



Journal of  
**Entomology**

ISSN 1812-5670



Academic  
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## **Role of Morphometric Description of Female Genitalia to Discriminate Phlebotomine Sand Flies Species (Diptera; Psychodidae; Phlebotominae) from Northern Part of India**

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### **ABSTRACT**

Phlebotomine sand flies are important in public health due to their ability to transmit certain pathogens, especially leishmaniasis. The male sand flies having a very unique and species-specific characters in their external genitalia. They have also almost identical morphological characters in the closely related species. Variability occurs in the females of phlebotomine sand flies which possesses highly fragile spermathecae with several important taxonomic characters which can be utilized for species identification. Due to their soft and contractile nature, there is a high probability that the morphology of the structure may change. Sand fly adults of both sexes can be collected by several methods, either while resting during the day or foraging at night. Sampling from resting sites, like walls of houses, animal dwellings, caves and tree holes, can be done by several forms of mouth aspirator; similarly, active catches with an aspirator can be done from bait animals or on human bait. They were mounted in the NC medium and identified using identification key. The results from the present work are expected to strengthen the foundation that the female external genital structure might be helpful in separating the females of very closely related species. Some more studies are necessary on Old World sand flies and New World sand flies for a better understanding of the validity of the structures as a possible identifying character in female external genitalia.

**Key words:** Phlebotomine sand flies, *Phlebotomus*, *Sergentomyia*, female genital structures, morphometric, leishmaniasis, taxonomy

### **INTRODUCTION**

Phlebotomine sand flies have long been incriminated as vectors of leishmaniasis in various parts of both the Old and New World in at least 80 countries (Alexander and Maroli, 2003). A fatal infectious disease, Kala-azar, in many Indian states, particularly in Bihar, West Bengal, Uttar Pradesh and Jharkhand, is caused by the protozoan parasite *Leishmania donovani* and transmitted by the sandfly vector *Phlebotomus argentipes*. The vector is distributed all over the country but the disease is confined to particular zones since before the last century and causing great deal of morbidity and mortality (Rao *et al.*, 2001). The study of this group of insects is important for further attempts at the control of leishmaniasis. In India, the sand flies with

*P. argentipes* were described by Annandale (1908). A series of work on different morphological aspects of the Old World sand flies were carried out by the Kala-azar Commission during 1900-1942. Sinton (1923, 1927) contributed the majority of the work dealing with morphological description of Indian sand flies. Mitra and Roy (1953) and Mitra (1959) emphasised on morphological variations of *P. argentipes*, collected from Maharashtra and Kashmir states of India, respectively. Kaul *et al.* (1973) described the phlebotomine sand flies in Rajasthan state with *Sergentomyia* (Sintonius) *sirohi* sp. nov. Young and Perkins (1984) and Young and Duncan (1994) also emphasised the work dealing with the morphological descriptions of the New World sand flies. Morphological variation of the male genitalia and the female spermathecae in Phlebotomine sand flies is unique among the blood sucking Diptera. Ilango and Lane (2000) described the fine structures involved in mating for *Phlebotomus papatasi* (Scopoli) and suggested that these characters can be of use on specimens where the spermathecae have been lost or where cryptic species are concerned. The external genitalia of the male is considered as the important morphological characteristics used to identify between species are which consists of paired structures that percept the female during mating with the internal genitalia of the female, consisting of a common duct branching to paired individual ducts terminating in spermathecae (Singh and Phillips-Singh, 2010a, b). The aim of the study was morphometric description of female genitalia and its role to discriminate sand flies of very closely related species.

## MATERIALS AND METHODS

Sand flies were caught using sticky traps in human dwellings and wild environments both in urban and rural areas of Saharanpur, Almora in northern Uttar Pradesh from September and November 2011. Three species, *Phlebotomus argentipes*, *Sergentomyia punjabaensis* and *Sergentomyia babu* collected specimens were collected preserved in 70% alcohol and later processed for mounting between slide and cover glass. Passing the insects in phenol (85% for 24 h), potassium hydroxide (10% for 12 h), acetic alcohol (10% for 15 min), acid fuchsin (8-10 min), 70oGL, 90oGL, 95oGL and absolute alcohol (15 min each), oil of cloves (24 h) and dissection and mounting in NC medium (Cerqueira, 1943). 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 (Corradetti *et al.*, 1961; Rioux and Golvan, 1967; Dolmatova and Demina, 1971; Killick-Kendrick *et al.*, 1991). Photographs of the morphometric structures were taken with the Nikon ECLIPSE E100 Digital Camera fixed to the microscopes. Camera Lucida was used to draw images. Length of spermatheca body, width of spermatheca body, length of individual duct of spermatheca and length of circus plays a very important role in morphometric studies of female genitalia, to distinguish the very closely related species.

## RESULTS

One species belonging to the genus *Phlebotomus* and two species from genus *Sergentomyia* were identified:

- *Phlebotomus argentipes*, Annandale and Brunetti (Fig. 1)
- *Sergentomyia punjabaensis*, Sinton (Fig. 2)
- *Sergentomyia babu*, Annandale (Fig. 3)

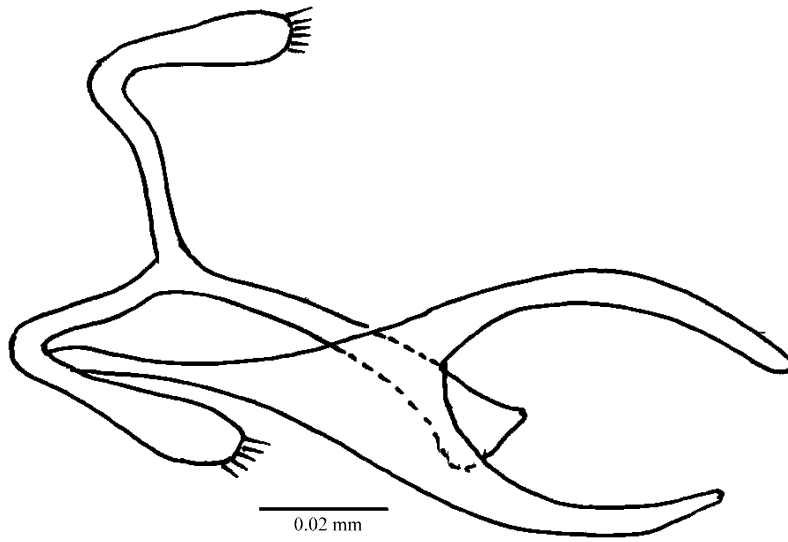


Fig. 1: *Phlebotomus argentipes* spermatheca and furca

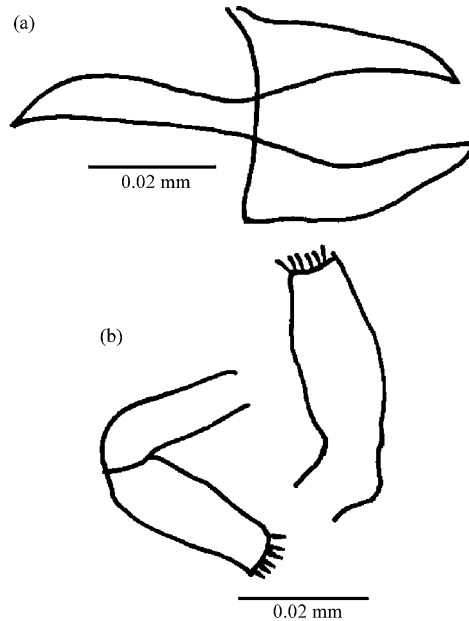


Fig. 2(a-b): *Sergentomyia punjabaensis*, (a) Spermatheca and (b) Furca

All the flies of genus *Sergentomyia* were with the presence of four style spines and the absence of erect hairs on tergal plates were observed in all flies. All female flies had a tubular spermathecae with uniform thickness. All flies had AFi; shorter than AFii+iii and labrum.  $R_2/R_{2+3}$  was less than 0.8. The lack of a standard to describe the species, the dissociation between the sexes, the large amount of synonymy and lack of knowledge exchange between taxonomists are factors impeding the proper identification of sand fly species. The majority of the descriptive studies have dealt with the importance of the male genitalia for identification and in some cases, a few features of the female mouth parts are considered. The principal morphological characteristics used to distinguish

between species are the external genitalia of the male, consisting of paired structures that grip the female during copula and the internal genitalia of the female, consisting of a common duct branching to paired individual ducts terminating in spermathecae (Fig. 4). Distinction between the

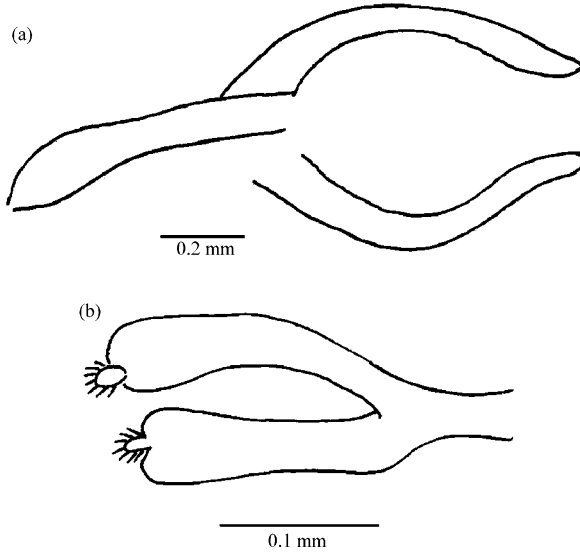


Fig. 3(a-b): *Sergentomyia babu* of (a) Spermatheca and (b) Furca

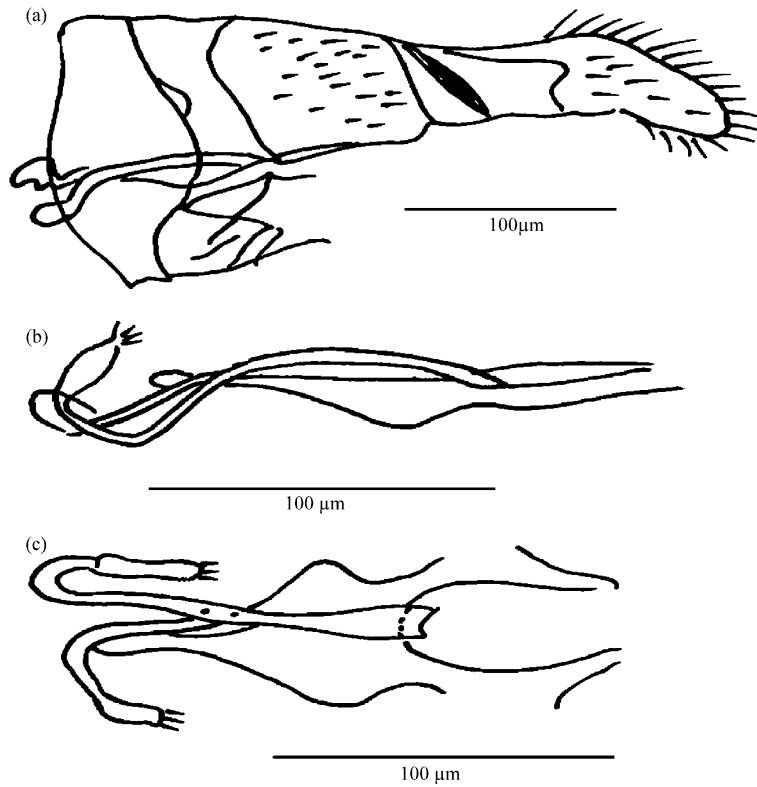


Fig. 4(a-c): Female genitalia (a) Its lateral view (b) Lateral view of spermatheca and genital fork and (c) Dorsal view of female genitalia showing genital spermatheca and ducts

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.

## DISCUSSION

Ilango and Lane (2000) noted that the common spermathecal duct of *P. argentipes* is very large and the male aedeagus correspondingly wide. Since females of *L. intermedia* have long and relatively large common ducts and those of *L. neivai* very short ones, it would be interesting to study the shape and dimensions of the male aedeagi and their relationship with the common ducts. There was a positive correlation between the lengths of the spermathecal ducts and aedeagal filaments, indicating that an evolutionary “arms race” occurs between males and females over the control of fertilization. The pattern of genitalia variation in phlebotomine sand flies also indicated that differences in the lengths of the spermathecal ducts and aedeagal filaments are distributed unevenly among closely related species. The ratio between the length of male genital filaments and that of spermathecal ducts of their co-specific females may be important to the lock-and-key mechanism of the beetle *Bambra invisibilis* (Dybas and Dybas, 1981). Some type of behaviour apparently designed to stimulate the female. In phlebotomines these could include vibration of the wings during copula and mechanical stimulation of the female abdomen by tufts and other structures on the copulatory apparatus of the males, as seen in some butterflies (Lorkovic, 1952). Eberhard (1985) discussed the reasons why male copulatory structures in many groups of animals exhibit such variation in morphology.

## CONCLUSION

The results of the present study concluded that female sand flies external genitalia structure are helpful in separating the female of very related species. More detailed studies on morphometric description of the suspected vector species, fauna and parasitic infection of the potential reservoir hosts of the cutaneous leishmaniasis are suggested to find the epidemiological aspects of the disease in this county.

## ACKNOWLEDGMENTS

Financial assistance from University Grants Commission (U.G.C.), New Delhi, India, through Project No. F. No. 40-362/2011 (SR) to the first author is gratefully acknowledged. Authors are also thankful to the Principal and Manager, Lucknow Christian College, Lucknow for providing necessary facilities.

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