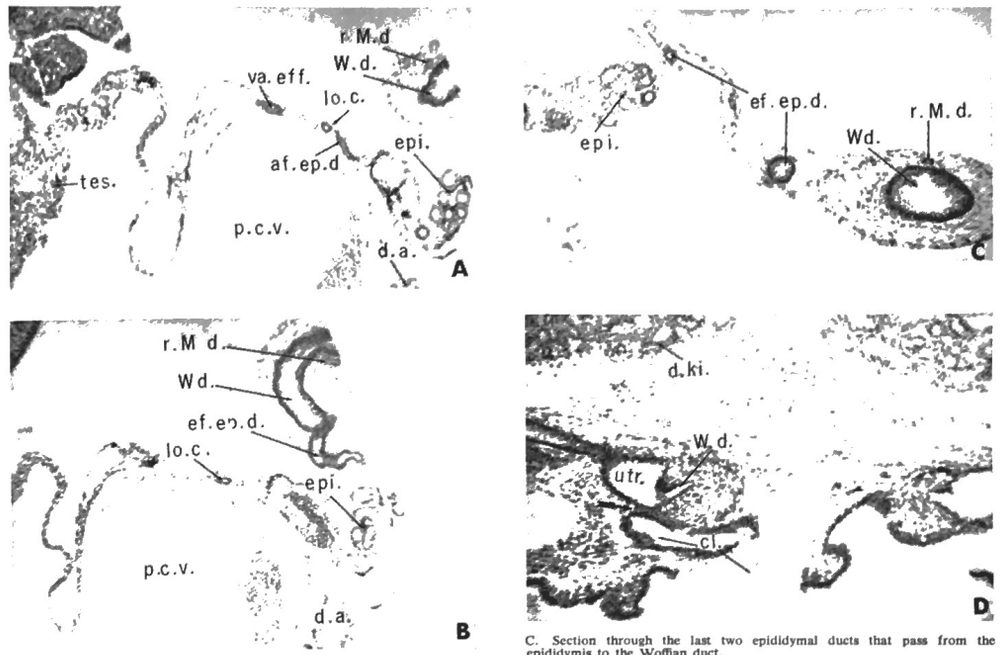
Discussion

The North American newts consist of two geographically separated and structurally distinct groups (Stebbins, 1954). The western group, *Taricha,* is confined largely to the Pacific Coast; the eastern one, *Diemictylus,* ranges from Pueblo, Mexico, and the tip of Florida to southern Canada and from eastern Kansas and central Texas to the Atlantic Coast. The European newts, as *Salamandra,* show an affinity with the eastern North American forms while the Asiatic newts, *Cynops,* are more closely related to our western species, *Taricha.* In the urogenital system the urogenital sinus of *Taricha* and *Cynops* support this relationship in contrast to separate openings into the cloaca in *Salamandra* (Francis, 1934) and *Diemictylus.*

The various species of *Ambystoma* also have the urogenital sinus (Taylor and Baker, 1964) similar to that of *Taricha* and *Cynops,* so the evidence indicates that *Diemictylus* and *Salamandra* are more advanced than other urodeles. A short ureter foreshadows the separate formation of a third kidney, the metanephros of amniotes (Witschi, 1956). The separate urinary papilla in *Diemictylus* is also evidence of this advancement.

The ambystomids are also similar to *Taricha* and *Cynops* in having the same general arrangement and number of nephrons in the epididymis, the same number of vasa efferentia, and the emancipation of the Wolffian duct from urine transport.

The rudimentary Mullerian duct, arising near the pectoral girdle, joins the Wolffian duct as it arches near the proximal end of the epididymis to enter the first nephron. This Mullerian duct extends almost the entire



A. Typical section near the anterior end showing epididymis, testis, and Wolffian duct with the connecting ducts.
B. Section with an efferent epididymal duct entering the Wolffian duct.
C. Section through the last two epididymal ducts that pass from the epididymis to the Wolffian duct.
D. Section showing small Wolffian duct entering the ureter. Sixty micra posterior to this section the urogenital sinus opens into the cloaca (at arrow).

Feg. 4. Representative cross-sections through the urogenital system of **Taricha granulosa.**

length of the Wolffian duct, embedded in its tissue, and it ends just before the Wolffian duct terminates.

There is a definite demarcation between the epididymis and the definitive kidney as indicated by the distinctive type of nephric tubules in each portion. In each genera the epididymis is approximately two-thirds of the entire opisthonephros. The definitive kidney is confluent with the epididymis and gradually broadens distally. These kidneys remain distinct from each other with no union dorsal to the cloaca as is reported in the *Sirenia* (Willett, 1965).

Further consideration of the phylogeny of the various urodeles will be deferred until representatives of other families have been examined, especially the Amphiumidae, Proteidae, and Plethodontidae.

Summary

The urogenital system of the Salamandridae indicates a close relationship with that of the Ambystomidae. *Diemictylus* is more advanced in this respect than *Taricha* and *Cynops* by having separate urinary and genital openings into the cloaca in contrast to a urogenital papilla. This also indicates a closer relationship between *Taricha* of the Pacific Coast and *Cynops* of Asia in contrast to the similarities between *Diemictylus* of the eastern United States and the *Salamandra* of Europe.

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