

OBSERVATION ON THE URINOGENITAL ORGANS OF
MICROHYLA ORNATA (DUM. AND BIBR.)

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INTRODUCTION

Since the publication of a comparative account of the urinogenital system of amphibians by Spengel (1876), only a few accounts of this system have appeared, especially of Salientian forms. Gaupp (1904) gave a detailed account of the urinogenital system of *Rana* in which he elucidated many terms used in the descriptive morphology of the system. The genera *Bufo* and *Rana* have been more or less thoroughly investigated, and the differences between their urinogenital organs recorded. The urinogenital system of frogs and toads, particularly of males, shows many remarkable modifications (Gadow, 1901; Noble, 1931; Hoffman, 1932; Bhaduri, 1932). The male urinogenital ducts or ureters of the female in several unrelated species of Salientia have been shown to unite before opening into the cloaca (Gilchrist and von Bonde, 1922; de Villiers, 1934; Bhaduri and Banerjee, 1939). The possibility of a correlation between the modifications of urinogenital organs and the breeding habits was indicated by Bhaduri (1932) in *Rhacophorus*. Further, vestiges of Müllerian ducts are often present in the males of many genera and species of Salientia, and are, so far as we know, always present in the genus *Bufo*, very interesting observations having been made by Eggert (1926), Starks and Howard (1929), Koch (1934) and Bhaduri and Banerjee (1939) on the course and fate of these ducts in several species of *Bufo*.

On a study of the literature we find that with the exception of Spengel (1876), who examined one spirit-preserved male specimen of '*Hypopachus Seebachii*' (= *H. variolosus* (Cope), vide Parker, 1934, p. 112), nobody has attempted to investigate the urinogenital organs of any other members of the Microhylidae as defined by Parker (1934). We have, therefore, investigated the urinogenital organs of a widely distributed narrow-mouthed toad *Microhyla ornata*, which presents interesting modifications of its urinogenital ducts.

ACKNOWLEDGMENTS

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MATERIAL AND METHODS

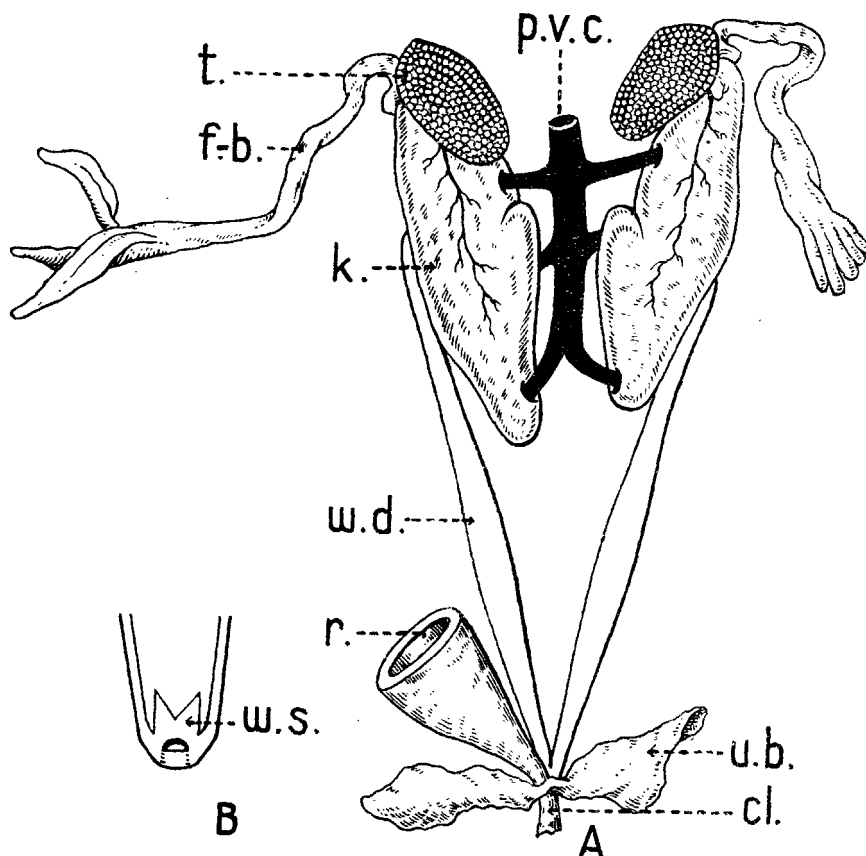
At the instance of the senior author, Mr. S. K. Das collected several specimens of *M. ornata* from his native district Mymensingh in East Bengal, during the month of July, 1938. The collection consisted of a few pairs which were caught in amplexus, and a number of fully mature breeding males and females. Besides, we received a pair of *M. ornata* from Dr. L. S. Ramaswami, who informed us that the pair was caught in amplexus by his Assistant, Mr. A. N. Rao, at Bangalore on September 17, 1939. In all cases

specimens were fixed in Bouin's fluid after giving an abdominal incision, and were in an excellent state of preservation.

The urinogenital organs were carefully dissected under a binocular dissecting microscope; next, serial transverse sections were cut to verify the correlation of various structures, especially of the ducts opening into the cloaca. As *M. ornata* does not attain a length of more than 22.5 mm. in male and 25.0 mm. in female (Parker, 1934), sections were found very useful in studying the urinogenital organs.

OBSERVATIONS

1. In the male the two kidneys (Text-fig. 1, *k.*) are dorsoventrally flattened and are situated in the posterior part of the coelom attached to the dorsal body-wall. The



TEXT-FIGURE 1 (A)—Diagrammatic ventral view of the urinogenital organs of a male *Microhyla ornata*. $\times 8$. (B)—Diagrammatic ventral view of the urinogenital ducts of the same showing the urinogenital sinus and its opening into the cloaca.
cl.—cloaca; *f.b.*—fat-body; *k.*—kidney; *p.v.c.*—posterior vena cava; *r.*—rectum (cut); *t.*—testis; *u.b.*—urinary bladder; *w.d.*—urinogenital duct; *w.s.*—urinogenital sinus.

outer border of each kidney is more or less semilunar in shape, while the inner border has a deep notch about the middle of its length. The two kidneys are asymmetrically placed, the left lying slightly anteriorly to the right. The male specimens were, on an average, about 22.0 mm. in length; the measurements of their kidneys are given below:

Measurements in millimetres.

Serial No.	Length of kidney.		Breadth of kidney.	
	Rt.	Lt.	Rt.	Lt.
1	5.0	5.0	2.0	2.0
2	5.0	5.0	2.1	2.0
3	5.3	5.1	2.5	2.0
4	4.8	4.8	2.0	1.9

The kidneys measure, on an average, 5.0 mm. by 2.0 mm. It may be noted that the ventral surface of the anterior portion of each kidney is slightly depressed, presumably owing to the lodging of the testis at that place.

The testes (*t.*) are more or less oval in shape and are attached to the kidneys by the mesorchia. The right and left testes are almost similar in shape and size, their average measurements being 2.0 mm. by 1.3 mm. The measurements of testes of the same four specimens, of which the kidney measurements are given above, are appended below:

Measurements in millimetres.

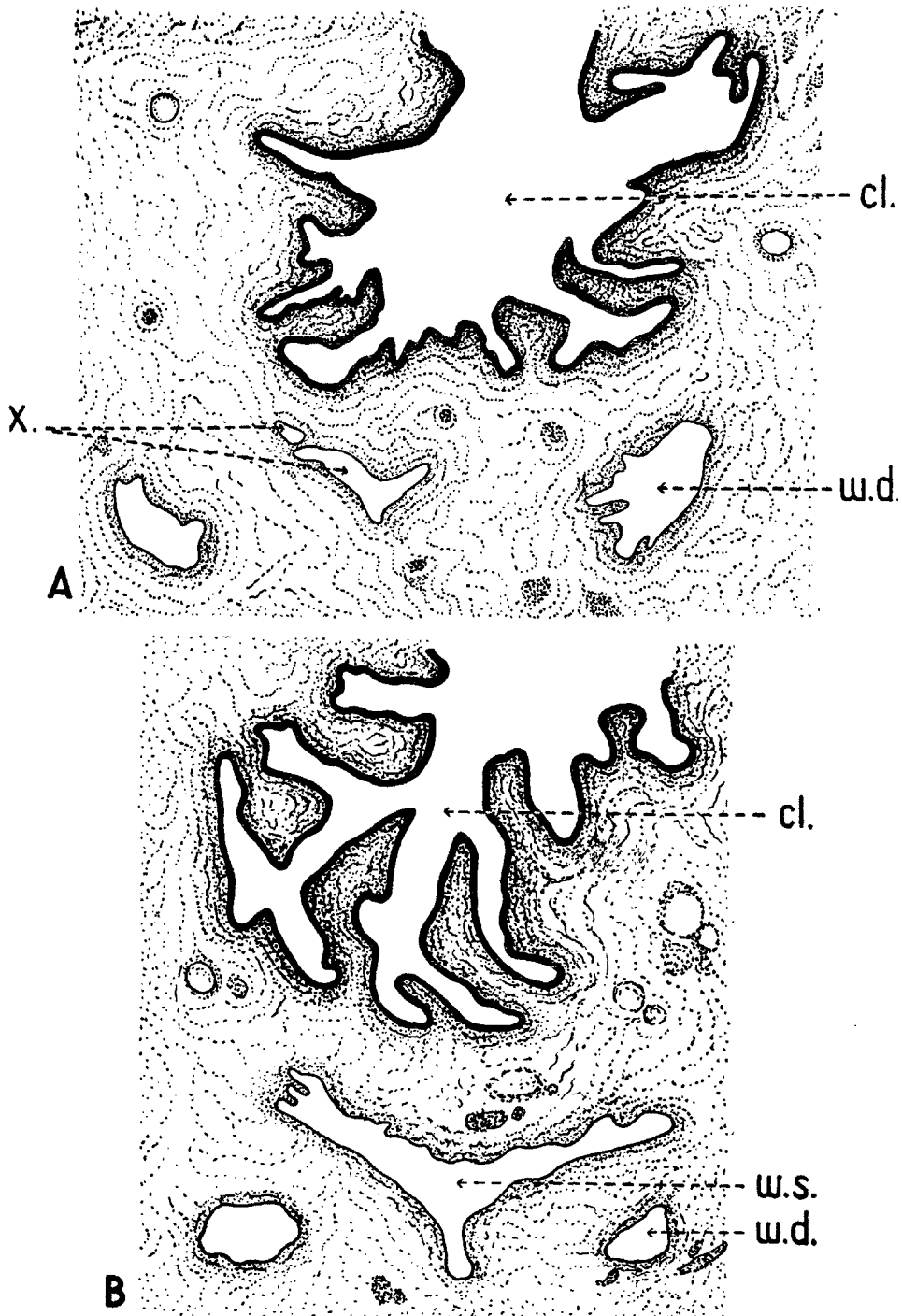
Serial No.	Length of testis.		Breadth of testis.	
	Rt.	Lt.	Rt.	Lt.
1	2.0	2.0	1.5	1.0
2	2.0	2.0	1.3	1.3
3	2.0	2.0	1.5	1.5
4	1.9	1.9	1.1	1.5

The vasa efferentia pass through the mesorchia from the inner side of each testis to the kidney of its own side. Since serial sections did not help us very much in determining the number of vasa efferentia given off by each testis, we have relied only on our dissections, and have found that there are, on an average, four to five unbranched vasa efferentia entering each kidney.

The fat-body (*f.-b.*) is attached to the anterior end of each testis (*t.*), and is closely applied also to the surface of the kidney (*k.*). It is not a massive structure like that of *Bufo* or *Rana*, but forms a long narrow ribbon-like stalk with three to five blunt finger-like short processes at its free end. It has not, however, been noted whether the fat-bodies show any seasonal variation in size, as generally happens in frogs and toads. The condition described may be taken as that found generally during the breeding season.

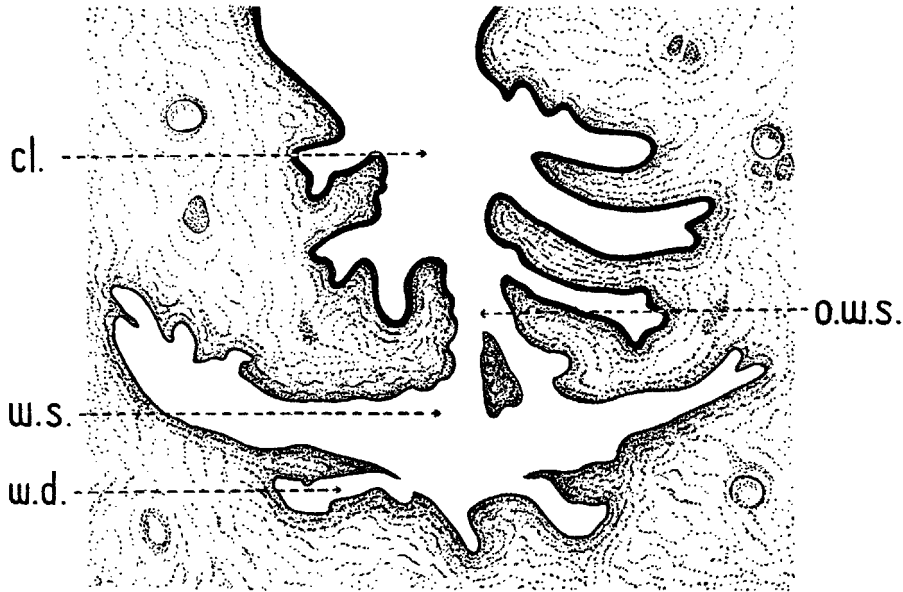
The urinogenital duct* (*w.d.*) starts from about the middle of the outer margin of each kidney, and runs alongside it up to its posterior end; on leaving the kidney it slightly increases in diameter as it courses backwards, but gradually decreases again as it approaches the cloaca. While coursing further backwards it runs in close apposition to, but quite separately from, its fellow of the opposite side. Each duct gives off a short conical diverticulum on its inner side, about 4.7 mm. behind the lower end of the kidney. The diverticulum of each side runs forward, and fuses with that of the opposite side forming an 'M'-shaped sinus in between the two urinogenital ducts, as shown diagrammatically in text-figure 1 B. The diverticulum is short, about 0.37 mm. in length, and 0.34 mm. in breadth, at the point of origin from the urinogenital ducts. Text-figure 2 A represents a transverse section showing the two tips of the diverticula (*x.*) only slightly separated from each other, and situated between the two urinogenital ducts (*w.d.*). In text-figure 2 B, the diverticula have coalesced to form a median sinus (*w.s.*), although the urinogenital ducts (*w.d.*) remain separate from it even at this level, and lie slightly dorsally to it. The section in text-figure 3 shows the urinogenital ducts (*w.d.*) becoming confluent with the transversely expanded urinogenital sinus (*w.s.*), and opening into the cloaca (*cl.*) by a single mid-dorsal aperture (*o.w.s.*). Thus a study of serial transverse sections shows that the urinogenital sinus is a minute sac-like structure, lying at the terminal

* We have followed Bhaduri and Banerjee (1939) in adopting the term urinogenital (Wolffian) duct, rather than the shorter term 'ductus deferens' used by Gaupp (1904).



TEXT-FIGURE 2.—Transverse sections through two succeeding regions of urodaeal parts of the cloaca of a male *M. ornata* to show (A)—the two tips of the diverticula of the urinogenital sinus (*x.*); and (B)—the confluence of the diverticula forming a urinogenital sinus (*w.s.*). $\times 126$. (Letterings as in text-figure 1.)

portion of the urinogenital ducts (Text-fig. 1 B). The wall of the urinogenital sinus shows the same general histological structure as that of the urinogenital duct.



TEXT-FIGURE 3.—Transverse section passing through the region succeeding that of the text-figure 2 (B), to show the confluence of the urinogenital ducts (*w.d.*) with the urinogenital sinus (*w.s.*) and the opening of the latter (*o.w.s.*) into the cloaca (*cl.*). $\times 112$.

A vesicula seminalis is not present, but a slight dilatation of the middle portion of the urinogenital duct (Text-fig. 1 A, *w.d.*), as found in *Bufo melanostictus* (Bhaduri and Banerjee, 1939), is also observed here. Mention may be made of the fact that in one series of sections clusters of sperms were found inside the crypts of the urinogenital ducts, but not in the urinogenital sinus, although that is the terminal passage through which the sperms descend into the cloaca.

Gilchrist and von Bonde (1922) in *Xenopus*, and Bhaduri and Banerjee (1939) in *Bufo melanostictus* have already pointed out that the urinogenital ducts unite together terminally, and open by a single orifice into the cloaca. *M. ornata* shows the same phenomenon, but here the urinogenital ducts, instead of uniting together, form terminally a bicornuate sinus which opens by an aperture into the cloaca.

De Villiers (1934), in dealing with the phallic organ of a primitive Salientia, *Ascaphus truei*, as an intromittent structure, observes that a urinogenital sinus is formed on the dorsal wall of the cloaca, and that the united portion of the urinogenital ducts opens into this sinus. He further points out that the urinogenital sinus, which is absent in *Rana*, is a constant and prominent feature in the Gymnophiona. Unfortunately he has not given a detailed description of the course and fate of the ducts and the sinus. His text-figure 9 A on page 38 may be compared with our text-figure 2 B. There is no doubt that he has also observed a urinogenital sinus, which may not be bicornuate as described above in *M. ornata*. De Villiers (*op. cit.*, p. 39) further expresses the opinion that the urinogenital sinus in *A. truei* 'is to be looked upon as a cloacal derivative', since it 'has an epithelial lining agreeing in most respects with that of the cloaca'. We have, on the other hand, described the urinogenital sinus as a portion of the urinogenital ducts also on the ground of a general histological agreement between the two. It would seem *a priori* that this difference of opinion is morphologically a very slight one, but it is hoped that it could be easily settled by a study of the development and formation of the urinogenital sinus.

In Spengel's account of *Hypopachus variolosus* there is no mention of a structure like the urinogenital sinus, while the disposition of its urinogenital ducts is more or less similar to that of the ordinary ranids.

The urinogenital sinus, therefore, becomes a remarkable anatomical feature in Salientia, especially because of the fact that it has been observed in two different genera, *Microhyla* and *Ascaphus*, belonging to two unrelated families. It would appear that there is some physiological reason for the provision of a urinogenital sinus at the terminal part of the urinogenital ducts. It is possible that the urinogenital sinus is an improvisation for storing and holding the sperms that descend through the urinogenital ducts, as the vesicula seminalis provides in some frogs (*Rana*). We have already pointed out that a seminal vesicle, like that of *Rana* described in many text-books, is not present in *M. ornata*. It may be noted that we have found clusters of sperms in the crypts of the urinogenital ducts in only one series of sections, there being no sperms in any of the several series that we have studied. This means that either the sperms have been discharged completely from the sinus or have not been stored in it so far. In Holmes' *Biology of the Frog* (1926, p. 49) it is noted that in the copulating specimens of *Rana temporaria*, of which a large number had been examined, the seminal vesicles contained no traces of spermatozoa.

The Müllerian ducts, which are found in very many species of male Salientia as vestigial structures, are totally absent in *M. ornata*. *Hypopachus* also does not show this structure (Spengel, 1876). It may incidentally be pointed out here that the Müllerian ducts, though shown to be present by Noble (1931, p. 274, Fig. 99, M.D.) in *Ascaphus truei*, have been noted by de Villiers (1934) to be absent. In this respect also *A. truei* and *M. ornata* resemble each other.

The urinary bladder (Text-fig. 1 A, *u.b.*) does not show any special feature. It is more or less like that of the ordinary frogs and toads. It is a bilobed sac with thin walls lying ventrally in the abdominal cavity, and opening by a single aperture into the ventral wall of the cloaca opposite the single opening of the urinogenital sinus.

2. In the female of *M. ornata* the urinogenital organs (Text-fig. 4) are built on the usual ranid pattern. The female specimens dissected by us were only slightly larger than the males, the average length being 22.3 mm.

The chief differences between the kidneys of the male and those of the female concern their relative size and position. The kidneys (*k.*) are slightly smaller than those of the male measuring, on an average, 4.5 mm. by 1.5 mm. The measurements of the right and left kidneys of three female specimens are given below:

Measurements in millimetres.

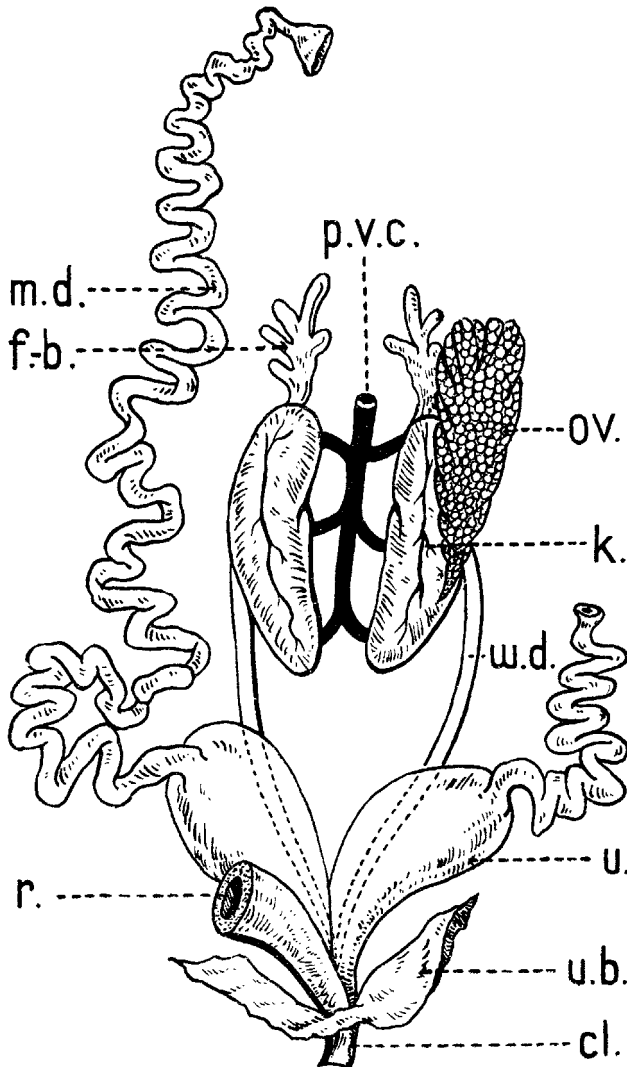
Serial No.	Length of kidney.		Breadth of kidney.	
	<i>Rt.</i>	<i>Lt.</i>	<i>Rt.</i>	<i>Lt.</i>
1	5.0	4.0	1.2	1.2
2	4.6	4.5	1.8	1.8
3	4.5	4.4	1.5	1.4

The kidneys, however, resemble those of the males in shape as well as in having a notch at about the middle of the inner border. The right kidney is very slightly larger than the left one, and both the kidneys are situated more or less at about the same level, unlike the condition in the male where they are slightly asymmetrical in position.

The paired ovaries are large and more or less lobulated, and are supported by the mesovaria from the mesial side of the kidney. They resemble those of *Rana* in general structure and are enclosed in an ovisac which ruptures to allow the ripe ova to escape into the body-cavity. The shape of the ovaries (*ov.*), at least in the breeding season, is different from that of *Rana* and *Bufo* in having a more or less triangular outline. The ripe ova are pigmented as in frogs and toads, and measure about 1.0 mm. across their diameter.

The fat-body (*f.-b.*) is markedly different from that in the male, there being no ribbon-like stalk characteristic of the fat-body of the male. As in ordinary toads and frogs it

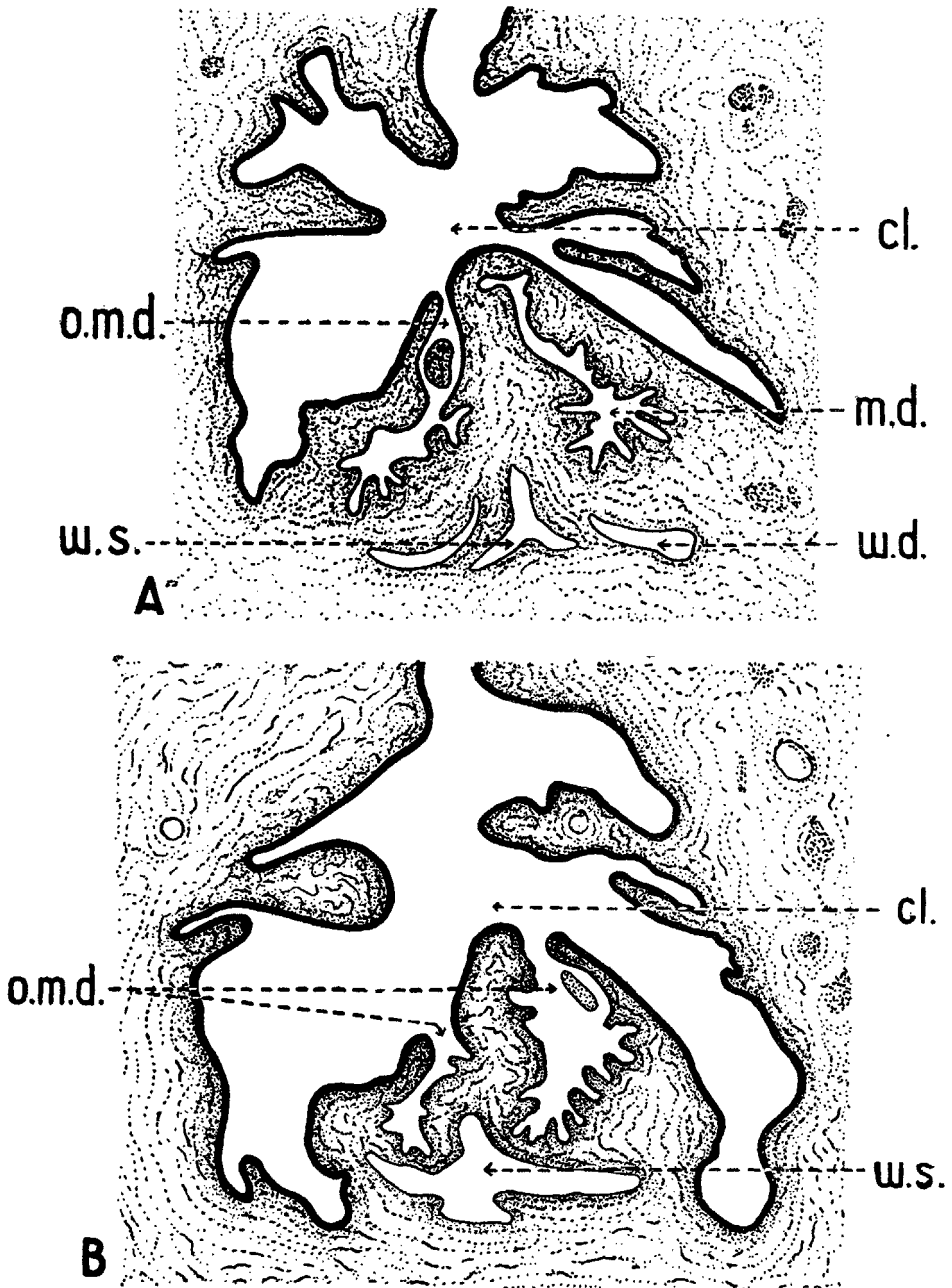
consists of a short basal stump from which four to six irregular and blunt finger-like processes branch off.



TEXT-FIGURE 4.—Diagrammatic ventral view of the urinogenital organs of a female *Microhyla ornata* (right ovary not shown and the left oviduct not fully represented). $\times 8$.
cl.—cloaca; *f.b.*—fat-body; *k.*—kidney; *m.d.*—Müllerian duct or oviduct; *ov.*—ovary;
p.v.c.—posterior vena cava; *r.*—rectum (cut); *u.*—uterus; *u.b.*—urinary bladder; *w.d.*—
 ureter (or Wolffian duct).

Each ureter or Wolffian duct (*w.d.*) follows a course similar to that of the urinogenital duct of the male. It does not show any dilatation after leaving the kidney, as is found in the male, but maintains a uniform diameter throughout its length, but the bicornuate sinus is present here also between the ureters of the two sides. This sinus resembles the urinogenital sinus of the male in its formation, but is much smaller in size. Transverse sections through the terminal portion of the ureters clearly demonstrate the presence of the sinus as well as the two separate tips of the diverticula. Text-figure 5 A represents a

transection showing the formation of the urinary sinus (*w.s.*) by the confluence of the diverticula which are, however, still separate from the ureters (*w.d.*). Further backwards



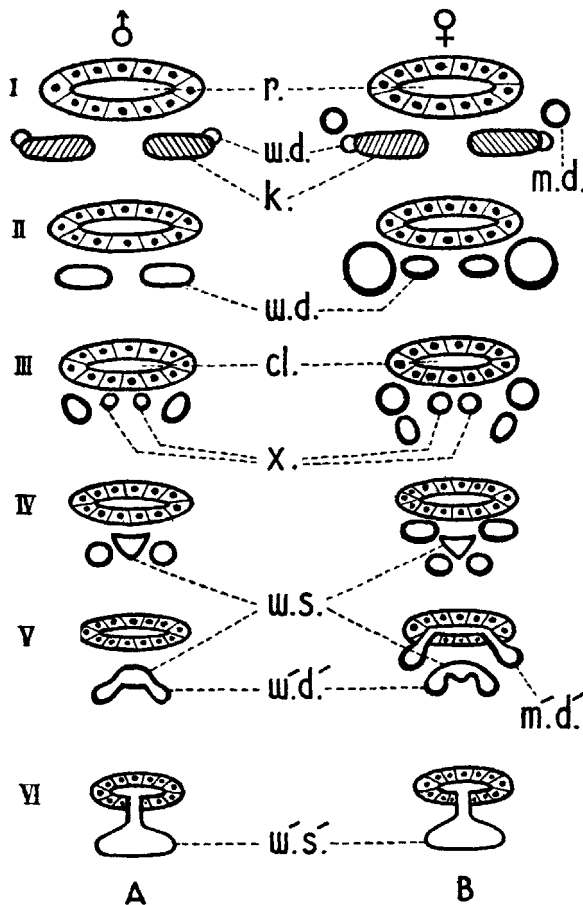
TEXT-FIGURE 5.—Transverse sections through two succeeding regions of the urodaeal parts of the cloaca of a female *M. ornata*, to show (A)—the formation of the urinary sinus (*w.s.*) by the confluence of the diverticula of the ureter (*w.d.*); and (B)—the confluence of the ureters with the urinary sinus (*w.s.*). $\times 140$.
o.m.d.—openings of the Müllerian ducts (uterine portion). (Other letterings as in text-figure 4.)

the ureters become confluent with the urinary sinus (*w.s.*) forming a transverse sac as shown in the text-figure 5 B, which opens by a single aperture into the cloaca, lying posteriorly to the openings of the uteri (*o.m.d.*).

So far we have had no knowledge of such a structure being present in any female Salientia. De Villiers (1934) studied only male *Ascaphus truei* and, therefore, we have no information about the condition in the female of this form.

In the case of males it has been suggested that the urinogenital sinus probably functions as a vesicula seminalis. Difficulty is felt in assigning a probable function to the urinary sinus in the females, but since it is considerably smaller in size than that of the males, it may be surmised that it is very likely a regressive structure.

The Müllerian ducts or oviducts (Text-fig. 4, *m.d.*) show a similar course and structure like those of the ranids. They are narrow, convoluted tubes situated one on each side of the vertebral column along the whole length of the abdominal cavity. The coelomic opening of each oviduct is a small funnel-shaped structure situated laterally to the base of the lungs, and this in turn expands into a large thin funnel bounded by the mesentery covering



TEXT-FIGURE 6.—Schematic figures (I-VI) of cross-sections passing through regions posterior to the kidneys of a male (A) and a female (B) of *M. ornata*, to show the relation, course and fate of the urinogenital ducts. (Urinary bladder and its opening not represented.)
cl.—cloaca; *k.*—kidney; *m.d.*—Müllerian duct (including uterine portion); *m'.d'*—Müllerian duct opens into the cloaca (V); *r.*—rectum; *w.d.*—Wolffian duct; *w'.d'*—Wolffian ducts in confluence with the Wolffian sinus (V); *w.s.*—Wolffian sinus; *w'.s'*—Wolffian sinus opens into the cloaca (VI); *x.*—two tips of the diverticula of the Wolffian sinus (III).

the liver and the lung of that side. The uterine portion of the oviduct is highly distensible, and contains mature ova ready to be laid. The openings of the uteri resemble those of *Rana* in that the uteri open separately into the cloaca (Text-fig. 5, *o.m.d.*). These openings are situated a little anteriorly to the single opening of the urinary sinus of the ureters.

The urinary bladder (Text-fig. 4, *u.b.*) shows no essential difference from that of the males.

3. For a better and easy understanding of the facts given in the foregoing pages, a series of schematic drawings of cross-sections of the Wolffian and Müllerian ducts commencing from the posterior level of the kidneys are given in the accompanying text-figure 6 A and B, showing the relation, course and fate of these ducts in both the sexes.

SUMMARY

(1) The kidneys differ in size in the two sexes, and show an asymmetrical position in the male.

(2) The testes, ovaries, oviducts and the urinary bladder are more or less of the ranid type.

(3) In the male the fat-body has a remarkably long ribbon-like stalk.

(4) The Müllerian ducts are absent in the males.

(5) The Wolffian ducts possess a small bicornuate sinus distally, opening by a single aperture into the cloaca. This sinus is compared with the male urinogenital sinus of *Ascaphus truei*, a primitive Salientia (de Villiers, 1934), as well as with the vesiculæ seminales of ranid frogs in function.

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