

Histomorphological study of the male reproductive system in the Indian drone Honeybee, *Apis cerana indica* (Hymenoptera)

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Manuscript details:	ABSTRACT
<p>Available online on http://www.ijlsci.in</p> <p>ISSN: 2320-964X (Online) ISSN: 2320-7817 (Print)</p> <p>Editor: Dr. Arvind Chavhan</p> <p>Cite this article as: Sawarkar AB and Tembhare DB (2015) Histomorphological study of the male reproductive system in the Indian drone Honeybee, <i>Apis cerana indica</i> (Hymenoptera), <i>International J. of Life Sciences</i>, Special Issue, A3:63-66.</p> <p>Acknowledgement The authors are grateful to UGC-Western Zone, Pune for providing financial assistance under minor research project (47-1506/10).</p> <p>Copyright: © Author, This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derives License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p>	<p>The internal male reproductive system in <i>Apis cerana indica</i> was consists of a paired testis, vasa deferentia, mucus gland and a median ejaculatory duct. It was observed that each testis internally packed with seven tube-like follicles compactly filled with the cysts. The spermatogenic stages mainly occurred in the pupal stages. The seminal vesicle represented by a distal large sac-like region of the vas deferens. It consists of an inner epithelial and outer thick muscle layers. The epithelial layer was consists of tall, columnar cells with brush border towards the lumen filled with a large mass of sperm bundles. The paired mucus glands were large, sac-like structures representing a peculiar type of male accessory glands. The epithelial layer of mucus gland was composed of tall, columnar glandular cells which showed their secretory nature. It was noticed that the mucus gland and the seminal vesicle has mesodermal in origin. The mucus glands open into a common ejaculatory duct via lateral ejaculatory ducts. The ejaculatory duct was a long slender tube opens distally into an aedeagus. The wall of the ejaculatory duct was differentiated into outer broad epithelial layer and inner cuticular layer. The inner thin cuticular layer bears elongated spines in the lumen. The presence of cuticular layer showed that the ejaculatory duct is ectodermal in origin.</p> <p>Keywords: drone honeybee, <i>Apis cerana indica</i>, male reproductive organs</p> <p>INTRODUCTION</p> <p>In social hymenopterans (ants, bees and wasps), the reproductive system in males has received little attention when compared to that of queens and workers on the basis of colony performance. They play the major role in colony formation, but still neglected (Bishop, 1920; Snodgrass, 1956). The male reproductive system in these social insects constitutes paired testes opens into the male gonopore through variable modified reproductive tract. Certain glands also associated to the reproductive tract, which produce secretions that are helpful to the sperm (Chen, 1984; Davey, 1985; Gillot, 1988; Chapman, 1998). Also in bees, the seminal vesicle and the sex accessory gland represents the primary sperm storage bag where the sperms are stored until mating. Their secretion may affect physiological and</p>

behavioral changes in mated female (Bishop, 1920; Snodgrass, 1956; Ferreira *et al.*, 2004). Physiological and biochemical studies of reproductive glands in hymenopterans have shown variety of basic features in spite of the morphological diversity of these glands (Davey, 1985; Happ, 1992; Baer *et al.*, 2003; Sawarkar and Tembhare, 2010; 2014).

Ferreira *et al.* (2004) noticed that the internal reproductive organs of 51 bees are variable histomorphologically. On the basis of origin, morphological pattern and physiological role, the reproductive apparatus differentiated into Type I, Type II and Type III.

As per the focus on recently demonstrated importance of the male secretory substance and their role associated to the sperm and female tract, the present study undertake the histomorphological differentiation of the internal reproductive organs of drone honeybee in *Apis cerana indica*.

MATERIAL AND METHOD

During the present study, the adult drones of *Apis cerana indica* were collected from the hive established at the premises of the CES College, Chalisgaon, Dist-Jalgaon (India) during the year 2010 to 2013. The internal reproductive organs of the drone honeybees were dissected in the insect Ringer solution. The tissues then fixed immediately in Bouin's fixative for 18-24 h, dehydrated in ethanol, cleared in xylene and then embedded in paraffin wax at 58-60^o C. The sections were cut at 4-6 μm thickness. The sections were stained with Ehrlich's Haematoxylin Eosin (HE) and Heidenhain's Iron haematoxylin-orange G (Fe-H) histological techniques (Tembhare, 2006).

RESULT AND DISCUSSION

The internal male reproductive system in *Apis cerana indica* consists of a pair of testes, a pair of vasa deferentia, a pair of accessory sex glands and a median ejaculatory duct (Fig. 1). It is also observed in other honeybees as *Apis mellifera* (Bishop, 1920; Snodgrass, 1956; Woyke, 1958; Simpson, 1960; Koeniger, 1986), *Apis dorsata* (Paliwal, 1993) and *Apis florea* (Koeniger *et al.*, 1989).

The testes are creamy, oval-shaped bodies lying at anterior side of the mucus glands and situated in between the 2nd and 3rd abdominal segments. The numbers of testicular follicle are seven in *A.c. indica* and are variable in number in other

hymenopterans (Snodgrass, 1956; Wheeler and Krutzsch, 1992; Duchateau and Mariën, 1995; Ferreira *et al.*, 2004). Each follicle is composed of an inner layer of epithelial cells and an outer layer of muscle fibres. At the inner side consist of number of cysts with full of spermatogenic stages (Fig. 2,2a). Each follicle had opened posteriorly into the vas eferens and then into a long, coiled vas deferens. It is differentiated into three regions, the apical short coiled tube, middle cylindrical seminal vesicle (SV) and distal straight duct. The apical part of vas deference consists of outer circular muscle layer and inner epithelial layer with large lumen (Fig. 3). The distal part opens into the basal region of the mucus gland (MG) with the muscular valve (Fig. 4). The SV represents distal large sac-like region of the vas deferens which bears an inner epithelial and outer thick muscle layers and externally covered with a thin peritoneal sheath. The muscle layer is composed of outer longitudinal and inner circular muscle layers. The epithelial cells are tall and columnar with brush border towards the lumen. The lumen is filled with a large mass of sperm bundles, keeping their heads towards the wall and tails at the center of the lumen (Fig. 5, 5a).

The paired mucus glands (MG) are large, kidney-shaped, sac-like structures representing a peculiar type of male accessory glands in the bee. Each gland consists of larger distal and a narrow proximal part with a constriction in between. Each gland is tapering basally and opens into the ejaculatory duct (ED) through a well-defined valve. The wall of the MG is composed of an inner epithelial layer and outer thick muscle coat and externally covered with thin peritoneal sheath. The epithelial layer is formed of tall, columnar glandular cells. The muscle coat is formed of three sublayers, the outer longitudinal, middle circular and inner longitudinal muscle layer. The lumen of the MG is filled with a variable amount of secretory material (Fig. 6, 6a).

The MG opens into the paired, lateral ED and then into a long slender common ED. The wall of the ED is composed outer broad epithelial layer and inner cuticular layer. It does not represent outer muscular layer. The epithelial layer is composed of squamous epithelial cells bearing nuclei at the base and scanty cytoplasm towards the perikarya. The inner thin cuticular layer bears elongated spines in the lumen. The lumen is large measuring about $289.7 \pm 2.763 \mu\text{m}$ in diameter and filled with a mass of sperms in the mature drones (Fig. 7, 7a).

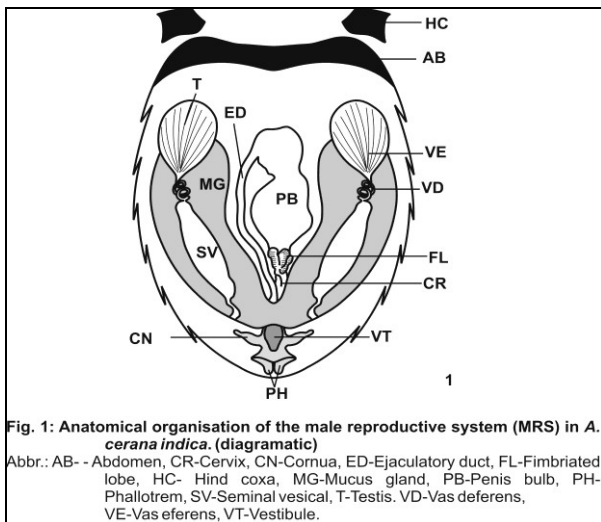


Fig. 1: Anatomical organisation of the male reproductive system (MRS) in *A. cerana indica*. (diagramatic)
 Abbr.: AB- Abdomen, CR-Cervix, CN-Cornua, ED-Ejaculatory duct, FL-Fimbriated lobe, HC- Hind coxa, MG-Mucus gland, PB-Penis bulb, PH-Phallosome, SV-Seminal vesicle, T-Testis, VD-Vas deferens, VE-Vas eferens, VT-Vestibule.

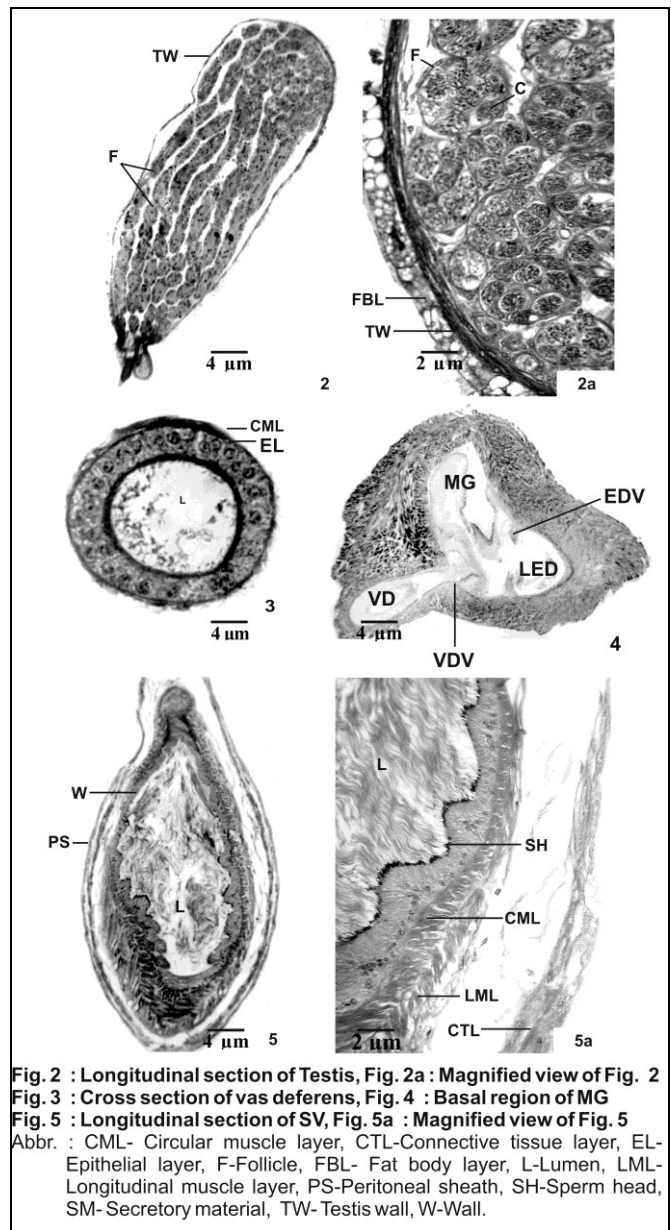


Fig. 2 : Longitudinal section of Testis, Fig. 2a : Magnified view of Fig. 2
 Fig. 3 : Cross section of vas deferens, Fig. 4 : Basal region of MG
 Fig. 5 : Longitudinal section of SV, Fig. 5a : Magnified view of Fig. 5
 Abbr. : CML- Circular muscle layer, CTL-Connective tissue layer, EL- Epithelial layer, F-Follicle, FBL- Fat body layer, L-Lumen, LML- Longitudinal muscle layer, PS-Peritoneal sheath, SH-Sperm head, SM- Secretory material, TW- Testis wall, W-Wall.

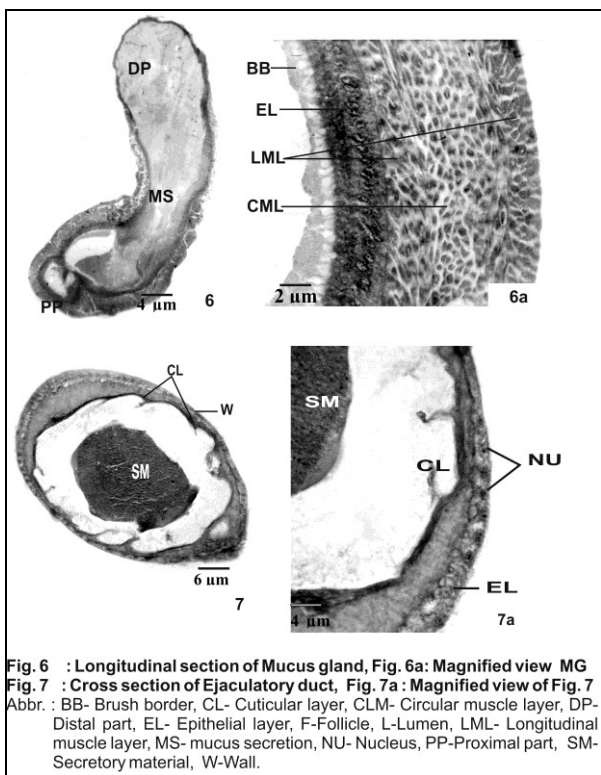


Fig. 6 : Longitudinal section of Mucus gland, Fig. 6a: Magnified view MG
 Fig. 7 : Cross section of Ejaculatory duct, Fig. 7a : Magnified view of Fig. 7
 Abbr. : BB- Brush border, CL- Cuticular layer, CML- Circular muscle layer, DP- Distal part, EL- Epithelial layer, F-Follicle, L-Lumen, LML- Longitudinal muscle layer, MS- mucus secretion, NU- Nucleus, PP-Proximal part, SM- Secretory material, W-Wall.

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The histological structure of the vas deferens (VD), seminal vesicle (SV) and mucus gland (MG) suggests their mesodermal origin while that of the ejaculatory duct (ED) as the ectodermal origin in *A. c.indica*. The epithelium of the SV and MG of *A. c. indica* is secretory and the inner surface bears the brush border, similar to that in *A. mellifera* (Bishop, 1920; Snodgrass, 1956) and *A. dorsata* (Paliwal, 1993). It is also noted that the wall of SV as well as MG in *A. c.indica* becomes thick and muscular due to the presence of longitudinal and circular muscle fibres which may initiate rhythmic contraction to facilitate transport of spermatozoa

towards the ejaculatory duct. The opening of MG and median ED are moreover, provided with well-defined valves in *A. c. indica*, which seems to be a common feature in the honeybees (Snodgrass, 1956; Paliwal, 1993) in order to control consecutive release of semen as well as mucus gland secretion.

CONCLUSION:

It is concluded that in *A. c. indica* the internal male reproductive system consists of a paired testis, seminal vesicle, mucus gland and an ejaculatory duct.

The testes are elongate, oval-shape, creamy white bodies observed during the pupal stages and consists of seven follicles.

The testis becomes shrink due to complete release of spermatozoa shown in adult drones. It is also noticed that the size of the SV and the MG increases in adults which may associated to the storage of sperms until the mating period. While the secretion of the mucus glands which may involve in the longevity of sperm and sperm storage. The SV and the MG consist of outer muscle coat and inner smooth columnar epithelial layer. While the ejaculatory duct composed of outer epithelial and inner cuticulin layer which shows that the SV and MG are mesodermal and ED is ectodermal in origin.

It may helpful to understand the reproductive physiology of drone in this species and other related Hymenoptera species.

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