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Chonopeltis australis (Crustacea: Branchiura); the female reproductive system

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ABSTRACT

The female reproductive system has been described for *Dolops ranarum* (Stuhlman, 1891) and various *Argulus* spp. but, there is no description of the reproductive system for *Dipteropeltis* spp. Calman, 1912 or *Chonopeltis* spp. Thiele, 1900. This paper describes the female reproductive system and egg laying behaviour of *Chonopeltis australis* Boxshall, 1976 using histology, light microscopy, scanning electron microscopy and observations. The histological study of six specimens showed that the organ structure is similar to that of *Argulus* spp. and *D. ranarum*. The oocytes therefore develop in the gonocoel, are ovulated into the lumen of the ovary which is continuous with the functional oviduct and eventually the gonopore. Females of *C. australis* deposit eggs on the surrounding substrate while the fish is at rest, without leaving the host, thereby mitigating the risk of not locating a host again.

1. Introduction

In Branchiura, the female reproductive system performs three functions; produces eggs with shells; stores sperm; and inseminates eggs prior to deposition. Most of the research done on reproductive systems in this group has been on *Argulus* Müller, 1785; either as part of species descriptions (Jurine, 1806; Leydig, 1850), or as one aspect of anatomical studies (Claus, 1875; Grobben, 1908; Martin, 1932; Ikuta and Makioka, 1997).

The first description of the female reproductive system in Branchiura is provided for *Argulus foliaceus* (Linnaeus, 1785) by Jurine (1806) as an ovary that is located in the "abdomen" above the alimentary canal; it extends from the origin of the "stomach" to the base of the "tail" and ends in a very short oviduct. The oviduct opening is between the fourth pair of thoracic legs (Jurine, 1806). The dorsal surface of the ovary was described as being covered by angular black spots arranged in longitudinal lines (Jurine, 1806).

Years later, Leydig (1850) added to the description by stating that the ovary is a simple tube that lies on the midline of the body, with the membrane of the ovary lined with striated muscles. Within the ovary, the eggs develop in stalked pouches which contribute to the berry-like appearance of the ovary (Leydig, 1850). Egg development starts as round cells with round nuclei and multiple nucleoli; that gradually enlarge and become oval (Leydig, 1850). Thereafter, fat deposits which become yolk granules accumulate within the ooplasm (Leydig, 1850). Finally, the mature egg has a homogeneous substance deposited around it (Leydig, 1850). He added that the female reproductive system also consists of seminal receptacles in the abdomen and that these are round and encased in a capsule. Furthermore, a duct that exits the capsule leads to papilla-like projections, carrying blind-ended attachments at their midway point. The oviduct opens into the projections. Additionally, the seminal receptacles and, by extension, the ducts and papillae receive sperm from the male during copulation (Leydig, 1850; see Fig. 1A for orientation).

Thereafter, Claus (1875) argued that the ovary is actually a solid strand that has numerous attachments on the periphery, each attachment being an egg chamber into which eggs develop. The development of eggs was described as being similar to that noted by Leydig (1850); however, Claus (1875) named the membrane that develops around the mature egg, the chorion, and stated that the blind-ended attachments of the ducts of the seminal receptacles do not exist. He also suggested that in the absence of a micropyle on the eggs after deposition, the chitinous papillae (projection viz. Leydig, 1850) at the end of the ducts of the seminal receptacles must be used to inseminate the eggs as they exit the ovarian duct. Wilson (1902) agreed with this suggestion.

Later, Grobben (1908) reviewed the previous work and added that the ovary is placed asymmetrically within the thorax, with a pair of oviducts, one being functional and the other not.

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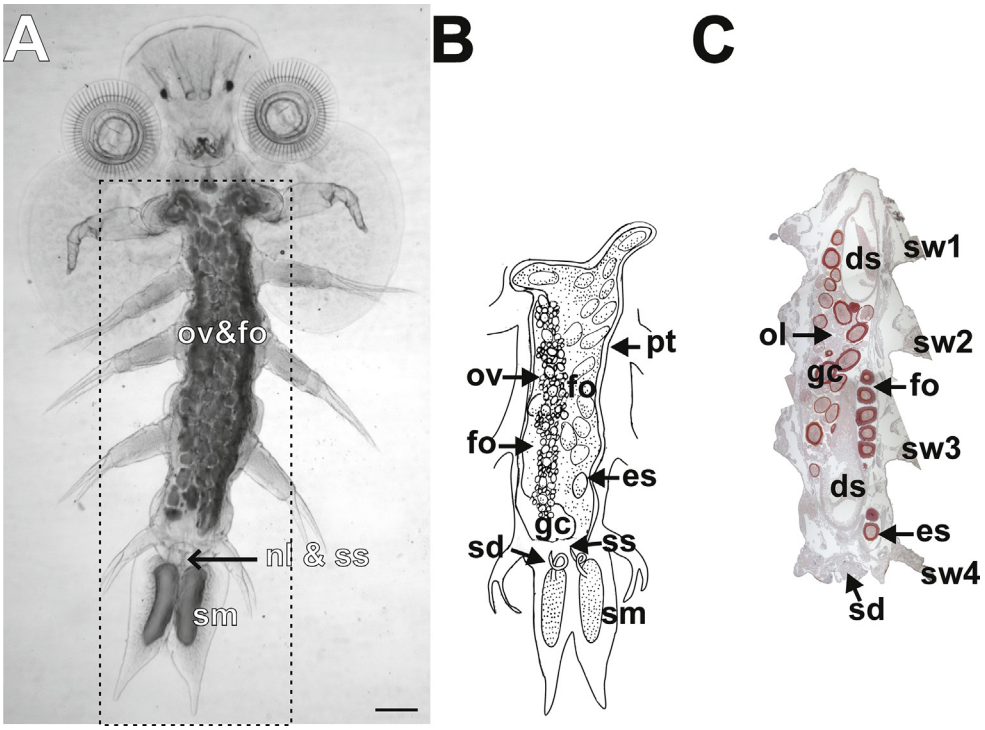

Fig. 1. *Chonopeltis australis* female. A. Light micrograph of a female specimen cleared in lactic acid. Scale bar 500 μm. block indicates Figure 1B. B. Schematic representation of the female reproductive system within the gonocoel. C. Composite micrograph of frontal section of female thoracic region. ds digestive system, es egg with shell, fo functional oviduct, gc gonocoel, nl natatory lobes, ol ovary lumen, ov ovary, pt peritoneum of gonocoel, sd spermathecal duct, sm spermathecae, ss spermathecal spines, sw swimming leg.

The functional oviduct is joined to the anterior end of the ovary and first makes a blind-sac prior to returning in a posterior direction to the base of the thorax (Grobben, 1908). Furthermore, the ovary and oviducts are enclosed in the circumgenital body cavity between the aorta and intestine (Grobben, 1908) and the homogenous secretion present around the mature eggs is secreted by the ovary wall.

Thereafter, Martin (1932) defined the circumgenital body cavity described by Grobben (1908) as homologous with the gonocoel of other Crustacea. In addition, she noted that the germinal ridge of the ovary occurs below the aorta, with the youngest eggs found dorsally attached to the lobulated ovary, while the mature eggs are near the tips ventrally. The mature eggs are shed into the gonocoel before entering the functional oviduct (Martin, 1932).

In a recent description, Ikuta and Makioka (1997) used *Argulus japonicus* Thiele, 1900 as the test organism and described the system as consisting of a sac-like ovary, with a ventro-laterally folded ovary wall. They confirmed that oocytes are attached to the outer surface of the ovary wall facing the haemocoel or extra-ovarian space, to develop as previously described until the primary egg membrane or eggshell is deposited. At this point the egg (secondary oocyte) is "ovulated" into the ovary lumen through the ovary epithelium and transported to the oviduct where final maturation occurs. This description thus suggested that egg development occurs in two stages, one on the outside of the 'ovary wall', and the remaining maturation within the functional oviduct after entering through the ovary wall.

With regard to the other Branchiura; the female reproductive system of *Dolops* Audouin (1837) was described for *Dolops ranarum* (Stuhlmann, 1891) by Avenant-Oldewage and van As (1990). Their description echoed that of Martin (1932), adding that the germinal ridge occurs along the length of the ovarium dorsally (Avenant-Oldewage and van As, 1990). In the genus *Dipteropeltis* Calman, 1912, only females have been described for the two species (Calman, 1912; Neethling et al., 2014); nonetheless, there is no information regarding the reproductive system for this genus. Interestingly, there is also no description of the female reproductive system of *Chonopeltis* Thiele (1900). Thus, the female reproductive system of *Chonopeltis australis* Boxshall, 1976 is described using light and scanning electron microscopy as well as histology, and egg deposition is described from observation.

2. Material and methods

During a survey of the fishes of the Vaal Dam and the Vaal River (26°49′14.73″S 28°3′52.49″E) in September 2009, specimens of *C. australis* Boxshall, 1976 were collected by Ms. Yolandi Cloete from *Labeo capensis* (Smith, 1841). These specimens were fixed in steaming hot aceto-formaldehyde-alcohol (AFA), transferred to 70% ethanol for preservation, prior to histology. Additional samples were collected between November 2011 and March 2013 from *L. capensis*; some samples were fixed in AFA and stored in 70% ethanol, and the rest were kept live. The live parasites were placed and kept on wild caught *L. capensis* in a glass tank to start a breeding colony and observe behaviour.

2.1. Whole mount light microscopy

Fixed female specimens of *C. australis* were cleared in 80% lactic acid for whole mount viewing with a Zeiss Axioplan2 compound microscope mounted with a Zeiss Axiocam HRc camera. Micrographs were obtained using the Axiovision program version 4.7.2 (12-2008).

2.2. Whole mount scanning electron microscopy (SEM)

Twenty-four female specimens of *C. australis* were used for SEM. Prior to chemical processing for SEM, all but four of the specimens