On the reproductive system of *Dolops ranarum* (Stuhlmann, 1891) (Crustacea: Branchiura)

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Serial sections of *Dolops ranarum* were used to reconstruct the female reproductive system. The histology of the different regions is described and it is concluded that no permanently open connection exists between the ovary and oviducts. Histological information on the testes is given.

Dolops ranarum-wyfies se geslagstelsel is gerekonstrueer met behulp van seriesneë. Die histologie van die verskillende streke word beskryf en daar word aangetoon dat geen permanente verbinding tussen die oviduk en ovarium bestaan nie. Inligting aangaande die histologie van die testes word ook verskaf.

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Although *Dolops ranarum* (Stuhlmann, 1891) was described almost a century ago, little has been done to describe the anatomy of this African parasite and other *Dolops* species. Structures that could be observed though the integument were described by Heller (1857), Stuhlmann (1891) and Bouvier (1898) while Maidl (1912) made histological sections but noted that his material was not well preserved. New technology such as resin embedding makes it possible to describe the reproductive system more accurately.

Methods

Material was obtained from fish hosts in rivers in the Limpopo Drainage System in the Transvaal, South Africa. Some specimens were fixed in 10% phosphate buffered neutral formalin but better results were obtained by using Gilson's fixative (Humason 1979). Specimens were dehydrated in ethanol (mercury deposits being removed by a few drops of tincture of iodine in 70% ethanol during this process), infiltrated under vacuum and polymerized at room temperature in a glycol methacrylate resin. Serial sections were cut with a dry glass knife at 2 or 3 μm and transferred onto water in a bath at room temperature, releasing the sections before they touched the water. Sections were collected on precleaned glass slides and air-dried before staining in Mayer's haematoxylin, blued in running tap water and counterstained with eosin Y. Drawings from transverse sections were made with the aid of a drawing tube attachment and graphic reconstructions made as described by Pusey (1939).

Results

Female reproductive system

The single ovary lies almost medially in the thorax. Various authors have questioned whether it is single or double in origin. In *Argulus* sp., Claus (1875) refers to a single anlage in the larva located on either the right or the left side of the body, while Grobben (1908) concludes that a single ovary is derived from paired anlagen, which fuse before the animal reaches maturity — a conclusion made by comparison with the homologous, paired testes and from the fact that two oviducts (of which only one is functional) are present. Martin (1932) indicates that the ovary in *A. foliaceus* has a bilobed appearance, but this was not observed in *D. ranarum* whose ovary appears rather like a bunch of grapes. In adults its size depends on the number as well as the size of eggs present. It is often large. The heavily yolked eggs are obovate in shape, with greatest and least diameters of 612–670 μm and 410–446μm respectively. A 10,0 mm female was observed to deposit 566 eggs (Fryer 1959). The eggs are heavily yolked.

The ovary lies in a cavity, called the circumgenital cavity by Leydig (1850) and Grobben (1908), completely cut off from the remainder of the haemocoel. According to Martin (1932) this is homologous to the gonocoel in other crustaceans. The wall of the gonocoel consists of strands of connective tissue, with pigment cells interspersed and envelops the ovary and oviducts.

Two oviducts originating anteriorly from a common oviduct, proceed posteriorly. Two anterior and two lateral horns are present on the common oviduct (Figure 1). These horns fill with eggs and subsequently extend into the carapace lobes and into the region between the thorax and cephalon. Here the oviduct wall consists of a thick, smooth muscle layer embracing columnar epithelial cells whose nuclei are situated apically (Figure 2).

Sections of the ovary in different regions show eggs in all stages of development, interspersed with columnar epithelium. In *Argulus* the columnar cells disappear completely during spawning (Martin 1932). This observation was not made in *D. ranarum* and eggs of all stages appeared to be present throughout the reproductive period, which lasts several months. The germinal ridge lies dorsally (Figure 3), with immature eggs surrounding it, while the larger eggs are placed ventrally. The germinal ridge almost reached the total length of the ovarium. Maidl (1912), however, shows that the germinal ridge occupies only the antero-dorsal part of the ovary in *D. longicauda*.

The ova are at first enveloped by a delicate vitelline membrane, which becomes considerably thicker before

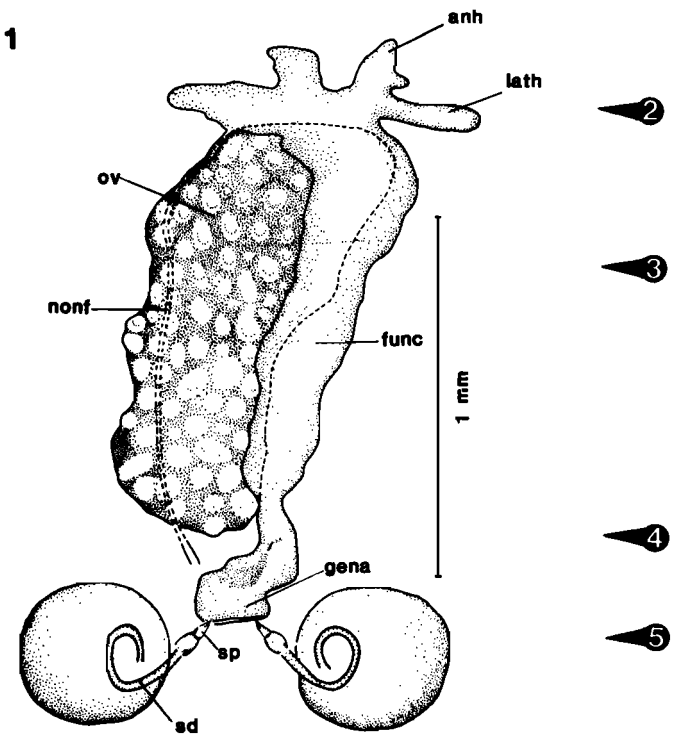

Figure 1 Graphic reconstruction of the female reproductive system. The lobulated ovary is overlain by the oviducts: anh = anterior horns of oviduct, gena = genital atrium, func = functioning oviduct, nonf = non-functioning oviduct, lath = lateral horn of oviduct, ov = ovary, sd = spermathecal ducts, sp = spermathecal spine. Arrows indicate approximate level of sections from which Figures 2–5 were drawn.

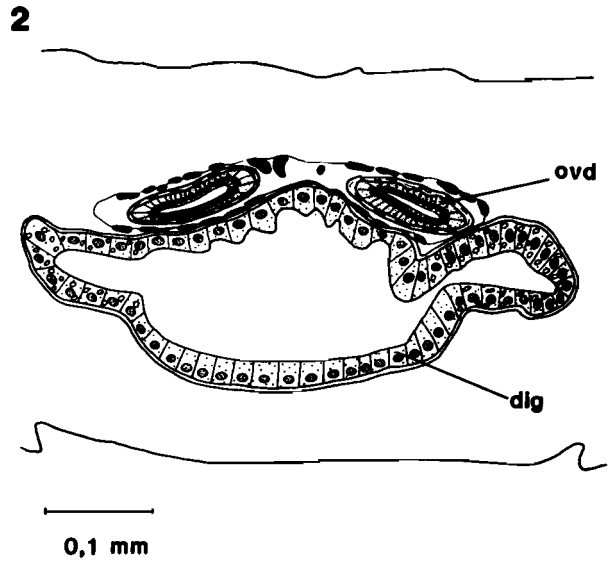

Figure 2 Section through anterior horns of unpaired oviduct. Note the thick muscular layer surrounding the oviduct and the position of the nuclei in the epithelial cells lining the oviducts (ovd): dig = digestive tract.

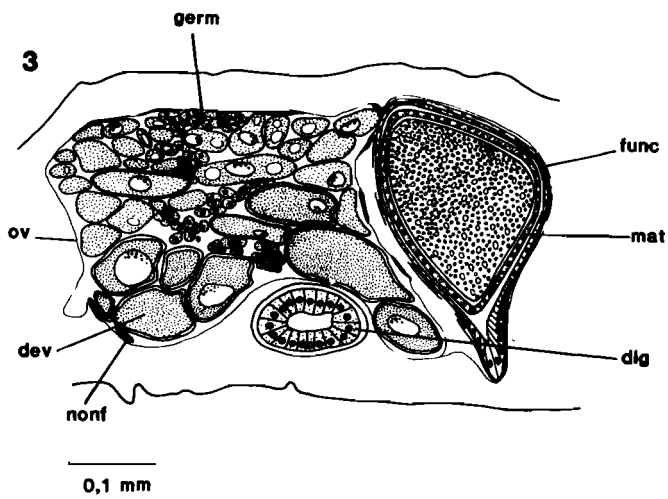

Figure 3 Section through ovary (ov) and oviducts: dev = developing eggs, dig = digestive tract, func = functioning oviduct, germ = germinal ridge, mat = mature egg, nonf = non-functioning oviduct.

they pass into the oviduct (Figure 3). Two egg membranes were described in *D. longicauda* by Heller (1857), an inner, the '*Dotterhaut*' (yolk membrane), and an outer membrane formed in the oviduct, the '*Schalenhaut*'. Maidl (1912), however, observed two separate membranes constituting the shell in *D. longicauda*. In the present study the shell was found to consist of a single layer of unevenly staining material.

Eggs in different stages of meiosis are present in the ovary. The translucent, nuclear area is surrounded by granulated deutoplasma that becomes increasingly granular and eventually forms yolk platelets in eggs which have progressed to the oviduct.

No connection was detected between the oviduct and ovary but the membrane separating the ovary and oviduct is characterized by discontinuities in the muscular layer surrounding the oviduct. Eggs in the oviduct are extremely soft and deformable and are often deformed to fill small spaces in the ovary.

Ova are never found in both oviducts at the same time. In the specimens examined (n = 50), approximately equal numbers contained eggs in the left and right oviduct. Anteriorly, the non-functioning oviduct is not obliterated and a lumen is present, though much smaller than in the functioning oviduct. Posterially it becomes ill defined and difficult to detect. Sometimes increased pigmentation is the only indication of its presence. Near the genital atrium, it widens again and a lumen becomes visible. Although this oviduct connects with the genital atrium, its lumen is blocked and it does not open into it.

Both oviducts lie in close proximity to the dorsolateral wall of the gut and consist, in the functioning oviduct, of bands of cylindrical, smooth muscle embracing a layer of columnar epithelium that tends to become cuboidal in a distended oviduct. No pigmentation was observed in the epithelium of muscle layers, but a layer of pigmented connective tissue surrounds the oviduct (Figure 4).

The functioning oviduct opens into the bag-like genital atrium (Figure 1) whose wall is similar to that of the oviduct, but is surrounded by a continuous muscle layer

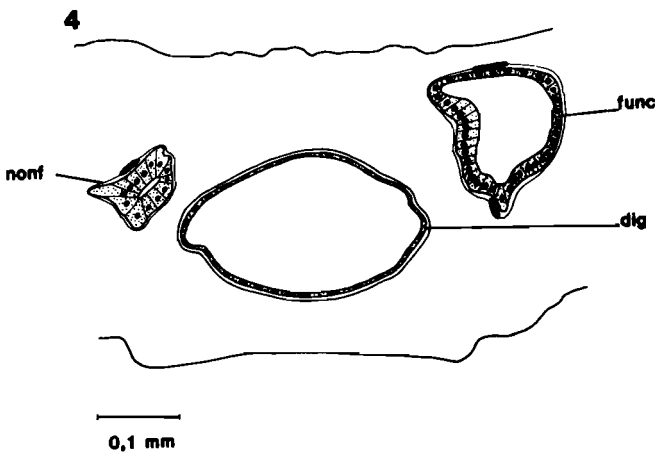

Figure 4 Section through oviducts immediately anterior to the genital atrium: dig = digestive tract, func = functioning oviduct, nonf = non-functioning oviduct.

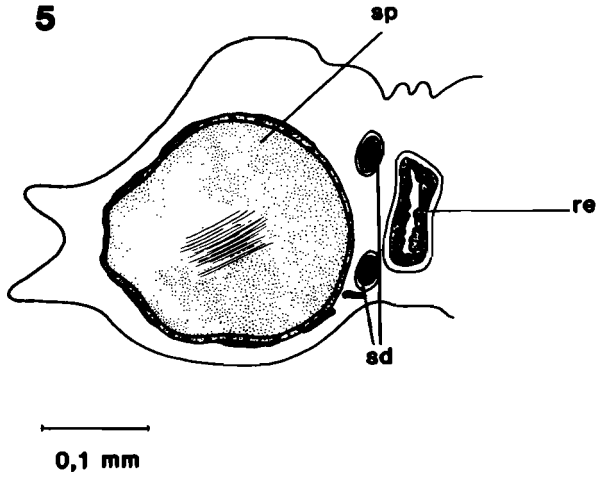

Figure 5 Section through one female abdominal lobe showing the structure of the spermatheca (sp) and spermathecal duct (sd): re = rectum.

and is lined by a cuticle. It opens at the anterior extremity of the abdomen in a centrally situated gonopore.

Two spermathecae are present in the abdomen. Their position and shape in a variety of species were described by Heller (1857), Stuhlmann (1891) and Bouvier (1898, 1899) and the anatomy and histology of *D. longicauda*'s and *D. ranarum*'s spermathecae were described by Maidl (1912) and Fryer (1960) respectively. Spermathecal ducts connect the spermathecae with the spermathecal spines, situated ventrally on the abdomen. A connective tissue layer, containing pigment cells, surrounds the spermathecae and spermathecal ducts. Cuboidal epithelium lines the spermathecae and ducts. The ducts are surrounded by a smooth muscle layer.

Apically, the epithelium is covered by a cuticle similar to, but thinner, than that covering the body (Figure 5). The spermathecae are not connected to the remainder of the reproductive system and sperm is injected into each egg during deposition, as has long been inferred in *Argulus* sp. and as deduced from anatomy and observations on egg laying in *D. ranarum* by Fryer (1960) (see also Avenant, Van As & Loots 1989).

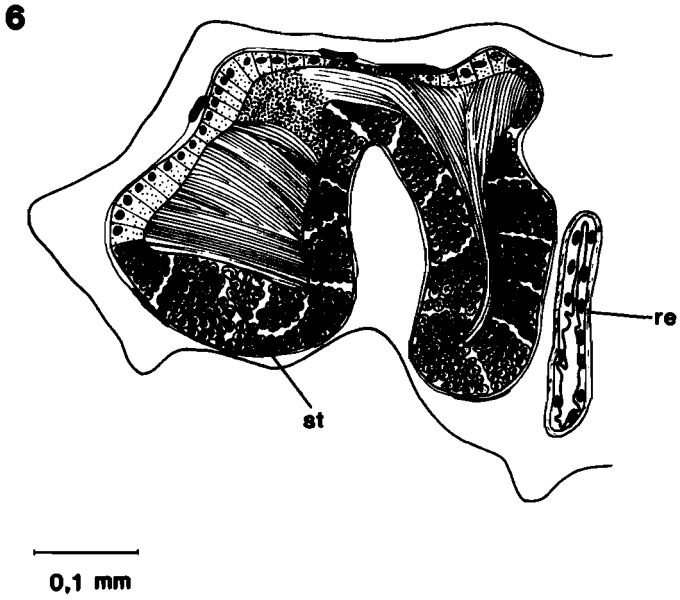

Figure 6 Section through one male abdominal lobe to illustrate the structure of the testes lobes: re = rectum, st = spermatid.

Male reproductive system

Except for casual statements by nineteenth-century investigators referring to such details of the reproductive organs as could be discerned through the integument, only two accounts of the male reproductive system of *Dolops* are available. The first was that of Maidl (1912) who being unaware of the presence of spermatophores — described later by Fryer (1958, 1960) — was unable to give a functional interpretation of certain structures observed.

Fryer (1960) detailed the position, structure and histology of the male reproductive system and described spermatophore formation. The present authors have nothing to add but the following remarks.

Three testicular lobes are present in each abdominal lobe. The testes occupy two-thirds of the length of the abdomen and bulge ventrally (Figure 6) indicating that they are very full. The lobes are covered, especially dorsally, by strands of pigmented, connective tissue. Cuboidal and columnar epithelium line the testes dorsally and spermatocytes are present ventrally. Much of the lumen is filled by maturing spermatozoa orientated in all directions (Figure 7a) while spermatozoa in the reproductive ducts lie parallel to the ducts. Spermatocytes and spermatids in all stages of spermatogenesis occur throughout the testes (Figure 7b).

Discussion

Two oviducts, but only one ovary are present in *D. ranarum* as is the case in *D. longicauda* (Maidl 1912) and *A. coregoni* (Martin 1932). Grobben (1908) showed that only one of these oviducts is functional, while Martin (1932) indicated that the non-functioning oviduct

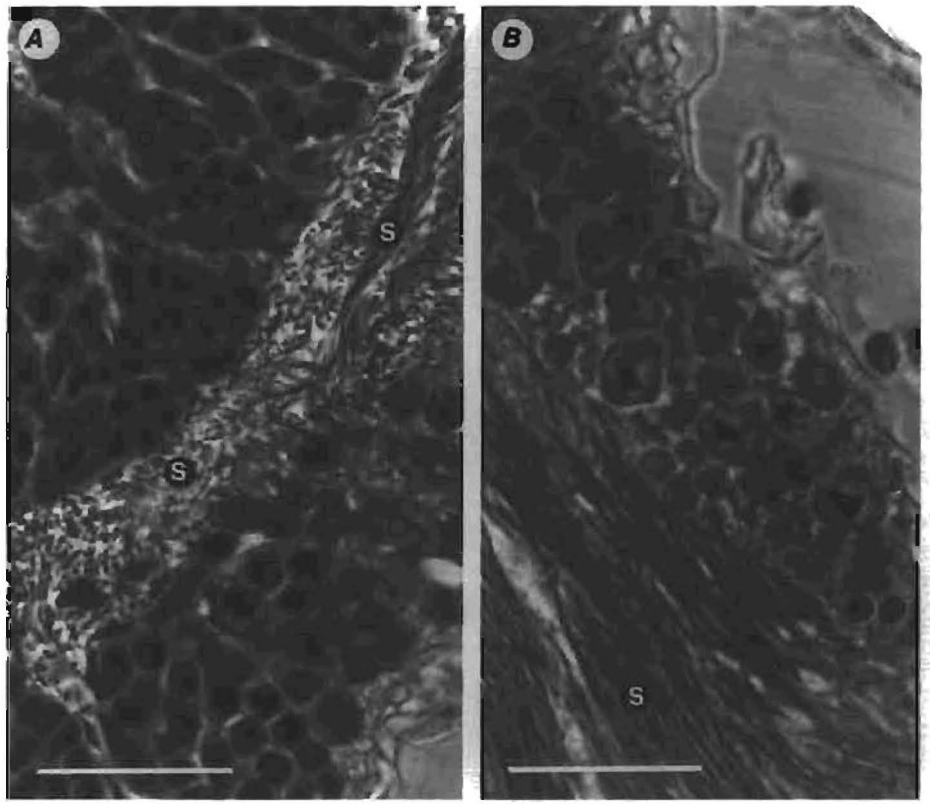

Figure 7 (a) Micrograph of section through lobulated testis. Sperm (s) fills the lumen. (b) Spermatids in different stages of development: s = sperm. Scale bar = 0,05 mm.

in *A. coregoni* is not obliterated and proposed that the oviducts function alternately or periodically. This appears probable in *D. ranarum*, since the non-functioning oviduct is occluded. Even the functioning oviduct shows a distinct flattening of cells in the proximity of eggs, similar to the non-functioning oviduct. Thus it appears that the ducts function alternately to allow restoration of cellular material in the oviducal lining.

By comparison with the homologous paired testes and from the fact that there are always two oviducts, Grobben (1908) deduced that the single ovary is derived from paired anlagen which fuse before maturity. Martin (1932) reported a bilobed appearance of the ovary in *A. coregoni*, but this was not observed in *D. ranarum*. Elucidation calls for embryological study.

Although Martin (1932) was of the opinion that eggs are shed into the gonocoel and migrate via the unpaired part of the oviduct to the functional oviduct in *Argulus* sp., it is not clear how eggs pass into the oviduct. In *D. ranarum*, as in *D. longicauda* (Maidl 1912), we found no connection between the oviduct and ovary. Furthermore, the unpaired part of the oviduct is surrounded by a thick continuous muscle layer. The functioning oviduct, however, is surrounded by bands of muscle. Although we have no evidence, we suggest that eggs leave the ovary anteriorly. The close juxtaposition of the ovary and oviduct in this area suggests that a connection is most probable but since no evidence is available the matter remains unsolved.

Acknowledgement

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