

Mating behavior and structural aspects of spermatophore of two Indian scorpion species of the genus *Heterometrus* (Scorpiones: Scorpionidae)

Pratyush P. Mohapatra^{1*} and Pragya Pandey²

¹Central Zone Regional Centre, Zoological Survey of India, Jabalpur - 482002, Madhya Pradesh, India

Email: pratyush.m@zsi.gov.in

²P&T Colony, Kali Bari Marg, New Delhi - 110001, India

Abstract

The spermatophores of *Heterometrus swammerdami* Simon, 1872 and *H. bengalensis* (Koch, 1841) were studied based on the samples collected from the captive population and were compared with that of *H. phipsoni* and *H. mysorensis* as per the information provided in the literature. In India, studies on spermatophore of scorpions are still at a preliminary stage. Basic morphometric information on the spermatophore of these species is provided for a better understanding of the natural and sexual selection among Indian scorpion fauna for future studies.

Keywords: Holosolenos, India, Morphology, Scorpion, Taxonomy

Introduction

Scorpions like some other groups of arachnids namely Chelonethi, Uropygi, Schizomida, Amblypygi and Acarina exhibit an indirect process of sperm transfer to avoid desiccation of spermatids (Savory, 1977). The taxonomic characters of hemispermatophore were first used as diagnostic characters in scorpion families such as Buthidae and Scorpionidae by Vachon (1952). Further studies by Bücherl (1956), Alexander (1956, 1957), Zolessi (1956), Shulov and Amitai (1958), Koch (1977), Maury (1980) and Angermann (1995) on hemispermatophore and spermatophore of various species of scorpions highlighted the importance of male genitalia in taxonomy. However, a description of the structural aspect of spermatophore or hemispermatophore of Indian scorpions had not been carried out in much detail except a few works by Bastawade (1992, 1994). Reproductive biology of *Heterometrus phipsoni* Pocock, 1893 was described with a brief description of the post-insemination spermatophore of the species by Mirza and Sanap (2009). Later Monod *et al.* (2017) gave a detailed account of the male sexual apparatus in scorpions and stressed on use of their functional morphology as a tool in phylogenetics.

Two basic types of spermatophores have been described in scorpions; namely flagelliform, found only in the family Buthidae and lamelliform, found in all other families (Franke 1979). Spermatophore of scorpions of family Scorpionidae is an elongated lamelliform structure and has a lamella at the distal end of the lamina. The spermatophore is secreted in the paraxial organ (gland) of the two sides of the genital operculum of the male scorpion as two mirror halves or the hemispermatophores. The hemispermatophores of both the sides get cemented after they are secreted and slowly extruded from the genital operculum till the end of lamella by the male. During this process the spermatophore gets glued to the substratum by the judder action of the male and the entire spermatophore is placed at a particular angle. The spermatophore thus stuck at a suitable place on the ground becomes available for the female mating in pair with the male.

The genus *Heterometrus* Ehrenberg, 1828 is represented by 38 species, of which 23 species are reported from India (Kovářík, 2004).

Material and Methods

A colony of 29 individuals of *Heterometrus swammerdami*, comprising sub-adult and adult males and females, was

* Author for correspondence

collected from Bhubaneswar on 25th May 2009. All the individuals were kept in a terrarium of 100cm X 60cm X 30cm size with 7cm soil substratum, collected from the same termite mound. Additionally, eight wandering males were collected from Bhubaneswar city from June 2009–August 2009 and were introduced to the same terrarium. The males were easily accepted by the colony without any conflict. Similarly, six pairs of *Heterometrus bengalensis* were collected from Kapilas hills of Dhenkanal district and Ghatgaon of Keonjhar districts during May–August 2009 and were kept in circular plastic containers of 20cm diameter and 8 cm height with 3 cm of soil substratum collected from the habitat of respective scorpions. Spermatophores of two species of scorpions were obtained from the captive breeding groups and were preserved in 70% ethyl alcohol. Photographs were taken to see the natural coloration. The measurements of different parts of the organs were taken by Mitutoyo digital caliper to the nearest 0.1 mm. The basic spermatophore terminologies are as per Monod *et al.* (2017) unless otherwise stated in the text.

Results and Discussion

Heterometrus swammerdami was observed living in colony, inside degraded or semi-degraded termite mounds, tree holes and also in abandoned rat or crab holes in the bunds of agricultural fields. Colonies of 8–56 individuals comprising sub-adults, adults of both the sexes were observed during the field study in Odisha. The males were found to be active during the summer season (April to July) and solitary individuals were observed out of their dens during that period. *H. bengalensis* was mostly seen in pairs during the breeding season in winter and summer months. Babies were seen with the mother until the third molting stage. This species lives below boulders by making burrow with an elliptical opening. The entrance of the burrow is smooth and wider, which then extends interiorly as a narrow tunnel ending with a wider semi-circular nest.

Two pre-insemination and thirteen post-insemination spermatophores of *H. swammerdami* and two post-insemination spermatophores of *H. bengalensis* were collected during August and September 2009. The mating dance or “promenade a deux” is associated with complex behaviors involving the holding of chela by the

male, shuddering, and stinging by both male and female, raising of the tail and raised pectines. After extruding the spermatophore, the male pulled the female and the female adjusts the opening of the genital operculum by finding the spermatic duct. The female then exerts pressure by pressing against the spermatophore, which changes the angle of truncal flexure and triggers the sperm to ooze out through the opening of the spermatic duct. The lamella is a long shaft like structure and acts as a lever to squeeze the stalk of the spermatophore containing semen.

The spermatophore looks soft and translucent when secreted by the male. It then turns hard and becomes sclerotized when comes in contact with air. The middle portion near the capsule is yellowish and darker with an overall lighter color. The spermatophore of the species of family Scorpionidae is an elongated structure with four distinct parts, viz. pedicel, stem, capsule, and lamella. The pedicel is the structure of attachment to the substratum. It looks translucent when secreted out from the body of the male. The ventral surface has a sticky end that gets glued to the ground (generally hard substratum), in a particular direction. The pedicel is attached to the stem by a flexure. The stem stores the spermatids and is lightly sclerotized. It acts as a supporting pillar to make the spermatophore stand at a specific angle before the insemination of spermatids and its angular direction changes after the insemination. The portion between the stem and the lamina is called the capsule which is a complex structure and mediates the sperm to pass through the spermatic duct opening. The capsular region is provided with complex structural arrangements with carinae, sperm duct, basal hook and holosolenos for tight packaging and expelling of sperm. Morphometric data on spermatophore of *H. swammerdami* and *H. bengalensis* are provided in Table 1.

Spermatophore of *H. swammerdami* (n=15)

The total length of the spermatophore varied from 17.8 mm to 21.5 mm (mean length = 19.9 mm). The pedicel is broader towards the distal end. The capsular region is with prominent distal carinae and a distinct holosolenos of 1.9 to 2.7 mm length (mean length = 2.4 mm). The laminar hook is prominent and raised. The juncture of lamella to the capsule is deeply notched with a concavity formed by the lamina and the hook. The distal

Table 1. Measurements of spermatophores (in mm.) of two following Scorpionide species

Sl.	Pl	Sl	Cl	Ll	Total	Hl
<i>H. swammerdami</i>						
1	3.2	5.1	2.7	10.6	21.5	2.1
2	3.1	5.5	2.6	9.6	20.9	1.9
3*	3.1	5.3	2.6	10.1	21.2	2.2
4	2.7	5.5	2.8	10.2	21.1	2.1
5	3.8	5.0	2.8	8.7	20.3	2.6
6	3.4	5.0	2.8	8.3	19.5	2.6
7	2.8	5.1	2.8	8.9	19.7	2.8
8*	2.4	5.4	2.6	9.8	20.2	2.7
9	2.2	5.2	2.4	9.4	19.1	2.3
10	2	5.0	2.3	8.5	17.8	2.3
11	2.2	5.5	2.4	9.1	19.3	2.6
12	2.7	4.8	2.4	9.1	19.0	2.1
13	3	5.7	2.9	9.8	21.4	2.5
14	2.5	5.1	2.8	8.9	19.3	2.7
15	2.2	5.4	2.7	8.4	18.7	2.5
<i>H. bengalensis</i>						
1	2.4	3.9	2.1	8.3	16.7	0.1
2	2.1	3.3	2.0	8.0	15.4	0.1

Abbreviation: Pl=basal piece length, Sl=stem length, Cl=capsular length, Ll=lamella length, hl = length of Holosolenos, * for pre-inseminate spermatophore.

Spermatophore of *H. bengalensis* (n=2)

The total length of the spermatophore varied from 15.4 mm to 16.7 mm (mean length = 16.0 mm). The pedicel is slightly broader towards the distal end. The stem is broader at the distal end than the proximal part. The capsule is distinct distal carinae and a small holosolenos of 0.1 mm length and is less prominent in pre-insemination spermatophore than the post-insemination one. The laminar hook forms an acute angle with the lamina. The lamina is almost of equal width throughout its length.

The spermatophore of *H. swammerdami* and *H. bengalensis* show definite structural differences in size, structural modifications in the capsule and shape of the lamella. In *H. swammerdami* the total mean length is 19.9 mm (n=15) whereas in *H. bengalensis* the total mean length is 16.0 mm (n=2). As per the morphometric information on the spermatophore of *H. phipsoni* provided by Mirza and Sanap (2009), the total length is 10.8 mm which is much smaller in comparison to these two species. The major differences between *H. swammerdami* and *H. bengalensis* + *H. phipsoni* can also be noted in having a prominent holosolenos in the former species, which is not prominent in the later ones. Furthermore, based on the information on the spermatophore of *H. mysorensis* (Figures 2,14,25 and 26 of Monod *et al.*, 2017), the



end of the lamina is distinctly broader.

Figure 1. Courtship in *Heterometrus swammerdami* Simon, 1872 (the brown one is female and the black is male).



Figure 2. Image of pre and post-insemination spermatophore of *Heterometrus swammerdami* Simon, 1872.

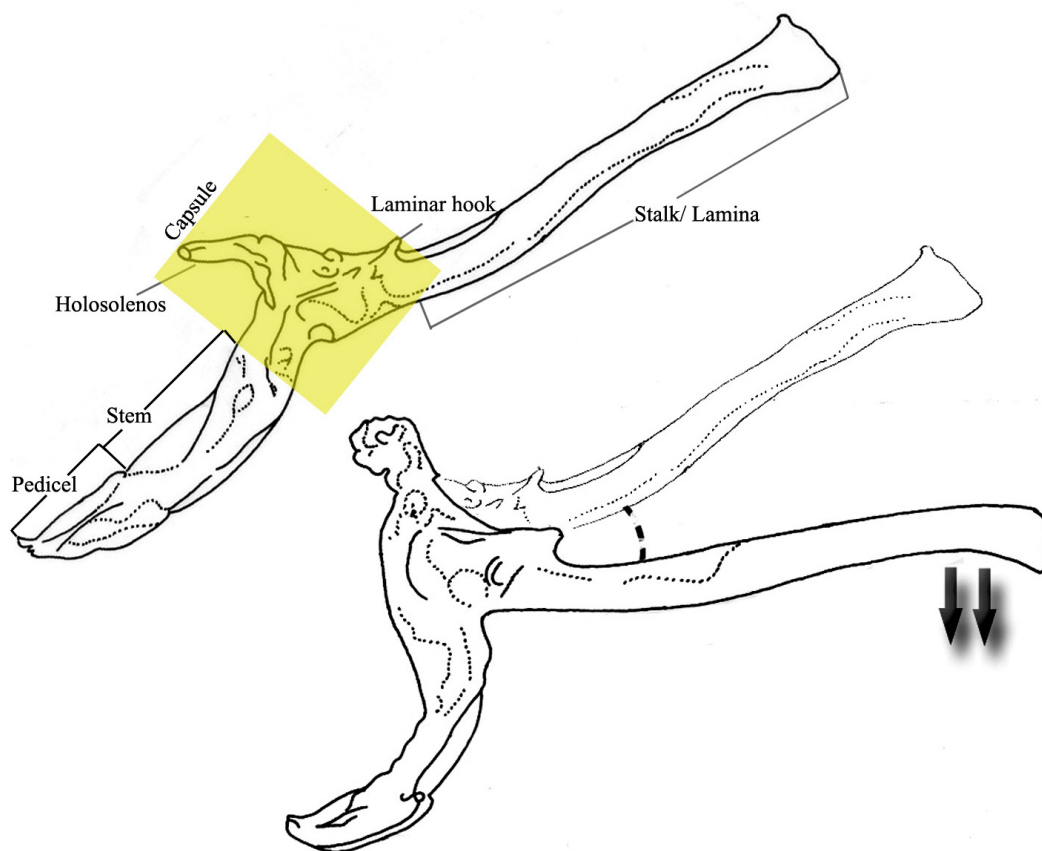


Figure 3. Schematic representation and labeled diagram of pre and post-insemination

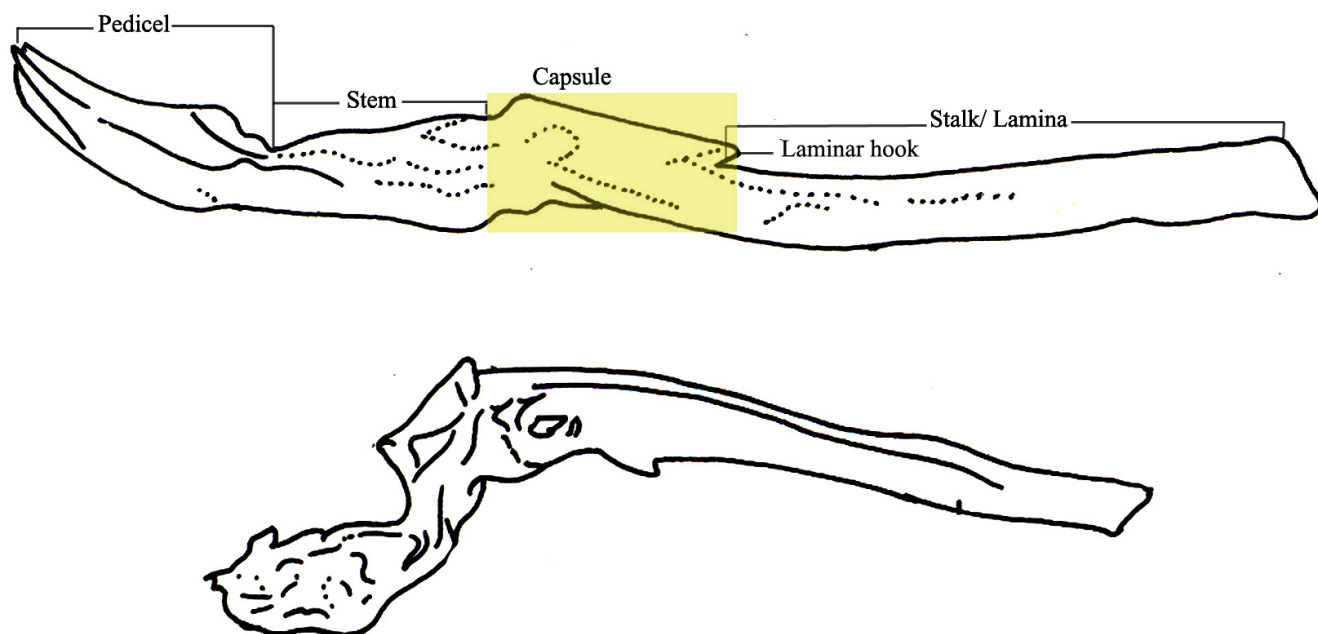


Figure 4. Labeled diagram of pre-and post-insemination spermatophore of *H. bengalensis* (Koch, 1841).

holosolenos is not prominent in the pre-insemination spermatophore. Based on the structure of lamella, it is wider towards the distal end in *H. swammerdami*, whereas it is more or less straight in *H. bengalensis* and *H. phipsoni*. Further studies on detailed morphological aspects will be useful in formulating suitable taxonomic characters in these species.

From the above observation and the structure of the post insemination position in these species, it may be hypothesized that presence of holosolenos is an advantageous character in terms of secure sperm transfer and the species do not have such structure (such as *H. bengalensis*, *H. phipsoni*, and *H. mysorensis*) seems to be exerting more pressure to have more acute angle to release the spermatids stored in stalk region. More studies on the morphology of male as well as female sexual apparatus can throw lights on evolution of mechanism of sperm transfer in scorpions and can also be used as important characters in taxonomy as well as phylogeny.

Acknowledgments

We would like to thank Dr. Kailash Chandra, Director, Zoological Survey of India for his constant encouragement. We are extremely thankful to Dr. S. Sambath, Scientist-D and Officer-in-Charge, Central Zone Regional Centre, ZSI, Jabalpur and the scientific staff members of CZRC, ZSI for support. Thanks, are also due to Dr. D. B. Bastawade, Zoologist (retired), Zoological Survey of India, Western Regional Station, Pune, Maharashtra for his generous help and encouragement and Dr. N. P. I. Das, Scientist, Environment and Safety Division, Indira Gandhi Centre for Atomic Research, Kalpakkam for drawing of the spermatophore structures and his encouragement in all steps for preparing the manuscript. We would also like to thank Dr. P.M. Sureshan, Scientist-E, ZSI-WGRC for his valuable comments

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