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OBSERVATIONS ON THE SEX OF CAMPELOMA (GASTROPODA: VIVIPARIDAE)

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IN THE course of many years of collecting in the fresh waters of northern United States (i.e., east of the Rockies) and Canada, it has been impossible to find males of *Campeloma*. Consequently, when a series of *Campeloma ponderosum coarctatum* (Lea) was collected at the mouth of Birdsong Creek on Kentucky Lake above New Johnsonville, Tennessee, in November, 1963, it came as a surprise to find that about half of the 34 specimens were males (Plate I). This scarcity of males in northern regions is also amply documented in the literature. H. J. Van Cleave and Dorothy Altringer (1937) studied the life history of *Campeloma rufum* (Haldeman), and in the four years of sampling they examined over 1500 specimens without seeing a single male. Norman Mattox (1938) studied the morphology of that same species and reported that among the 750 specimens he examined from Illinois, Kentucky, and Wisconsin, he too never saw a male. J. C. Medcof (1940) studied *Campeloma decisum* (Say) in the Speed River in Ontario and, although he made observations on the genitalia of 450 specimens, he never encountered a male. These observations and others, not published, reinforce the significance of the work of Norman Mattox (1938) who indicated that practically all *Campeloma* throughout northern United States are parthenogenetic.

Parthenogenesis is relatively uncommon in mollusks. Joseph Jacob (1957) indicated that A. E. Boycott (1919) was the first to prove that a parthenogenetic condition existed in *Paludestrina jenkinsi* (Smith) when he sectioned animals that were producing young in isolation. Males of *Campeloma* were known since 1888 when R. E. Call published on the gross anatomy of the genus and sketched the male genitalia. Many years later F. C. Baker (1928:53) gave a good figure of the male genital system of *Campeloma integrum* (Say). About thirty years ago this figure served to convince some of the parasitologists who were

studying a parasite (*Leucocholoridiomorpha constantiae*) that commonly appears as metacercaria in the uterus, that males do occur in this genus. However, Baker's figure had to suffice since among many thousands of specimens used in those life history studies, not a single male was ever discovered.

The parthenogenetic condition of *Campeloma rufum* (Haldeman) demonstrated by Norman T. Mattox (1937; 1938) places us in his debt for much of what is known concerning the morphology, cytogenetics, and functional anatomy in this group. In a more recent survey of parthenogenesis in animals, Esko Suomalainen (1950:196) gives a classification that places these northern *Campeloma* as "thelytoky" in which the eggs develop into females. The type of parthenogenesis found in this genus is further classified as "apomictic" in which chiasma formation occurs and it is followed by reduction in chromosomes. The normal diploid number is restored when the nuclei again fuse in what is called "automictic" fusion. Suomalainen listed a number of invertebrate animals that fall into this category and he indicated that no chromosome pairing had been found in any of those groups. These data are important in the sense that they may help to explain the very unsatisfactory state in which we find the systematics of *Campeloma* at present.

To determine the chromosome number, C. M. Patterson in our laboratory used gonadal tissue from three male and three female individuals of *Campeloma ponderosum coarctatum*. With the acetic-orcein squash technique (La Cour, 1941), it was determined that the diploid complement consists of 28 chromosomes (2n=28) as seen in mitotic metaphase cells. This observation is in agreement with the number reported by Pollister and Pollister (1943). More basic studies are necessary particularly among groups in which normal males and females are common. Hitherto, it was not generally appreciated that colonies with males occur mainly in the southern states. Consequently, bisexual material will be available only in streams of our southern states. Collections from those areas show that males are common among such other southern species as *Campeloma lewisii* Walker in the southwest and *Campeloma geniculum* (Conrad) reported from Alabama, Georgia, Florida, South Carolina, and North Carolina.

It has generally been conceded that the systematics of the species in the genus *Campeloma* are in a chaotic state. Such an unusual life history in which some of the species are parthenogenetic while others, mainly those in the south, are bisexual with normally functioning males, may, in part, account for the difficulty in delineating species.

The *Campeloma ponderosum coarctatum* (Lea) considered here is a case in point in that it appears to have a southern distribution and should not be confused with *Campeloma subsolidum* (Anthony), the animal of which was figured by Bryant Walker (1918:25). In his Catalog (*Ibid.*:129) he stated: "*Campeloma subsolidum* (Anthony) is a valid species. Whether the *Paludina exilis* of Anthony is a sexual form as believed by Lewis and others or an individual or local mutation is unsettled. The fact that it has not been found in southwestern Michigan, where the species is a common one, would seem to cast doubt on its being a sexual variation." Further, with respect to the distribution of *C. ponderosum coarctatum,* Calvin Goodrich (1944) in his study of the operculates of the Coosa River indicated that this subspecies as redefined by Bryant Walker "is the common form of the Coosa from streams above Rome, Georgia, to Wetumpka, Elmore County, Alabama, the colonies apparently populous. Collections have also been made in sidestreams of Etowah, St. Clair and Talladega counties, Alabama, but so near the discharges that probably all should be considered riverine mollusks." Walker (1893) quoted DeCamp to stress that *Campeloma ponderosum* is a southern species and not found in Michigan. All subsequent work in Michigan has borne out this contention.

The ecology of *C. ponderosum coarctatum* has been unusual in the sense that the animals are local in their distribution. In the fall of 1963 the water level was very low and specimens were fairly common at the mouth of Birdsong Creek. They were obtained by John M. Bates and myself by feeling for them in the soft mud in 1 to 4 feet of water. The following spring, John Condor kindly attempted to obtain a series to be used to compare gonad activity between the two seasons. The water level in the spring of 1964 was so high that he was able to get only a single specimen by diving into water well over his head. This specimen was sectioned but unfortunately was too heavily parasitized to yield any normal gonad tissue.

In August, 1964, John Bates and I again tried to collect another series of *C. ponderosum coarctatum* at the same station (Birdsong Creek); we were able to get only half the number collected the previous fall, although the conditions for collecting were about as favorable. Since our main objective was to obtain mussels, it was necessary to collect in mud flats over an extensive area along both sides of the Kentucky Lake impoundment in this region of the Tennessee River. While fine silt and mud flats—conditions which usually appear ideal for *Campeloma*—were extensive both above and below New Johnsonville, the only colony of *Campeloma* found was that at the mouth of Birdsong Creek.

It remains an open question whether this localized pattern in its distribution is related to the feeding habits of these snails. Studies such as those by Allison (1942) indicate that *Campeloma* is a carrion feeder. The presence of a clammer's camp at the mouth of the creek may have provided a source of decaying mussel meats which could have served as food and may account for the concentration of *Campeloma* there. Allison trapped them with chicken manure in cloth bags; Bovbjerg (1952) and Medcof (1940) also emphasized the tendency for *Campeloma* to form aggregations. Similar colonies are commonly observed in lakes and streams in Michigan.

The live specimens collected in the Tennessee impoundment at Birdsong Creek were relaxed in sodium nembutal and then fixed in Bouins fluid. The relaxing process in this group is slow and it is not unusual to subject these animals to anaesthesia for three days before they are ready for fixation. While Lever *et al.* (1964) give a rapid method for relaxing mollusks, it is not a method easily applied in the field. Nevertheless, our procedure provided well-relaxed specimens. Several of these animals were later prepared as whole mounts (Plates II and IV) using Guyer's borax carmine; they were then cleared in methyl salicylate. Other specimens were subjected to the usual paraffin technique and sectioned to observe gonad activity (Plate V) and the structural relations of the organs. Whole mount preparations served to illustrate the gross morphology of the male and female reproductive systems. Since the male genital system has not been previously studied in detail, this system will be particularly stressed.

Male Genital System.—The recognition of a modification of the right tentacle to serve as a verge or penis often permits a quick and easy separation of male and female specimens. At times, the modified right tentacle may be twice the size of the unmodified left one (Plate I). However, it came as a surprise after separating a series of specimens, using the appearance of the right tentacle as the criterion, to discover that serial sections made of specimens which appeared to be females turned out, under microscopic examination of the gonad tissues, to be males. In this connection it is also of interest to note the wide variation found among the verges of males in one series (Fig. 1). The problem as it concerns the shape of the verge appears related to the age of the specimen in such a way that the young of the year are not apt to have the right tentacle clearly modified. Small specimens from this colony, presumably the young of the year, showed little difference in the appearance of the right and left tentacles. It was only when some of these smaller specimens were stained and cleared that the slight modifi-

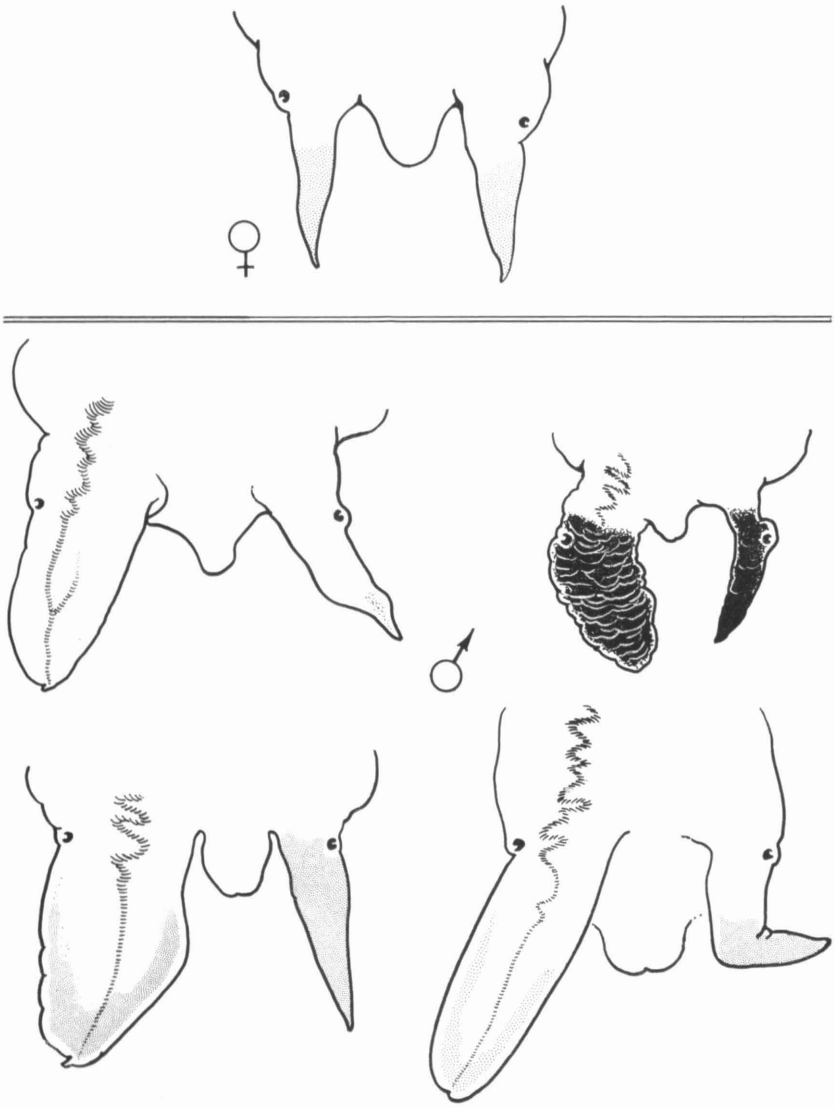

FIG. 1. Modification of the verges among a series of male specimens of *Campeloma ponderosum coarctatum* (Lea). Top: a normal female. Bottom: differences in size, shape, the amount of pigment, and the prominence of the small papilla normally present near the opening of the vas.

cation of the right tentacle was evident; there was additional indication when the vas deferens could clearly be seen entering the right tentacle after the specimens were cleared.

In studies of the northern parthenogenetic forms, Medcof (1940) reported that reproduction in *Campeloma decisum* (Say) did not start until the second summer, and in most cases it was delayed until the third. He also observed that the size of the parent appeared directly related to the animal's reproductive capacity. Hence, colonies are maintained largely by the reproductive activity of the three-year-old specimens. However, Van Cleave and Altringer (1937) found that *Campeloma rufum* (Haldeman) was maintained for the most part by the two-year-old specimens. In both studies the mature embryos were observed to accumulate in the uterus during the winter. While it has not been studied in *Campeloma ponderosum coarctatum* (Lea), there appears to be a similar lag in the sexual development among these bisexual forms as seen in sections of gonads.

Structurally the male genital system is essentially as reported by Call (1888) and F. C. Baker (1928). The testis (Plates IV and V) is large and massive; its numerous acini form a thick layer surrounding the liver or digestive gland. Sperm leaving the testis pass to the prostate (Plate IV) which is a thickly coiled structure lying along the top of the last whorl. In cleared specimens the vas deferens appears as a long and loosely coiled tube connecting the prostate with the verge or penis terminating in the enlarged right tentacle. The verge is supplied with a prominent penial nerve; it also has a small but distinct papilla on the side of the penial aperture.

The size relation between the testis and the digestive gland is striking in that the testis covers the greatly reduced liver in the upper whorl and a half; the same relationship is shown in one of the serial cross sections (Plate V). An enlarged section of the testis of a specimen collected November 14, 1963, shows many acini with active spermatogenesis. The numbers of sperm produced are prodigious, and in this section there are all stages in spermatogenesis.

Female Genital System.—In contrast to the males with their enormous testis, the females have an ovary which is so small as to make it difficult to find. Several females were sectioned and the ovary was seen only in a young specimen (Plate V). As indicated by others who have studied viviparid snails, the eggs are usually very small. The eggs are retained in the uterus where they develop into large and well-developed young (Plate II). The retention of the eggs and the development of numerous young accounts, in part, for the difference in the shape of

the female as compared with the male specimens (Plates I, II and IV). This difference is seen both in the animals with and without their shell. The relation of the pallial organs is shown in Plate III.

HISTOLOGY.—In addition to the gross appearance of the organs of a male, a section of a specimen (Plate V) gives the general histological features of the testis, digestive gland, stomach, intestine, prostate, kidney and gill. In another serial section of a female (Plate V) the large and swollen bursa copulatrix and the adjoining seminal receptacles were observed packed with sperm as an evidence that copulation had taken place before the middle of November. It would be difficult among animals such as *Campeloma* which live completely buried in the mud or sand substratum to observe the process of copulation in nature.

While it was not possible to study the feeding of this species, the relatively small size of the buccal mass as well as the long and thin esophagus (Plates II and IV) indicates that the food would not be coarse particulate material. The large glandular and muscular development of the forward portion of the foot in both males and females suggests that there are some unusual feeding mechanisms in this group of snails. While they are known to be carrion feeders, the functional relation of this highly developed foot and the relatively undeveloped digestive system with a small stomach and comparatively small digestive gland, all suggest a highly specialized type of feeding. In normal activity these animals remain buried in the mud or sand and are usually not observed unless they are screened out of the substratum.

ACKNOWLEDGMENTS.—Particularly helpful in this work was the assistance and interest of John M. Bates both in procuring specimens and in providing information. The plates to show gross morphology were made by William L. Brudon; C. M. Patterson determined the chromosome number and also assisted in micro-photography. John Condor kindly attempted to obtain a spring collection under difficult and hazardous flood conditions. I am indebted to these and others who have made this study possible.

SUMMARY AND CONCLUSIONS

1. Most *Campeloma* from northern states and Canada are parthenogenetic; species such as *Campeloma ponderosum coarctatum* and *C. geniculum*, typically southern species, tend to produce both males and females.
2. Parthenogenesis in such northern forms as *Campeloma rufum* and *C. decisum* was clearly demonstrated by N. T. Mattox; eggs develop in females by an apomictic process and the normal diploid number is restored by automictic fusion

according to Suomalainen. The number of chromosomes in males of *C. ponderosum coarctatum* is 2n=28.

3. The chaotic state that exists in the determination of species in *Campeloma* may well be linked to the lack of uniformity in the way these animals reproduce.
4. The colonies of *C. ponderosum coarctatum* appeared to be sporadic, suggesting that aggregations occur where the habitat enables the animals to burrow and where there is a source of carrion food.
5. Males of this species not only tend to be smaller but they tend to be narrower than the females.
6. While the right tentacle becomes modified to form a verge or penis, its size and shape seem to vary sufficiently to make the use of this organ in systematics impractical. Young specimens show such a slight modification of the right tentacle as to make its use in sexing difficult.
7. The testis of males is large and covers the digestive gland; spermatogenesis was observed in November; the ovary is very small and almost impossible to find in many of the females.
8. Evidence that some mating occurred in the fall was found in a female with the bursa copulatrix full of sperm; the females of this species carry the young in the uterus into the winter.

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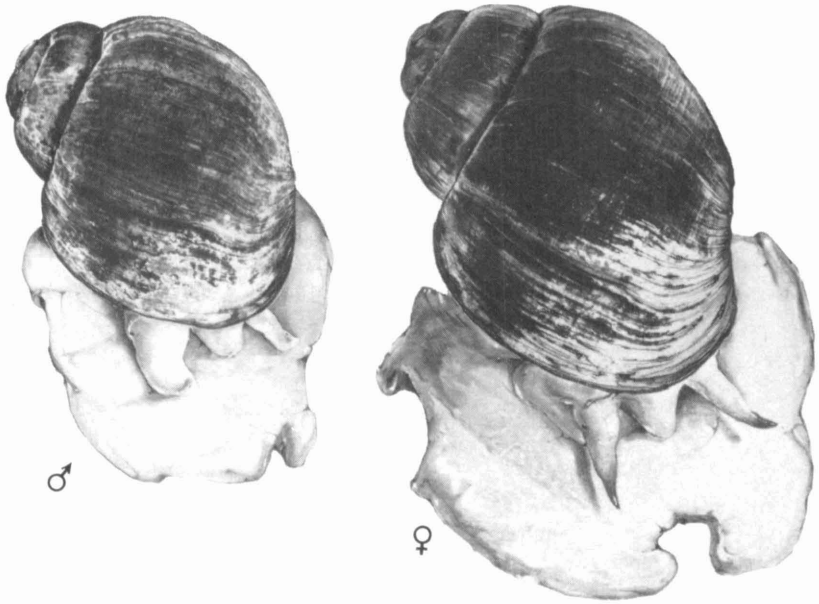


PLATE I

Male and female of *Campeloma ponderosum coarctatum* (Lea) collected at the mouth of Birdsong Creek on Kentucky Lake south of New Johnsonville, Tennessee; × 1.4.

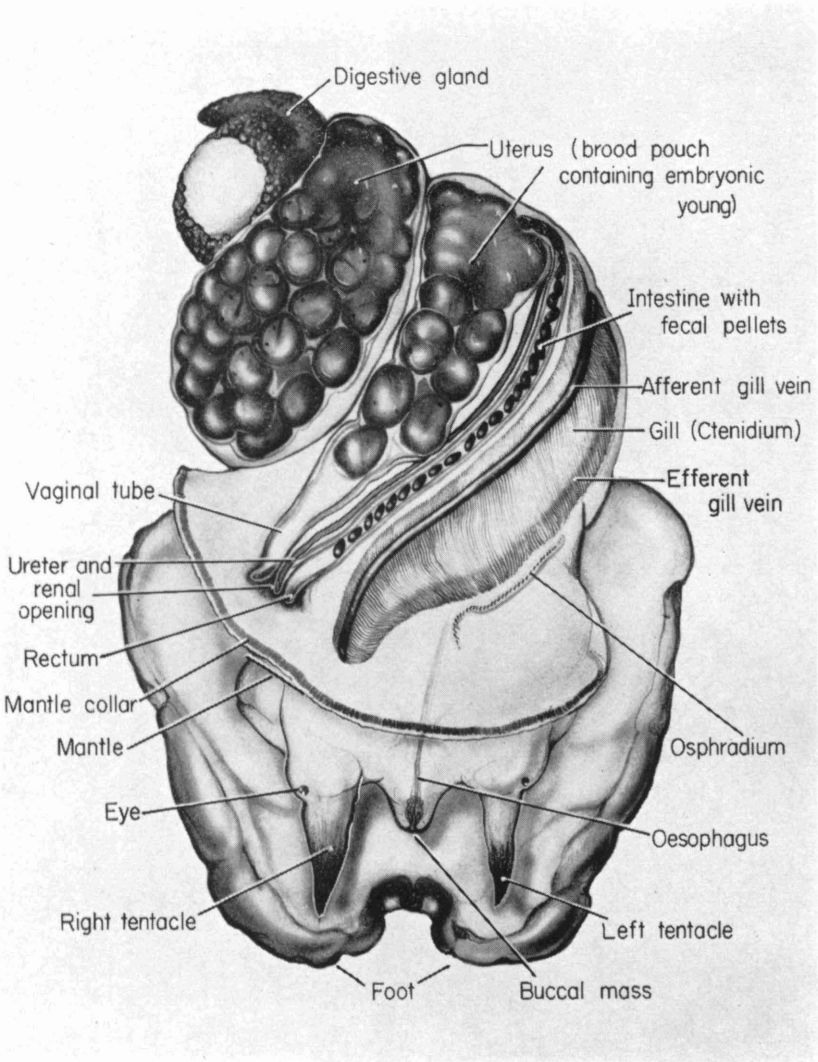
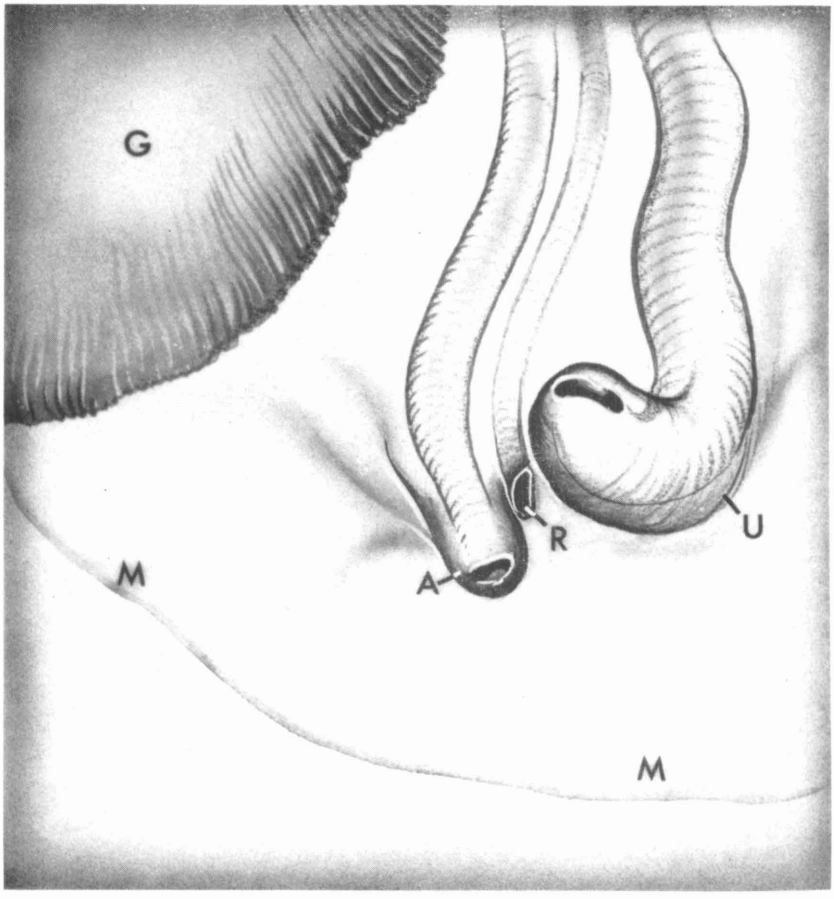


PLATE II

Female *Campeloma ponderosum coarctatum* (Lea) showing the structures visible in a cleared whole mount; particularly striking is the large number of young retained in the uterus of this specimen; collected in November, 1963; prepared as whole mount and stained with Guyer's borax carmine.



G = Gill, M = Mantle, A = Anus, U = Uterus & Vaginal tube, R = Renal Opening

PLATE III

Organs of the pallial cavity of a female *Campeloma ponderosum coarctatum* (Lea).

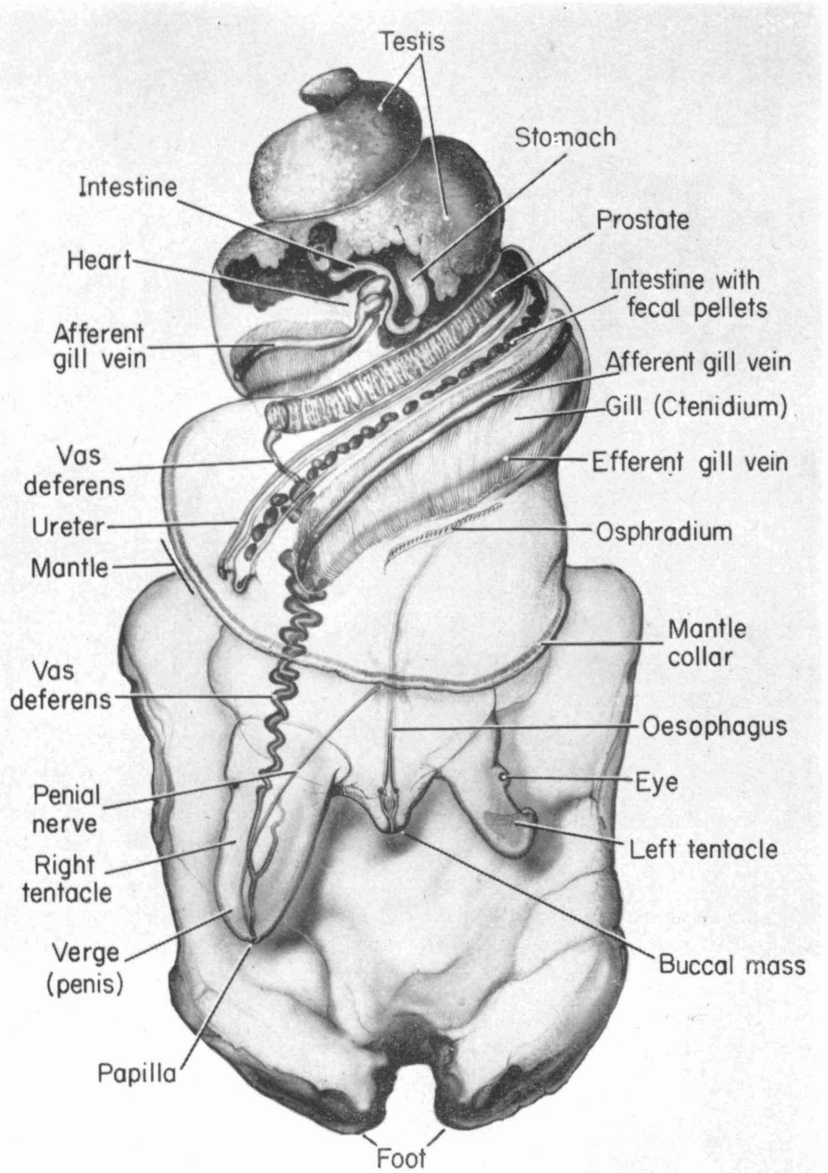


PLATE IV

Male *Campeloma ponderosum coarctatum* (Lea) to show the relation of the organ systems as seen in a cleared, whole mount. × 3.8.

PLATE V

Histological section of *Campeloma ponderosum coarctatum* (Lea)

UPPER LEFT: Low power view of section of a male specimen to show relation of testes, digestive gland, stomach, prostate, kidney, gill, and osphradium.

UPPER CENTER: Testes and digestive gland as seen in section through an upper whorl to show the relatively small amount of digestive gland and the massive male gonad.

UPPER RIGHT: Enlarged section of upper whorl to indicate the large number of acini in a male with very active spermatogenesis; these specimens were collected at the mouth of Birdsong Creek on November 14, 1963.

MIDDLE LEFT: An enlarged section of upper whorl of a male to show the digestive gland and acini with very active spermatogenesis.

MIDDLE CENTER: Several acini with mature sperm in the lumen and spermatogenesis in an active state in the walls of the acini.

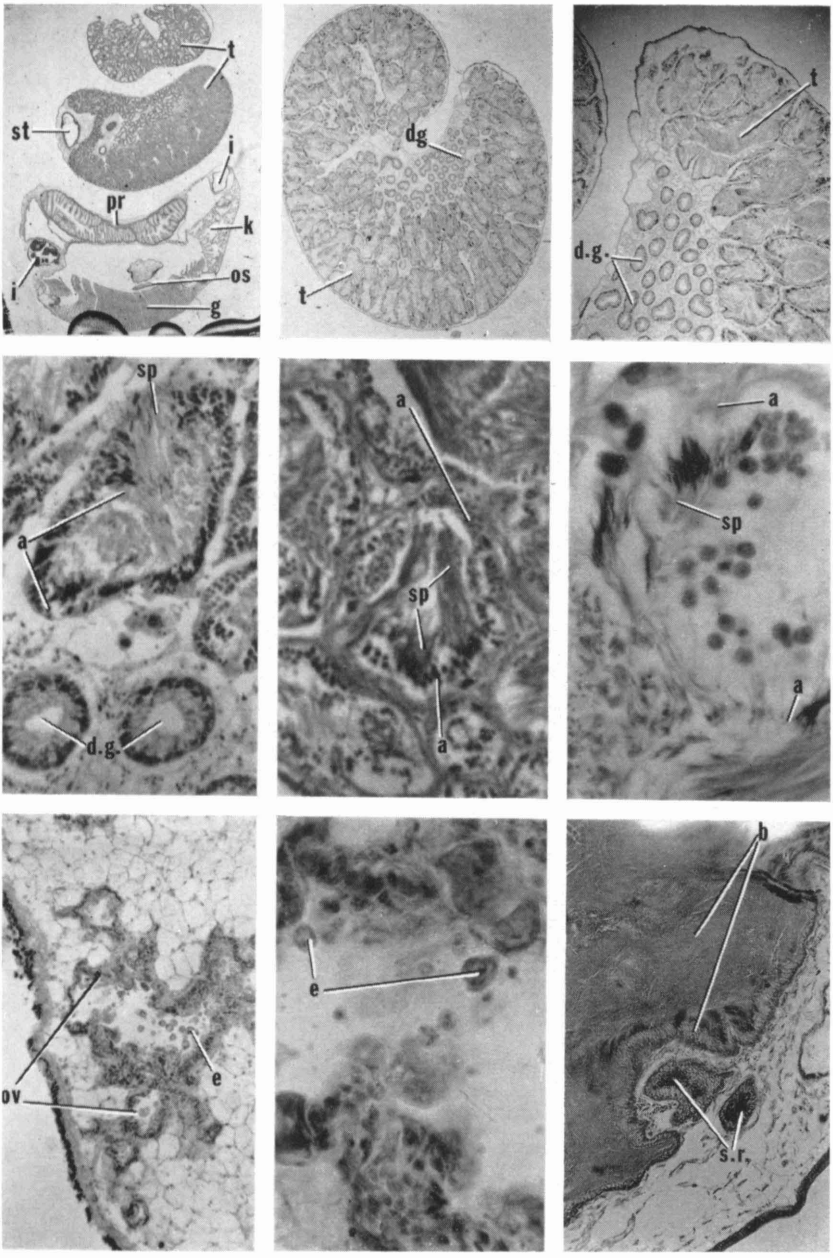
MIDDLE RIGHT: An enlarged acinus with various stages in spermatogenesis.

LOWER LEFT: Gonad of a female showing ovary; note the relatively small size of the eggs.

LOWER CENTER: An enlargement of the ovary with eggs free in the lumen and active oogenesis in the wall.

LOWER RIGHT: Bursa and seminal receptacle of female filled with sperm on November 14, 1963, indicating that copulation had taken place in the fall of that year.

ABBREVIATIONS: a—acinus; b—bursa copulatrix; d.g.—digestive gland; e—egg; g—gill; i—intestine; k—kidney; os—osphradium; ov—ovary; pr—prostate; sp—sperm; s.r.—seminal receptacle; st—stomach; t—testes.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