

SPERM PATHWAY AND SPERMATOPHORE FORMATION IN THE HERMAPHRODITE SNAIL *EREMINA EHRENBERGI* ROTH, 1839

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(Received August 9, 1992; revised May 16, 1993)

Storage and transport of sperm and spermatophore formation were studied in the hermaphrodite snail *Eremina ehrenbergi*. Sperm are found in the vesicula seminalis all the year round. At precopulation state, they pass down to enter the sperm-channel of the spermoviduct where they are mixed with three different secretions. At this time, the body of the spermatophore is under formation in the penis proper. At copulation state, single fully formed spermatophore is found in the genital atrium of the copulant. It consists of a very small head, a narrow neck, a comparatively large body and a long tail. The sperm mass is enclosed inside the body of the spermatophore and the empty tail has a highly folded wall.

Key words: Sperm, Spermatophore, Sperm channel, Penis proper.

Introduction

The morphology of the hermaphrodite genital system of some stylommatophoran pulmonates and its role in the production and conduction of the different gametes (sperm and ova) were studied [1-7]. The variation in the structures of the gonads in pulmonates open other studies on the process of spermatogenesis and the structure of the sperm [8-11] Bawab *et al.* [7, 12].

During copulation, the male tract of the reproductive system of gastropods discharges its product in one of three forms: a semen, an aggregation formed prior to ejaculation or a spermatophore [13].

In several stylommatophora, sperm transfer takes place by means of the spermatophores. The usual function of the spermatophore in groups such as annelides, arthropods and cephalopods which lack copulatory organs is to protect sperm during their transference from male to female. In stylommatophorans which have a well developed muscular penis, the spermatophore may have some other role. The function of the spermatophore in relation to the release and migration of sperm after copulation has been studied in *Helix pomatia* [13], however, in general, very little is known about the sequence of formation of the spermatophore during copulation.

Much work is still needed before we have a properly conceived view of the site of spermatophore formation and the number of spermatophores produced by each animal. The principal aim of the present work is to add to information already available in this field. The present study, however, also aims to determine the structure of the spermatophore in the snail *Eremina ehrenbergi* and the synchronization of its

formation with the process of copulation. As a first step, this requires a comprehensive study of the structure and function of the male tract and the copulatory organ.

Material and Methods

Eremina ehrenbergi Roth, 1839 is a desert snail found in Egypt. Live adult snails were collected bimonthly from El-Omayed, 80 km west of Alexandria. Each collection was transferred to the laboratory and some suitable care for the snail was continually offered so as to maintain a successful and permanent culture. In the laboratory, two couples were observed in the position of copulation described by Lipton *et al.* [14]. Other couples were observed preparing to copulate. Each faced its partner and the dart sac and penis of one of the two snails were protruded from the genital opening. Snail representative of these states were immersed in boiled water to facilitate the removal and dissection of the soft parts. The male reproductive tract of the dissected snails were then isolated and the parts of the male tract involved in the formation of the spermatophore were studied.

For histological study, the isolated parts were fixed in either Bouin's or Carnoy fixatives then dehydrated, cleared and embedded in paraffin wax. The isolated parts were serially sectioned so as to trace the location of the sperm in the different parts of the male gonoduct. Sections were cut at 6-8 μ .

The spermatheca of the partner, taken immediately after copulation, was incised and macroscopically examined for its contents. In addition, smear preparations of the contents were made.

Results and Discussion

The reproductive system of *Eremina ehrenbergi* is unpaired and described as a monaulic monotremy system

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(Fig. 1.). The proximal generative portions of its hermaphrodite gonadal tubules produce large numbers of sperm all years round with a maximum peak of production during Autumn [15].

Storage of the sperm. The produced sperm are discharged from the gonad through a long hermaphrodite duct (Fig. 1) whose function appears to be essentially conductive. This duct is differentiated into three distinct regions: proximal, middle and distal. In addition to the above function, the middle region (vesicula seminalis) stores the produced sperm. Sections taken through the hermaphroditic duct showed large numbers of stored sperm in the vesicula seminalis all the year round (Fig 2). The secretory activity of its epithelial lining was readily observed (Fig. 3). It secretes moderate amounts of fluid secretion 1 which accepts the red and green colours of Mallory's and Masson's stains respectively. Secretion 1 was mixed with the stored sperm and may be for nourishing them during the time of preservation.

Pathway of the sperm. Serial sections taken through the spermoviduct from its beginning revealed that the conduction of the stored sperm takes place through the talon which is embedded in the albumen gland (Fig. 1-AG). In spite of the complicated structure of the talon, sperm can pass directly from the hermaphrodite duct into the spermoviduct through the ciliated hood of the talon (Fig. 1).

In section, the spermoviduct has an oval outline, a very thick wall and a marked irregular lumen (Fig. 4). This lumen is differentiated into two unequal connected channels (Fig. 5); the egg (e.ch.) and sperm channels (sp. ch.). The sperm channel is represented by the narrow lumen. Histological examination shows that, before copulation, sperm when passing down through the sperm channel (Fig. 1) do not enter the egg channel because there is a functional separation of the two by epithelial folds (Fig. 6). These epithelial folds are termed the outer (o.v.) and the inner valves (i.v.).

In addition, the epithelial lining of the sperm channel is highly ciliated (Fig. 7) and a muscular layer surrounding its epithelial lining was observed (Fig. 6-m). Well defined glandular tissue (prostate gland) was observed outside the sperm channel (Fig. 5-pr.gl.) consisted of numerous prostatic acini (Fig. 9-pr.ac.) with fine prostatic ductules which open directly into the sperm channel lumen (Fig. 8). The prostatic cells in each acinus appear loaded with a fine granulated secretion (Secretion 2) (Fig. 9-S.2) which accepts the orange and green colours of Mallory's and Masson's triple stains respectively. The discharge of this secretion is by merocrine type. Underneath the epithelial lining of the sperm channel and between the prostatic acini other glandular cells were easily observed (Fig. 7-sm.g.) These glandular cells are termed the submucosal gland cells. They have no definite shape and appear

loaded with a copious granulated secretion (S.3) which stained the blue and green colours of Mallory's and Masson's triple stains. This secretion is discharged into the sperm channel lumen by holocrine mode. Other gland cells were also observed in the inner and outer valves. They were wholly occupied with patches of a fluid secretion (Fig. 6-S. 4) which stained violet to Mallory's. These gland cells are termed the valve gland and their secretion is Secretion 4.

At the time of copulation, the sperms are discharged through the lengthy sperm channel of the spermoviduct and are intermingled with and partially surrounded by secretions 2,3 and 4 of the sperm channel (Fig. 10).

Encapsulation of the sperm. The above histological picture concerning the sperm channel is the same along the whole length of the spermoviduct until the point of its bifurcation into the oviduct and the vas deferens. At this point, sperm are expelled by the aid of cilia in the sperm channel and enter the vas deferens which lies on the same side of the sperm channel and forms a natural continuation of its lumen (Fig. 1). Examination of serial sections taken through the vas deferens of different snails at different states showed that it always empty and it appears that it functions merely to expelled sperm. The thick muscular wall of the vas deferens of *E. ehrenbergi* provides evidence for this function.

Following the vas deferens is the penis which is the most distal part of the male tract (Fig. 1-p). It is the copulatory organ which consists of two distinct regions; the proximal penis proper and the distal praeputium. In section, the penis proper has a well defined muscular coat and an internal highly folded, epithelial lining (Fig. 11). If the dimensions are not taken into consideration, the praeputium has a similar histological structure to that of the penis proper, but with shallower folds and consequently, comparatively wide lumen. According to Mann [16] the spermatophore of pulmonates is formed during copulation. During the course of this study (about two years) no spermatophore were observed in any part of the male tract of snails post-copulation. On the other hand, fully formed spermatophores were found in snails in pre-copulation and in copulation states. Sections of male gonoducts of snails which had been dissected at these state showed single spermatophores in either the penis proper or the genital atrium of the partner.

In snails which were killed at pre-copulation state, almost fully formed spermatophores were found in sections of the penis proper. In addition, the secretory activity of the epithelial lining of penis proper was readily observed (Fig. 11). The used combined staining differentiated two different materials secreted by the epithelium of the penis. One secretion appears as a crystalline substance which accepts the colour of orange G of Mallory's stain. According to the sequence of the produc-

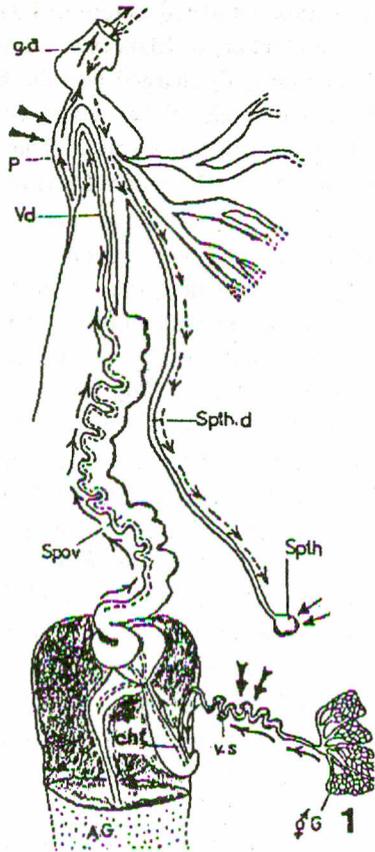


Fig. 1. Diagrammatic drawing for the reproductive system of *E. ehrenbergi* showing its different parts. X 3.

cht.: ciliated hood of the talon; q.g.: hermaphrodite gland; g.a.: genital atrium; p.: penis; sph.: spermatheca; sph. d.: spermathecal duct; spov.: spermooviduct; vd.: vas deferens; v.s.: vesicula seminalis.

- arrows indicate storage of sperm.
- arrows indicate sperm pathway.
- arrows indicate site of formation of spermatophore.
- arrows indicate pathway of introduced spermatophore.

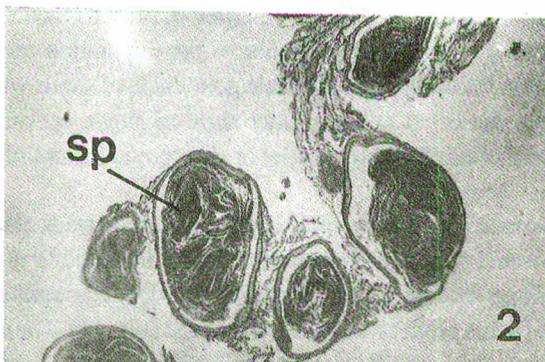


Fig. 2. Section through the vesicula seminalis of the hermaphroditic duct of *E. ehrenbergi* showing the preserved sperm. X 30.
sp.: sperm.

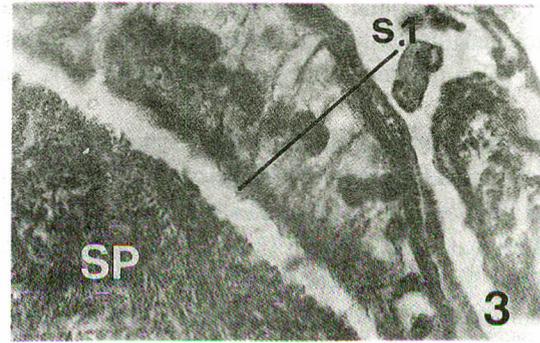


Fig. 3. Highly magnified portion of the vesicula seminalis showing its secretory epithelial lining. X 860.
sp.: sperm; S.1: secretion 1.

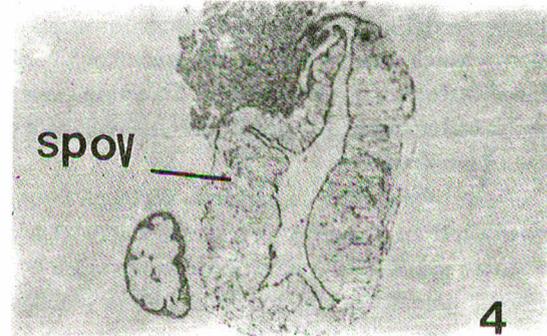


Fig. 4. A transverse section of the spermooviduct of *E. ehrenbergi* showing its lumen. X 28.
spov.: spermooviduct.

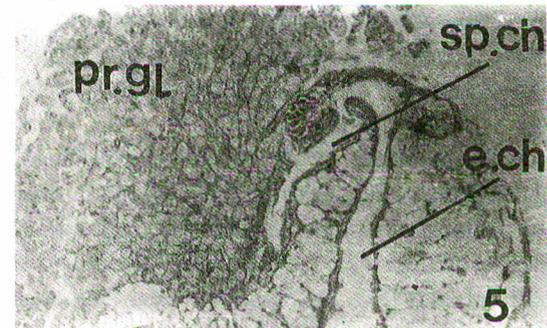


Fig. 5. A magnified portion of section of the spermooviduct showing the egg channel and the narrow sperm channel with the surrounding prostate gland. X 65.
e. ch.: egg channel; pr. gl.: prostate gland; sp. ch.: sperm channel.

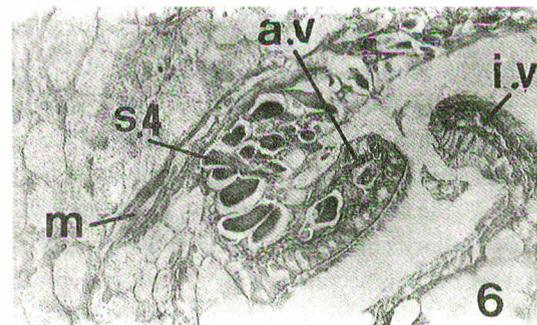


Fig. 6. A magnified portion of the spermooviduct showing the valves between sperm and egg channels. Notice the secretion of the valve gland. X 340.
a.v.: outer valve; i.v.: inner valve; m.: muscles; S. 4: secretion 4.

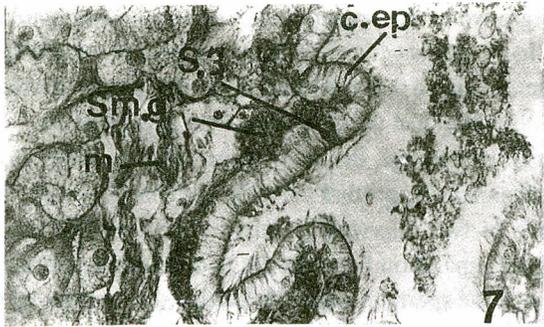


Fig. 7. A magnified portion of section of spermoviduct showing the ciliated epithelial lining of the sperm channel and the associated submucosal gland cells. X 450.

c.ep.: ciliated epithelium of the sperm channel; m.: muscles; Sm.g.: submucosal gland cells; S. 3: secretion 3 of the submucosal gland cells.

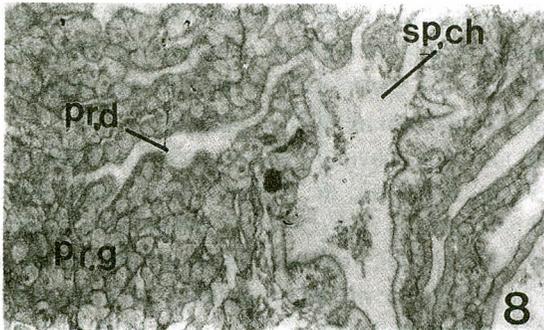


Fig. 8. A magnified portion of section of spermoviduct of *E. ehrenbergi* showing the prostatic tissue and the prostatic while opens into the sperm channel. X 100.

pr.d.: prostatic ductule; pr.g.: prostate gland; sp.ch.: sperm channel.

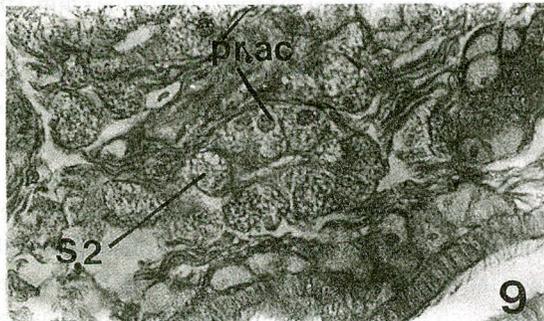


Fig. 9. A magnified portion of section of spermoviduct of *E. ehrenbergi* showing the acini of the prostate gland. Notice the granulated secretion of the prostatic cells (secretion 2). X 450.

pr. ac.: prostatic acinus; S. 2: secretion 2 inside the prostatic cell.

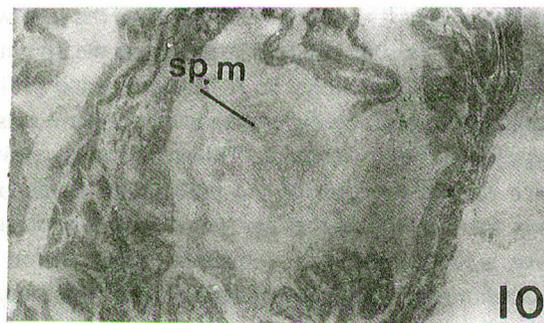


Fig. 10. A transverse section of spermoviduct showing the expelled sperm mass inside the sperm channel. X 250.

sp. m: sperm mass.

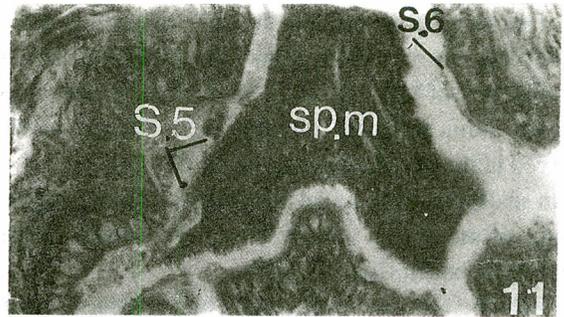


Fig. 11. A magnified portion of a transverse section of the penis proper of copulated *E. ehrenbergi* showing its epithelial lining and sperm mass in its lumen. Notice the crystalline secretion (secretion 5) and the fluid secretion (secretion 6). X 320.

sp.m.: sperm mass; S.5: Secretion 5, S.6: Secretion 6.

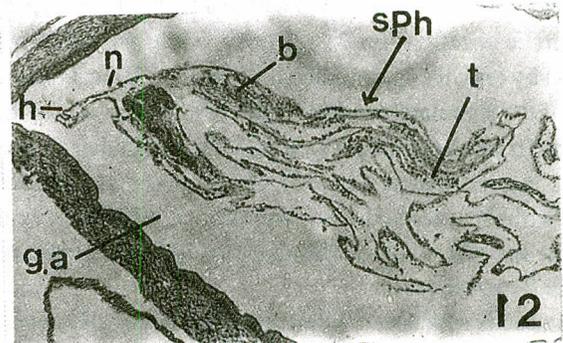


Fig. 12. A photograph of a longitudinal section of the genital atrium of the copulated snail *E. ehrenbergi* showing the spermatophore inside. X 75.

b.: body; g.a.: genital atrium; h.: head; n.: neck; t.: tail; sph.: spermatophore.

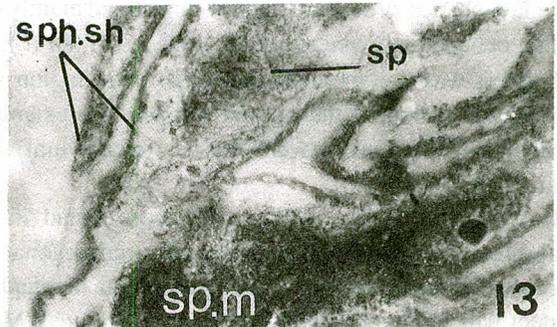


Fig. 13. A magnified portion of a longitudinal section of the spermatophore inside the genital atrium showing its body region with the sperm mass inside. Notice the folded spermatophoretic sheath. X 300.

sph. sh.: spermatophoretic sheath; sp. m.: sperm mass; sp.: sperm.

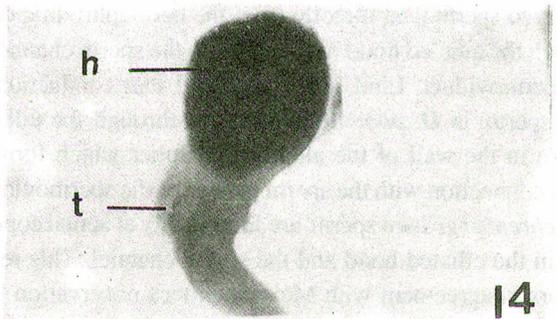


Fig. 14. Cleared spermatophore which was isolated from the spermatheca of the partner at the end of copulation. X 10.

h.: head; t.: tail.

tion of the secreted materials in the male tract, this is 'secretion 5'. The other, 'secretion 6' appears fluid and accepts the colour of aniline blue of Mallory's stain. In sections, a mass of sperm enclosed within noticeable amounts of secretions 5 and 6 was observed in the lumen of penis proper (Fig. 11).

In snails that were killed at copulation state, longitudinal sections of the genital atrium show fully formed spermatophores in its lumen (Fig. 12-sph.).

Fully formed spermatophore. The spermatophore of *E. ehrenbergi* consists mainly of a body and tail (Fig. 12-b.t.) In addition, the very small anterior region is differentiated into a head (h) and neck (n). The body, however, is formed mainly of an inner sperm mass enclosed within a highly folded spermatophoretic sheath. (Fig. 13-sph, sh.), while the tail is represented by only the folded sheath (Fig. 12). Critical examination of the spermatophoretic sheath reveals that it is made of two different secretions which are mixed together to form a single layered sheath (Fig. 13). Comparing these two secretions with those found in the penis proper, it becomes evident that the spermatophoretic sheath is formed mainly in the penis proper by its secretions.

In snails which were killed just after the end of copulation process, a fully formed spermatophore was always found in the spermatheca and could be easily removed. Under macroscopical examination, the isolated spermatophore appears as a small, claw-shaped structure (Fig. 14) constituted mainly of a rounded, opaque body and a curved, transparent tail (t.). To prove that it was the spermatophore, the body region was smeared on a slide and the opened capsule was examined microscopically. Live sperm were observed over several hours of examination.

The present results together with those found in the available literature show that the sperm produced by gonads of pulmonates are stored in the middle region of the hermaphroditic duct which is more wider and more convoluted. Many authors, Breucker [17], Lind [13], Bawab *et al.* [7, 12] referred to this region as the vesicula seminalis. The present results together with that of Bawab *et al.* [7, 12] showed that the preserved sperm pass directly from the hermaphroditic duct, through the ciliated hood of the talon, to the sperm channel of the spermoviduct. Lind [13] mentioned that conduction of 'own sperm' in *H. pomatia* takes place through the ciliated groove in the wall of the albumen chamber which forms a direct connection with the sperm groove of the spermoviduct. In *E. ehrenbergi* 'own sperm' are found, only at actual copulation, in the ciliated hood and the sperm channel. This result appears in agreement with Meisenheimer's observation [18] and subsequent investigators who have worked on the genital system of *Helix*. This was found not true in the experimental work of Lind [13] who observed that more than half of the

sperm groove of the spermoviduct of *H. pomatia* contained sperm in the snails which had not copulated. He stated that, it must be concluded that sperm are frequently expelled from the hermaphroditic duct at times other than at copulation.

In *E. ehrenbergi*, the 'own sperm' after passing into the sperm channel, do not enter the wide egg channel owing to the presence of two intervening valves. These valves act as a functional separation between the two channels. During the passage of the sperm through the lengthy sperm channel they are supplied and intermingled with the three secretions of the prostate gland, the sub-mucosal gland cells and the valve gland cells. Breucker [17] and Lind [13] mentioned that the prostate gland of *Helix* remains in an active secretory phase for a long period. Lind [13] added that the secretions from the glands of the gonoducts included products of the prostate gland and are secreted into the sperm groove, but did not indicate the number, origin or nature of these products.

The muscular coat of the sperm channel, the long cilia of its epithelial lining and the presence of secreted materials may all be important factors in the conduction of the sperm to the vas deferens. The muscular nature of the vas deferens wall may provide the means for pushing the sperm to penis. Lind [13] mentioned that in *Helix* the conduction of the sperm, at copulation, through the sperm groove and the vas deferens is very fast and takes few seconds and that it is certainly due to the action of the long cilia of the entire sperm duct and is doubtless also due to the mechanism of peristaltic movements. For the formation of the spermatophore Solem [19] state that specialization of the lower vas deferens into an "epiphallus" which produces a spermatophore is a specialized feature for most pulmonate land snails. Lind [13] found that the body of the spermatophore of *H. pomatia* was formed in the epiphallus while the canal of the spermatophore tails is formed in the flagellum. In the penis proper of *E. ehrenbergi*, continuous secretory activity of its epithelial lining causes the formation of the spermatophoretic sheath. The spermatophoretic sheath of *E. ehrenbergi* consists of a single layer derived from the two mixed secretions of the penis proper. The high secretory activity of the penis proper and the furrows in its wall may determine the folded nature of the spermatophoretic sheath. Very similar to *H. pomatia*, Lind [13] the praeputial lumen of *E. ehrenbergi* showed no sperm mass or any type of secretory material. This result may provide evidence for the function of the praeputium as an outer covering for the protruded penis proper during copulation. To give a conceived and complete picture about the spermatophore of *E. ehrenbergi* further studies should attempt to clarify the chemical nature of the spermatophoretic sheath so as to elucidate the method of preservation of the contained sperm.

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