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REPRODUCTIVE SYSTEM OF AN ISCHNOCERAN SPECIES, *ARDEICOLA EXPALLIDUS* INFESTING CATTLE EGRET (*BUBULCUS IBIS*)

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ABSTRACT
The morphological features of reproductive tract of an ischnoceran species *Ardeicola expallidus* infesting *Bubulcus ibis* are described. The male reproductive system comprises of a pair of testes, two pairs of very small vasa efferentia, a pair of vasa deferentia, vesicular apparatus and ejaculatory duct. Each testis consists of two elongated (pyriform) follicles which lie on lateral sides in 3rd to 8th abdominal segments, on either side of mid-gut. A thin and short vas efferens arises from the base of each testicular follicle and opens in the vas deferens. The vesicular apparatus is a complex organ incorporating the seminal vesicles and the accessory gland. Posteriorly, it opens into a prominent long tube ductus ejaculatorious. The female reproductive system consists of a pair of ovaries, a pair of lateral oviducts, a median oviduct, a vagina and a spermatheca. Each ovary consists of five polytrophic ovarioles located on either side of the gut in first to fifth abdominal segments. The lateral oviducts lying in V-VI abdominal segments are small thick tubes that connect the ovarioles to the median oviduct. Proximal portion of each lateral oviduct receives the pedicel of all the ovarioles of one side. The vagina is a spacious sac like structure, lying just beneath the hind gut, in the 7th abdominal segment. The point of union between the median oviduct and vagina is marked by the opening of spermathecal duct. The spermatheca is thin walled, sac like, situated dorsally above the median oviduct, opening into a very fine duct (spermathecal duct) terminating on the dorsal side of vagina.

INTRODUCTION

The avian lice, phthirapterans owe their economic importance due to their potency to cause anemia, detrimental immune reactions, irritability, dermatitis, skin necrosis, reduced weight gains, secondary infections, localized haemorrhages, blockage of orifices, inoculation of toxins and exsanguinations (Price and Graham, 1997). Survey of literature reveals that although taxonomic studies have been performed on cattle egret louse, *Ardeicola expallidus* there are hardly any reports on its anatomy.

Specific studies on the male reproductive tract of phthirapteran species have rarely been performed. Blagovestchensky (1955 and 1959) indicated the morphological features of reproductive tracts of selected ischnoceran and amblyceran lice. The anatomy and histology of male reproductive tract of three ischnocerans (*Columbicola columbae,* pigeon louse; *Bovicola caprae* and *Bovicola limbata*, goat biting lice) and two amblyceran species (*Trimenopon hispidum*, swan louse; *Eomenacanthus stramineus*, chicken body louse) were described by Schmutz (1955) and that of male reproductive tract of *Lipeurus lawrensis tropicalis* was described by Agarwal and Saxena (1980) and Saxena and Agarwal (1981, 1982). Studies on the female reproductive tract of some phthirapteran species were conducted by Saxena and Agarwal (1980, 1981) and Agarwal and Saxena (1982) and of two ischnoceran lice by Saxena *et al*. (2004). Workers like Eichler (1963), Smith (2001) and Price *et al*. (2003) reviewed the information on the anatomy of Phthiraptera.

Looking into the paucity of information on the reproductive system of *Ardeicola expallidus* infesting cattle egret (*Bubulcus ibis*), the present venture was undertaken and an illustrated description of the male and female reproductive systems of the louse is provided.

MATERIALS AND METHODS

Feathers bearing adult lice (*A. expallidus*) were gently cut from host body. Healthier adults were sorted from feathers or *in vitro* culture stock (maintained at 35 ± 1ºC, 75-82% RH, at a feather diet) and placed in a drop of insect Ringer's solution (on black paper fitted in dissection dish) with the help of fine camel hair brush.

The lice were dissected under Olympus Stereozoom Magnus MSZ-TR binocular microscope with the help of fine entomological German pins (further sharpened by rubbing on "0" number sand paper and combination stone). After making an incision in the terga (along mid line), a drop of aqueous bouins fluid was added to get proper differentiation of organs. The fat bodies were carefully removed, the visceral organs were exposed and taken out (Fig. 2A, B). The reproductive tracts were isolated, dehydrated by passing in different grades of alcohol, stained with acid fuschin, cleared in xylene or clove oil and mounted in DPX to observe the anatomy of the reproductive organs. The organs were examined, measured and photographed.

RESULTS

Reproductive system

A. expallidus shows sexual dimorphism, the females being longer than the males.

Male reproductive system

The male reproductive system of *A. expallidus* comprises of a pair of testes, two pairs of very small vasa efferentia, a pair of vasa deferentia, vesicular apparatus and ejaculatory duct (Fig. 1 a, 2a).

Each testis (0.57mmL, 0.08mm W) consists of two elongated (pyriform) follicles which lie on lateral sides in 3rd to 8th abdominal segments, on either side of mid-gut (Fig. 2 A). Two pyriform follicles remain fused together at their broader bases. Terminal end of each testicular follicle is not produced into fine short filaments. A thin and short vas efferens arises from the base of each testicular follicle and opens in the vas deferens (0.99mm L, 0.006 mm W) (Fig. 1 A).

The short vasa efferens arising from each testicular follicle are very fine and short and soon continue in the long tubular vas deferens, which runs downwards up to 8th segment then takes a U turn and ascends upwards to open into the vesicular apparatus (Fig 2 A).

The vesicular apparatus (0.6mm L, 0.15mm W) is a prominent, muscular, club-shaped structure lying beneath the gut (Fig. 2A). Morphologically it is a complex organ incorporating both the seminal vesicles and the accessory gland. The anterior portion of vesicular apparatus is narrow in comparison to the posterior (Fig. 2 C). One of the component may be regarded as seminal vesicle (inner one into which vasa deferentia opens) while the other as accessory gland. Distally, the vesicular apparatus opens into the ejaculatory duct (0.55mm L, 0.06mm W) (Fig. 1 A).

Posteriorly, the vesicular apparatus opens into a ductus ejaculatorious which is a prominent long tube, narrowing posteriorly. It remains prominently curved in the middle portion.

Female reproductive system

The female reproductive system of *A. expallidus* consists of a pair of ovaries, a pair of lateral oviducts, a median oviduct, a vagina and a spermatheca (Fig. 1 B, 2 B).

Each ovary (0.78mm L, 0.15mm W) consists of five polytrophic ovarioles located on either side of the gut in first to fifth abdominal segments. Each ovariole consists of four distinct zones; the terminal filament, the germarium, the vitellarium and the pedicel (Fig. 2 B)

The terminal filament is very thin, long and slender. The posterior ends of all the terminal filaments of ovarioles of one side unite together and fuse to form the lateral oviduct. The germarium is very small and indistinguishable from the posterior part of the filament. The vitellarium is the most conspicuous portion of the ovariole and contains two developing oocytes, arranged in a linear fashion. The oldest oocyte is situated near the pedicel and forms the bulk of ovarioles. The younger oocyte is situated anterior to the old oocytes and is small. The oocytes in all the five ovarioles of an ovary occur in different stages of maturation. Pedicel is the fine long duct which connects the ovariole to the lateral oviduct. The length of pedicel varies according to the stage of development of ova present in egg tube. The most developed ovariole contains large ova and the short pedicel while the least developed ovariole has a small ova and a long pedicel (Fig. 2 D).

The lateral oviducts (0.14mm L, 0.13mm W) are small, thick tubes that connect the ovarioles to the median oviduct. They lie in V-VI abdominal segments. Proximal portion of each lateral oviduct receives the pedicel of all the ovarioles of one side (Fig. 2 D). Distally each lateral oviduct opens into the median oviduct. Median oviduct (0.54mm L, 0.36mm W) is a pouch like structure located in V-VIth abdominal segments (Fig. 1 B). The latter then continues into the vagina which opens into the genital chamber by the female gonopore. The median oviduct is filled with a thick secretion which might be used by the lice to glue the eggs to the feather. Accessory glands were not detected (Fig. 1 B).

The vagina (0.42mm L, 0.52mm W) is a spacious sac like structure, lying just beneath the hind gut, in the 7th abdominal segment (Fig. 2B). The point of union between the median oviduct and vagina is marked by the opening of spermathecal duct (Fig. 1B). The vagina is supported by several muscle strands and opens into the genital chamber by female gonopore in the posterior part of last abdominal segment.

The spermatheca (0.27mm L, 0.07mm W) is a thin walled sac like structure situated dorsally above the median oviduct. It opens into a very fine duct (spermathecal duct) (0.21mm L, 0.02mm W) which terminates on the dorsal side of vagina (Fig. 1 B).

DISCUSSION

The pattern of male reproductive tract of *A. expallidus* resembles other ischnoceran species. Each testis comprises of two follicles which are joined together giving a bilobed appearance. However, in the amblyceran species, each testis is made up of three pyriform follicles which remain separate from one another and are joined together by vas deferens (Blagovestchensky, 1955, 1959; Schmutz, 1955; Srivastava, 1974). The two vasa deferentia connect the testes to the vesicular apparatus. The vasa deferentia of the two sides have the same diameter throughout their length. They reportedly widen at the point of connection in certain species (Blagovestchensky, 1959). The vesicular apparatus of the phthirapterans presumably acts as composite organ (in addition to providing space for collection of sperms, also secretes fluid for conveyance of sperms). The vesicular apparatus performs secretary as well as storage function. In certain species (*Menacanthus stramineus*), vesicular apparatus consists of two completely separate paired structures (inner one called vesicula seminalis and outer lateral tube) (Schmutz, 1955), in some species (*L. lawrensis tropicalis*), it remains divided for over half of its length (Agarwal and Saxena and Agarwal, 1980) while in others (*C. columbae, M. eurysternus* and *L. percnopteri*), the vesicular apparatus is completely undivided and dual nature is marked externally by median furrow (Blagovetchensky, 1955, Schmutz, 1955; Srivastava, 1974). The vesicular apparatus is apparently prominent, simple,

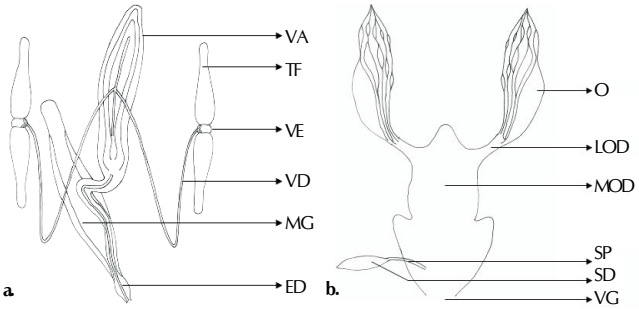

Figure 1:Camera lucida drawings of the reproductive system of *Ardeicola expallidus*; a: Male; b: Female; Abbreviations: ED- Ejaculatory Duct, LOD- Lateral Oviduct, MG- MaleGenitalia MOD- Median Oviduct, O- Ovary, SD- Spermathecal Duct; SP- Spermatheca, TF- Testicular Follicle, VA- Vesicular Apparatus, VD- Vas Deferens, VE- Vas Efferens, VG-Vagina

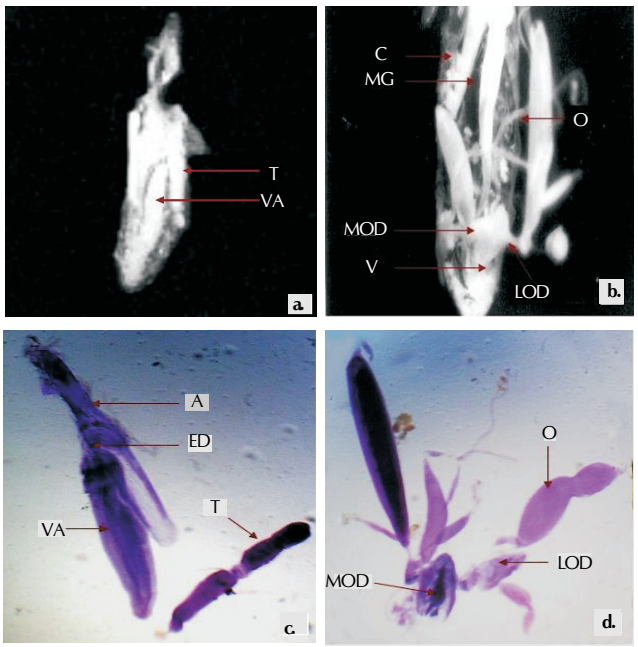

Figure 2: Microphotograph of reproductive system of *Ardeicola expallidus*; a: Dissected male (x 23); b: Dissected female (x 32); c: Whole mount preparation of male (x 51); d: Whole mount preparation of female (x 49); Abbreviations: A-Aedegus, C- Crop, ED- Ejaculatory Duct, LOD- Lateral Oviduct MOD- Median Oviduct, O- Ovary, T- Testis, V- Vagina, VA- Vesicular Apparatus

muscular and club shaped compact structure, having a median furrow.

The ejaculatory duct exhibits some variation in different phthirapteran species. It is a short muscular tube that connects vesicular apparatus to genitalia as in *M. stramineus*. In other species, *L. lawrensis tropicalis*, the ejaculatory duct becomes greatly swollen to make an appendix like bulbous structure.

The general pattern of female reproductive system of *A. expallidus* resembles other ischnoceran species. It consists of paired ovaries (each comprising of ovarioles) connected to conspicuous median oviduct through short thick tubes, the lateral oviducts. The wall of lateral oviduct of *L. lawrensis tropicalis*, reportedly bears protuberances (Saxena and Agarwal, 1981). The lateral oviduct is a small thick tube, having a smooth wall.

Furthermore, the vagina of *L. lawrensis tropicalis* is reported to be longer, divisible into three parts: anterior portion remains sunken into the posterior part; the space formed is used to collect the sperms whereas in *A. expallidus*, the vagina is a thin walled spacious sac like structure, lying just beneath the hind gut. The spermatheca (thin walled sac of varying morphology, from which runs a feebly sclerotized tube opening in dorsal wall of genital chamber) is present in certain species but absent in several others (Blagovestchensky, 1959; Eichler, 1963; Saxena and Agarwal, 1981; Smith, 2001). Spermatheca of *L. percnopteri, M. eurysternus* and *P. ocellatus* have been reported to be bilobed while *A. expallidus* possesses simple sac like spermatheca. Thus, the findings reported in this communication differ from the reports of the earlier workers and the reproductive system of *Ardeicola expallidus* is being reported for the first time.

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