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A Study on Genitalia of Phlebotominae Sand Flies (Phlebotomidae: Diptera) in Northern India: A New Tool for Detection of Species

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Abstract: The aim of this study was to study the genitalia of phlebotomidae sand flies for search of new species in various parts of India. The pattern of genitalia variation in phlebotominae sand flies also indicated that differences in the lengths of the spermathecal ducts and aedeagal filaments are distributed unevenly among closely related species. Mouth aspirators and small hand nets were used to capture phlebotominae sand flies form different localities. The results show that unlike most Diptera, including the other members of the family Psychodidae, the organs of sperm transfer in phlebotomines present a bipartite condition. The external genitalia of the male, consists of paired structures that grip the female during copulation and the internal genitalia of the female, consists of a common duct branching to paired individual ducts terminating in spermathecae. This study suggests that these characters can be used for identification of specimens where the spermathecae (an established tool for species identification in phlebotomines) have been lost or where cryptic species are concerned.

Key words: Phlebotominae sand flies, spermathecal ducts, aedeagal filaments, genetalia

INTRODUCTION

The subfamily Phlebotominae has a wide distribution throughout the world, mainly in the tropics and subtropics (Adler and Theodor, 1957; Singh and Phillips-Singh, 2009). Phlebotomine sandflies are small, blood-sucking dipteran insects. Only female sandflies feed on blood of various vertebrate animals such as reptiles and mammals including man and use the nutrients to develop eggs (Lane, 1987).

Insect vectors play an important role in the transmission of many major human diseases such as Malaria and leishmaniasis, which have a direct impact on human health. Phlebotomine sand flies are small with a body length seldom exceeding 3 mm. Their color ranges from almost white to black. The morphology of insect genitalia is often highly species-specific and its variation has been suggested as an important impetus for evolution (Joshi *et al*., 2009; Sivagnaname, 2006; Swaminath *et al*., 2006). Unlike most Diptera, including the other members of the family Psychodidae, the organs of sperm transfer in phlebotomines present a bipartite condition (Downes, 1968). The principal morphological characteristics used to distinguish between species are the external genitalia of the male, consisting of

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paired structures that grip the female during copulation and the internal genitalia of the female, consisting of a common duct branching to paired individual ducts terminating in spermathecae. The testes of the males are connected to a genital pump, which connects via the aedeagus to a pair of genital filaments actually ducts (Filho *et al*., 2009).

Distinction between the females of two species is based principally by characters of the spermathecae (total length, number of annulations, form and length of head and length of the common duct in relation to the individual duct) and head i.e., number of horizontal teeth in the cibarium (Marcondes, 1996). Marcondes and Borges (2000) were unable to distinguish between the males of these species with confidence on the basis of their morphology and could only separate them using morphometric studies and by analyzing artificial neural network. Muller *et al*. (2007) and Galati *et al*. (2007) also focused that the male-female gathering in the same taxon is based on ecological, morphological mainly length of male genital filaments and female spermathecal ducts and molecular criteria.

The purpose of this study was to obtain the information about the pattern of variation in the relative size of different parts of genitalia mainly, the length of ejaculatory pump and filament with the length of spermathecal ducts which provides support for the divergence within a complex of species and that could be considered as a satisfactory character for the identification of vector species of sand flies from the non-vectors.

MATERIALS AND METHODS

Sand flies were collected using sticky paper traps, CDC light traps and aspirators from outdoors (cow shelters, dog shelters and holes in rocks and caves) as well as indoors (stable and living rooms) from January to July 2009. Sticky paper traps (15×20 cm) installed after sunset were collected before sunrise from 20 catching sites. Collection by aspirators was done within houses. Female identification was based on the shape of spermathecae and the disposition of the teeth in the pharyngeal armature, whereas males were identified based on their genitalia (hypopygium), in particular the parameres and aedeagus, the shape, insertion, disposition and length of the hairs in the coxite and the spines in the style (Dolmatova and Demina, 1971; Killick-Kendrick *et al*., 1991). The specimens collected by light traps and aspirators were directly transferred to 96% ethanol in the field and labeled accordingly. Specimens caught by sticky paper traps were immersed first in 96% ethanol to remove the oil, transferred to 90% ethanol, cleared in lactophenol and mounted (Marcondes, 1998). All specimens were placed in Berlese medium on labeled slides for identification. Before mounting, the head and genitalia of the sand flies were cut, the body parts removed from each specimen with forceps and the wings were stained for easier viewing of veins (Singh and Ipe, 2005) for further morphometric analysis. The thoraces were ground up and homogenates were stored. Identification was based on the morphology of male and female genitalia using the identification keys of Lewis (1978).

RESULTS

The genitalia of the recorded species for the above studies show resemblance in the lengths of their structures and genital filament/spermathecal duct ratios which were highly significant. No significant differences were found in the genital structures. The proportion between these lengths could therefore be used as a character to differentiate between the species of sand flies (Marcondes, 1996; Marcondes *et al*., 1998a, b; Marcondes and Borges, 2000). Geographical variation with respect to this ratio should also be studied.

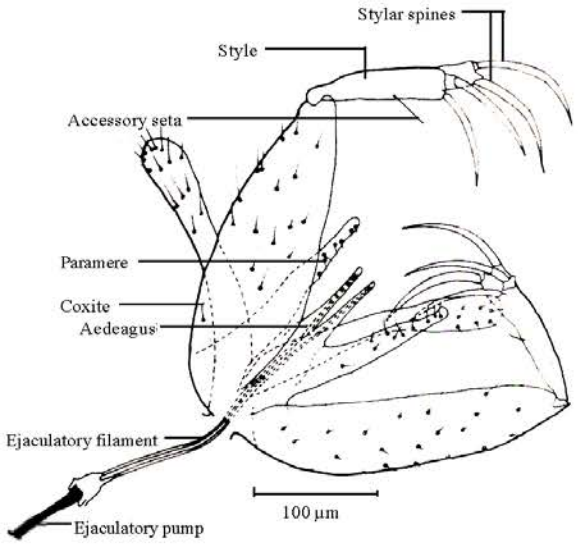

Fig. 1: Male genitalia

Ilango and Lane (2000) noted that the common spermathecal duct of *P. argentipes* is very large and the male aedeagus correspondingly wide. The present study also focused that the genital filaments are longer in comparison to the spermathecal ducts, as found for 25 of the 26 taxa examined by Ilango and Lane (2000). This greater length of genital filaments would allow sperm displacement by males (Ilango and Lane, 2000). These studies also contradict the conclusion that lengths of genital filaments and those of spermathecal ducts are similar in sand flies, as proposed by Williams (1988). The differences between the two species with respect to the lengths of the structures and genital filament/spermathecal duct ratios were highly significant (Fig. 1). The above study demonstrates that the male external genital characters are important characters as they are very unique, having species specific characters in most sand flies. However in some cases, the males of closely related species have almost identical morphological characters. In such situations, it is difficult to separate the females which are also morphologically very similar. So, an easily detectable and reliable female specific diagnostic character is very important for identification and determination of the epidemiological role of the insect.

DISCUSSION

The important morphological characteristics used to identify between species are the external genitalia of the male, which consists of paired structures that percepts the female during mating with the internal genitalia of the female (Fig. 2), consisting of a common duct branching to paired individual ducts terminating in spermathecae. A pair of genital filaments in the aedeagus is connected to the testis though the genital pump. These filaments penetrate the body of the female and deposit the spermatozoa, rather than the aedeagus as in most insects. The morphology of insect genitalia is often highly species-specific and its variation has been suggested as an important impetus for evolution. Structural variation of the male genitalia and the female spermathecae in phlebotomine sand flies is unique among the blood sucking Diptera.

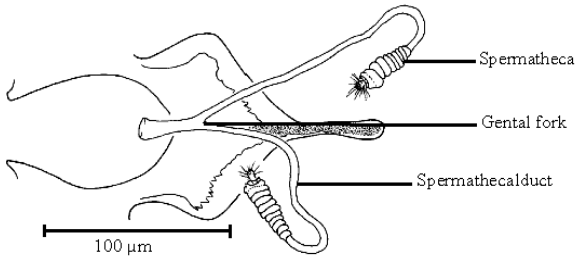

Fig. 2: Female genitalia

A copulating pair of *Phlebotomus perfiliewi* Parrot showing the genital filaments of the male entering the genital ducts of the female, with their extremities almost reaching the spermathecae was cleared and mounted (Hertig, 1949). The relationship between male genital filaments and spermathecal ducts has been also studied in few other sand fly species.

It was also noticed that a positive correlation between the lengths of the aedeagal filaments and spermathecal ducts, shows an importance for males and females over the control of fertilization. In the closely related genera and species, the pattern of minute structures in the genitalia indicates little difference in the lengths of the spermathecal ducts and aedeagal filaments. It is suggested that an illustration of the armature in the genital atrium will be useful for the descriptions of new species.

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