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Research Article

OCCURRENCE OF OVIDUCAL GLANDS IN TWO TROPICAL MARINE CALANOID COPEPODS *PSEUDODIAPTOMUS ANNANDALEI* AND *PSEUDODIAPTOMUS SERRICAUDATUS* – A NEW REPORT

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ABSTRACT

The female reproductive system of the calanoid copepod species shows species specific variation in organization. Occurrence of oviducal gland in *Pseudodiaptomus annandalei* and *Pseudodiaptomus serricaudatus* is reported for the first time in present study. In the case of *P. annandalei*, and *P. serricaudatus* fertilized eggs undergo embryonic development in an ovisac. In both the species secretory activity of the oviducal gland synchronizes with the vitellogenesis and maturation of oocytes, which take place in the oviduct. The release of secretory materials from the oviducal gland precedes the release of mature oocytes from the oviduct, and forms the membranous ovisacs in which fertilized eggs are deposited. The oviducal glands of *P. annandalei* and *P. serricaudatus* show resemblance with the oviducal glands of freshwater diaptomids, *Heliodiaptomus viduus* and *Sinodiaptomus (Rhinediaptomus) indicus* which suggests a closer phylogenic affinity of the *Pseudiaptomus* genus with freshwater diaptomids. The role of oviducal gland in the female reproductive system of calanoid copepods is discussed.

Keywords: Calanoid copepod, Reproduction, Oviducal gland, Ovisac.

INTRODUCTION

Copepods are sexually reproducing animals and sexes are separate. The gonads located in the cephalosome are typically median in position and unpaired in free living forms. In females the oviducts arise from the anterior portion of the ovary and extend back along the side of the body to the genital somite. The ducts open into a genital antrum. The antra are paired invaginations of the genital sternite that meet at the midline to form a transverse bilobed structure. Seminal receptacles also open into the antra. A variety of glands of unknown functions open into the antra (Boxshall, 1982). The ovary usually contains previtellogenic oocytes and vitellogenesis takes place when the oocytes are in the oviducts and diverticulae of oviducts. There are variations pertaining to the fecundity of copepods which is mostly related to the reproductive status of the female, food availability and environmental parameters.

Copepods constitute important primary consumer in all types of aquatic ecosystems and play vital role in the energy transfer from primary producers to secondary consumers. Though many reports are available on the taxonomy and distribution of planktonic copepods (Kasturirangan, 1963), only few reports available on their biology and reproduction. Important contributors on female reproductive system are by Lowe (1935), Fahrenbach (1962), Park (1965), Razouls *et al*. (1987), Dharani (1998), Sujatha (2000), Altaff (2003), Zehra (2000), and Gopikrishna (2004). The calanoid copepods have adopted two different spawning methods of gravid eggs, in the first type fertilized eggs are freely released in to the medium while in the second type fertilized eggs are deposited in the egg sac, which is attached to the genital pore until the nauplii hatch. The adoption of ovisac formation in some of the calanoid copepods has led to modify the distal region of the oviduct of the females in the oviducal gland.Occurrence of oviducal glands was reported in freshwater calanoid copepods, *Hemidiaptomus ingens provinciae* and *Mixodiaptomus kupelwieseri* (Cuoc *et al*., 1989), *Heliodiaptomus viduus* (Altaff & Chandran, 1994) and

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Sinodiaptomus (Rhinediaptomus) indicus (Dharani, 1998). In this paper occurrence of oviducal glands in two marine species, *Pseudodiaptomus annandalei* and *Pseudodiaptomus serricaudatus* and their role in the formation of egg sac is reported for the first time.

MATERIALS AND METHODS

Zooplankton were collected offshore of the Muttukadu and Ennore station. (About 5 nautical miles) using a motor driven dingy boat. Zooplankton were collected by towing a Bongo net (0.5m diameter mouth, 2.5m mesh cloth, made of bolting silk 50µm mesh size, which is fixed with 25cm bottom cup) for nearly half an hour for each sample during early hours of the day. The samples were fixed using 5% buffered seawater formalin. Zooplankton was sorted using different mesh sized filter cloth and different groups of copepods were separated. *P. annandalei* and *P. serricaudatus* were identified to species level following the taxonomic descriptions by Kasturirangan (1963), Huys & Boxshall (1991), Mazzocchi *et al.* (1995), Toda *et al.*(1994), Sewell (1999), Conway *et al.* (2003).

To view the reproductive system in situ, the method of (Pantin, 1964) was followed. The mature females of *P. annandalei* and *P. serricaudatus* were fixed in 70% ethanol, transferred to Borax–carmine and stained for 24 hours. The specimens were differentiated in acid alcohol till outer layer appear transparent and inner structures pinkish. Then the specimens were dehydrated in 90% ethanol, passed to absolute ethanol, cleared in xylene and mounted in DPX. To ascertain the correct shape of the reproductive organs, the borax carmine stained, acid alcohol differentiated specimens were dissected in a glycerol ethanol mixture under stereo dissection microscope and then observed under compound microscope. The different parts of the reproductive system were described following the terminology by Hopkins (1977) and Dussart & Defaye (1995).

For histology, mature females of both the species were fixed in aqueous Bouin's fluid for 12 hours and were washed in tap water for 1 hr. They were dehydrated in 50%, 70%, 90% and absolute ethanol, cleared in xylene, embedded in paraffin wax and serial sections (cross sections, longitudinal sections, vertical sections and transverse sections) were taken. The sections were then de paraffinized in xylene and hydrated in descending series of ethanol and finally with distilled water, stained with haematoxalin, and counter staining was done with 70% alcoholic eosin and then passed through 90% ethanol and absolute ethanol. After complete dehydration, xylene was used for clearing the alcohol and then mounting was done in DPX (Pantin, 1964). The reproductive system was observed compound microscope and photomicrographed under different magnifications.

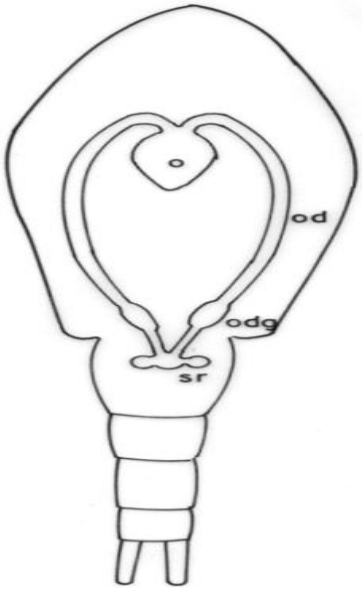
RESULTS

The female reproductive system of *P. annandalei* consists of a median ovary a pair of oviducts antrum, seminal receptacle and reproductive pore. Ovary is an elongate organ lying on the dorsal side of the anterior midgut. It has a wider anterior end and a narrow as well as rounded posterior end. A pair of oviducts originates from the anterior end of the ovary and takes a posterior course and then proceeds laterally through the perivisceral cavity towards the end of the prosome. The oviducts enlarge terminally to give rise to oviducal gland which extends into the genital segment and opens into the antrum (Figure 1). The antrum in turn opens to the exterior through female reproductive pore. A pair of spherical seminal receptacle occurs in the genital segment. In this species fertilized eggs are held in the ovisac where embryonic development up to naupliar stage takes place. The ovisac of this animal contains 23 ± 3 eggs. The female reproductive system of *P. serricaudatus* also consists of a single median ovary. A pair of genital ducts which opens through the lateral sides of the digestive tract to open to the exterior through a common gonopore on the ventral side of the first urosomal somite (Figure 2). However, species specific difference is observed in the shape of the ovary and oviducal glands. The ovary *P. annandaleiI* is oval in shape while that of *P. serricaudatus* has broader center and tapering anterior and posterior ends. The oviducal glands of *P. annandalei* show higher elongation than those of *P. serricaudatus.* Occurrence of oviducal glands in marine calanoid copepod is reported for the first time.

Histology of the posterior region of the oviduct shows distinct difference with regard to the thickness of its wall and glandular epithelium. During the process of maturation of oocytes in the oviducts, the wall of the oviducal gland becomes highly glandular and produces large quantity of secretory material which is stored in the oval shaped lumen. The wall of the oviduct is haematoxalin positive whereas the secretary material in the lumen of the oviduct appears eosin positive. The lumen leads into a narrow tube which enters into the genital duct and opens into the antra. The wall of the oviducal gland as it proceeds towards the posterior end of the prosome becomes thin and non glandular (Figure 3-5). It is interesting to note that the secretory activity of the oviducal gland synchronizes with the vitellogenesis and maturation of oocytes in the oviduct.

In the case of *P. serricaudatus* also the wall of the oviducal glands is thicker and glandular than the wall of the oviduct (Figure 6- 8). While oocytes undergo vitellogenesis to become gravid, the oviducal glands produce secretory material and stores in their lumens. When the mature oocytes are released from the oviduct, release of secretory materials from the oviducal gland precedes

and forms the membranous ovisac. The fertilized eggs are deposited in the ovisac and undergo embryonic development to become nauplii.



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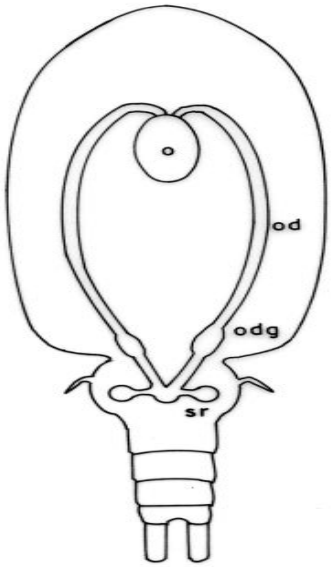

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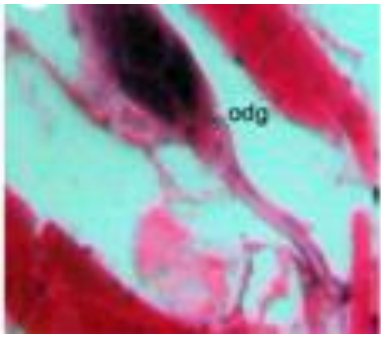
Figures 1 & 2. Female reproductive system of Calanoid copepods *Pseudodiaptomus annandalei* and *Pseudodiaptomus serricaudatus*. o-ovary, od- oviduct, odg-oviducal gland, sr-seminal receptacle.



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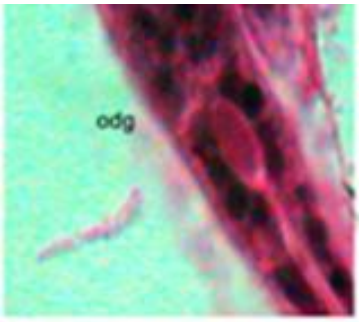

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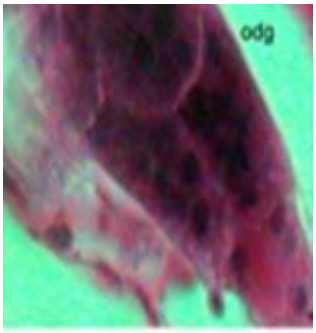

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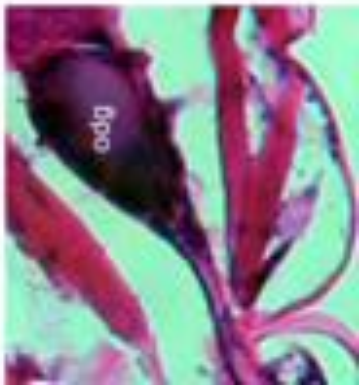
Figure 3-5. Histology of oviducal glands of *Pseudodiaptomus annandalei.*



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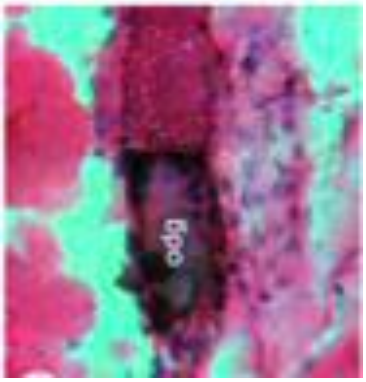

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Figure 6-8. Odg-Oviducal gland of *Pseudodiaptomus serricaudatus.*

DISCUSSION

With regard to the reproductive system, female of calonoid species have a single median ovary located dorsal to the gut in the posterior part of the cephalosome and first metasomal somite. Paired oviducts originate from anterolateral part of the ovary and extend forwards, as diverticula, in to the cephalosome and backwards on either side of the gut to the urosome. The oviducts open into a single chamber, the genital antrum. The genital antrum has paired dorsolaterally directed pouches, the seminal receptacles in which spermatozoa are stored. The organization of the female reproductive system shows generalized pattern in most of the calanoid species (Park, 1965; Corkett & McLaren, 1979; Blades *et al.,* 1984; Razouls *et al*., 1987; Marshall & Orr, 2013). Such an organization of female reproductive system is also observed in both the calanoid copepod species presently studied. However, variation is observed with regard to the size and shape of the ovary, the diverticula of the oviduct, and modification of the posterior region of the oviduct in to a distinct oviducal gland, structure of the antrum and structure of the seminal receptacles in different species.

It is interesting to note that the posterior region of the oviduct occupying the distal region of prosome is modified into oviducal gland in *P. annandalei* and *P. serricaudatus*. This gland is quite conspicuous and distended compared to other regions. The proximal spherical shape and narrow distal region even though common for both *P. annandalei* and *P. serricaudatus*, specific variation is observed with regard to the shape of the gland in these species. Such an oviducal gland is not being described in any other marine calanoids copepod. Nevertheless, occurrence of such an oviducal gland is reported in some of the freshwater calanoids copepods. In the case of *H. ingens provinciae* and *M. kupelwieseri* (Cuoc *et al*., 1989) described ultrastructure of the posterior region of the oviduct which is highly glandular in nature. Occurrence of well - developed oviducal gland with an elastic sac in it was reported for the first time in the planktonic freshwater diaptomids, *H. viduus* by Altaff & Chandran (1994). Subsequently, occurrence of such a gland is also reported in another freshwater diaptomids, *S.* (*R.*) *indicus* by Dharani (1998). The oviducal glands of *P. annandalei* and *P. serricaudatus* show resemblance with the oviducal glands of freshwater diaptomids. This suggest that a closer phylogenic affinity of the *Pseudiaptomus* genus with freshwater diaptomids.

Eggs of copepods are either carried by the female attached in a mass to the genital opening or laid freely in the water column. It seems that some of the calanoid copepods produce eggs without any adhesive material which are broadcasted to the medium individually. There is difference of opinion regarding the nature of egg sac in different egg brooding calanoid copepods. In egg carrying *Eurytemora velox*, Huys & Boxshall (1991) reported that while spawning the secretions of the outer membrane of eggs glued them in to the egg mass and the same is attached to the genital segment. Further, they observed that the egg mass is often called an egg sac, but in calanoids there is no evidence that the eggs are contained in a membrane. Based on the studies on the eggs carried by species such as *Euchaeta* and *Paraeuchaeta* (Mauchline, 1998) also reported that the egg mass is often referred to as an egg sac but there is little evidence that the eggs are carried in a bag.

Contrary to these observations, (Kosobokova *et al*., 2007) provided clear evidence of the presence of enclosing egg sac membranes in the Euchaetidae, Augaptilidae and several Aetideidae. These observations are in accordance with the observations by Corkett & McLaren (1969) and Hopkins & Machin (1977) on the presence of an outer egg sac membrane surrounding the egg masses in *Paraeuchaeta norvegica*. The studies (Altaff & Chandran, 1994) on freshwater calanoid *H. viduus* and (Dharani, 1998) on *S.* (*R.*) *indicus*, clearly indicated occurrence of specialized oviducal glands in the distal part of the oviduct and their role in producing a membranous egg sac. These thick walled and multicellular glands form an elastic sac within itself filled with secretary material (Altaff & Chandran, 1994). When the oviduct contained previtellogenic oocytes the elastic sac was found attached throughout the inner wall of the oviducal glands. When the oocytes became fully developed, the elastic sac detaches from the wall of the oviduct and released into the distal part of the oviduct in the genital segment. After fertilization of the eggs the elastic sac transformed into an ovisac, in which eggs were enclosed. Histochemical tests indicated the direct involvement of the elastic sac secretion in the formation of the ovisac (Altaff & Chandran, 1994). Such a function could be attributed to oviducal glands of both *P. annandalei* and *P. serricaudatus.*

CONCLUSION

The female reproductive system of the calanoid species shows species specific variation in organization of the different regions. Occurrence of oviducal gland in *P. annandalei* and *P. serricaudatus* is reported for the first time in present study. The secretary activity of the oviducal gland synchronizes with the vitellogenesis and maturation of oocytes in the oviduct which enables embryonic development of fertilized eggs in the ovisac. This brooding strategy of *P. annandalei*, *P. serricaudatus* ensures higher survival of the nauplii compared to the species which broadcast eggs directly to the medium. The structural and functional resemblances of oviducal glands of *P. annandalei*, *P. serricaudatus* with those of freshwater diaptomids, *H. viduus* and *S.* (*R.*) *indicus* suggest close phylogenic affinity between them.

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