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A STUDY OF MALE AND FEMALE GENITALIA: A TAXONOMIC TOOL FOR THE IDENTIFICATION OF SAND FLY (Diptera: Phlebotomidae)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author NB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GM managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Sand flies are minute blood sucking flies and are of considerable medico-veterinary significance as these are the vector of several diseases viz., Leishmaniasis, Bartonallosis and viral diseases such as Sand fly fever, Chandipura Encephalitis etc. They are found throughout the world's tropical and subtropical region. The correct identification of the species of this fly is very important for its control and epidemiological study. The structure of male and female genitalia is species specific and show extraordinary diversity. Thus the male and female genitalia are an important source of diagnostic features to separate the specimen at generic as well as specific level. Male genital armature of *Phlebotomidae* has occupied a right place in the studies of *Phlebotomidae* taxonomy but female genitalia is neglected by taxonomist as they used only the internal female genital structure in the Phlebotomid taxonomy. From the present investigation it has been revealed that both male and female genitalia show a large number of variation both externally and internally due to this these genital characters in the family *Phlebotomidae* are very reliable tool for taxonomic studies and these characters can easily be used in the formulation of dichotomus keys for identification of different species of sand flies. The present investigation will be significant for the search and description of new species of India.

Keywords: *Diptera*; *Phlebotomidae*; taxonomy; genitalia; leishmaniasis.

1. INTRODUCTION

Phlebotomine sand flies are a group of *Diptera* belongs to suborder Nematocera and family *Phlebotomidae*. These are small insect, long legged and light or dark brown in colour. They are smaller than mosquitoes and their bodies and wings are densely covered with hairs. They are usually found in microhabitat i.e. cavities or holes in the walls of houses and dark places in the corner. In India sand

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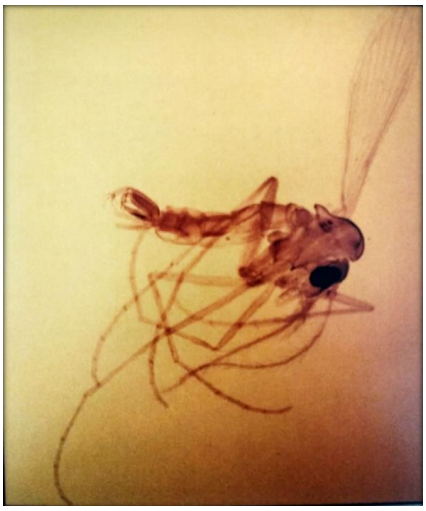
Male



Female

fly is reported throughout the year [1]. Their density increases with the onset of warm weather with humidity, About 700 spp. Of sand flies exist in the world and about 70 spp. are responsible for the transmission of Leishmaniasis, Bartonella or Arboviruses in the old and new world. Most importantly, sand flies are the vector of leishmanial parasites that cause the death of thousands of humans and animals each year in more than 90 endemic countries. Only female species is haematophagus and it require a blood meal every third or fourth day for oviposition. So the female sand fly is the transmitting vector of many diseases to humans and other animals.

Some species of sand flies do not attack human but feed on animals and birds. All species that feed on human being are not the vector of diseases. This is also an important fact that different species of sand flies have a different susceptibility to Leishmania and other diseases. The control of sand flies is important for the prevention of diseases. All method of control has to be directed against certain species. Thus the species of sand fly should be correctly identified [2,3].

Their identification depends basically on the microscopic examination of key morphological characters of head, thorax and abdomen. The genitalia of this fly is species specific and bears many unique diagnostic characters that can be used by taxonomist for identification of species and phylogenetic study [4,5].

Genitalia of Phlebotomine sand fly exhibit morphological variation. The principal morphological characteristic used to distinguish between spp. of sand fly are external genitalia of male and internal genitalia of female [6,7,8]. Now more than 40 characters of genitalia have been identified as taxonomic character i.e. number of spine on style, morphology of paramere, ratio length of genital filament/ length of pump, shape of aedeagus, opening of spermathecal duct etc. Spermathecal complex has an important role in reproduction because the sperms are stored in it, hyper activated and modified in some way so that they can fertilize ovum. Spermathecal complex shows a great variation among subgenera due to this it is a relevant feature for taxonomy and phylogenetic systematics [9,10,11].

The aim of this study was to obtain the information about the pattern of variation in the different parts of male and female genitalia which provide support for the divergence within a complex of species and that considered as a reliable tool for the distinction between different species and genera of sand fly.

2. MATERIALS AND METHODS

The material, Phlebotomine sand flies, was collected from various parts of the country particularly from the state of U.P., Rajasthan, Bihar, Himachal Pradesh, Tamil Nadu, Jammu & Kashmir etc. in appropriate seasons considering the seasonality shown by the flies. The adults of Phlebotomine sand flies were collected with the help of pooter or Aspirator. Sometimes collection was easily made directly by alcohol rinsed brush from the cracks and crevices of the buildings. The another important method which was used for adult phlebotomines collection is the light trap method and by *Diptera* sweeping net. The

preservation of material was done in the 70% ethanol or Pampel's fluid in collection tubes.

The collected insect were dissected and mounted on micro slides by using methodology of Saether (2002). The specimen was dissected with the help of two micro needle under the Zoom Stereoscopic Binocular Microscope. After passing through the ascending alcoholic series the material was mounted in Canada balsam under micro coverslip. Terminologies for male genitalia have been followed of Lewis (1978) and female genitalia have been followed of Saether (2002). Photography was done by Digital microscopic camera.

3. RESULTS AND DISCUSSION

Being a vector of many fatal diseases like Leishmaniasis, the Sand flies have great epidemiological importance. Some spp. of sand flies are morphologically similar which make their identification quite difficult so it is necessary to identify these spp. accurately to control its population and for epidemiological studies. According to Biological concept of species, Species are the group of interbreeding organism and genital organs are the main reproductive part of species. So the structure of male and female genitalia are an important tool to discriminate different species of Sand fly. Keeping this point in mind I have studied genitalia of different species of sand fly and found out that male and female genitalia of sand fly bear many specific diagnostic features that is useful to identify these fly at specific level.

In the present study, two major genera of Sand fly have been studied namely *Phlebotomus* and *Sergentomyia*. During the past few years numerous morphological characters have been employed for the delineation of sand fly taxonomy. This wide range of variable characters includes the structure of head, wing, thorax, legs, male genitalia and internal structure of female genitalia. Lewis included only the internal structure of female genitalia such as spermathecal for identification of sand fly. He ignored other characters of female genitalia except spermatheca for sand fly systematics. In the present investigation it has been revealed that both external and internal structures of female genitalia provide very reliable diagnostic features for the distinction between different species and genera of sand fly. It is also clear that male and female genital characters may prove more reliable tool in separating the specimens of *Phlebotomidae* than the other characters.

Now a days numerous morphological characters have been used for the phylogenetic study of sand fly. The wide range of variable characters in male genitalia include presence of basal lobe and tuft of bristles on the coxite, Morphology, position and number of spines on Style and Paramere, Number of spines on Surstyle, shape of Aedeagus, Ratio length of Genital filament/ Genital pump etc.

In the case of female genitalia shape and ornamentation of Spermatheca, opening of spermathecal duct, Spines on genital armature have a significant role in identification of sand fly. So it has been revealed from this study that genitalia of sand fly exhibit extraordinary diversity and we can easily identify the correct species of fly by studying the genital characters.

3.1 Basic Structure of Male Genitalia of Sand Fly

Last two abdominal segments IX and X is involved in the formation of male genitalia of *Phlebotomidae*. It bears many diagnostic features to separate the different genera and species of Sand fly. The main component of male genitalia are as follows: Epandrium and Hypandrrium, Gonocoxite, Gonostylus, Paramere, Aedeagus, Surstyle, Proctiger and Genital pump [11-13].

3.1.1 Epandrium and hypandrium

The tergite of IX segment forms the epandrium and sternite forms hypandrium. Epandrium forms the principal dorsal sclerite of the genital segment and connected with hypandrium by pleural membrane. These together form the basal ring or genital ring.

3.1.2 Gonocoxite

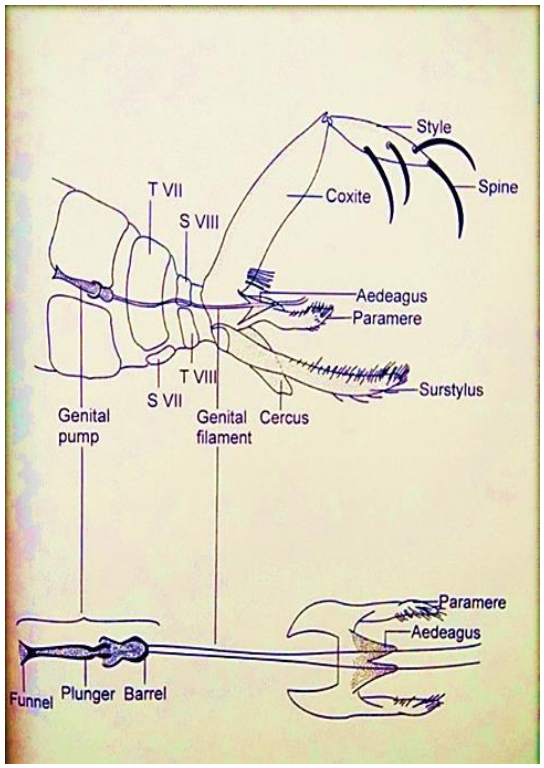
It is the basal part of principal outer genital clasper or major genital appendage. It articulates with the posterior margin of the hypandrium. The coxite is covered with scales and hairs.

3.1.3 Gonostylus

It is the distal part of principal and outer genital claspers. Style have a variety of shape from a simple appendage to cylindrical, oval or oblong. These are paired and have several spines.

3.1.4 Paramere

A pair of unsegmented paraphallic processes situated between the posterolateral base of aedeagus and the dorsomedial base of Gonocoxite. They are paired appandages of different shape and sizes.



Male Genitalia

3.1.5 Aedeagus

This is the male intermittent resulting from combination of gonapophysis IX and surrounding phalli. It is short, conical or elongated in the form of long slender rods or oblong plates. In some species a pair of spines present near to the aedeagus.

3.1.6 Surstyle

These are reduced tergite X which is closely associated with tergite IX to form a lateral lobe the Surstyle. These are paired long, cylindrical, slightly curved appendages, covered with hairs on ventral side.

3.1.7 Proctiger

The vestigial tergite and sternite XI form a proctiger bearing the cerci and anus. Cerci are paired, soft and hairy appendages which are situated between the surstyle. The anus is irregular or elongated in shape and opens between the cerci.

3.1.8 Genital pump

It is situated in abdomen and attached to a pair of genital filament passing throughout the aedeagus. It is also known as ejaculatory pump.

3.2 Taxonomic Peculiarities of Male Genitalia of Sand Fly Species

3.2.1 *Phlebotomus papatasi*

Phlebotomus papatasi

- Tergite VIII and Sternite VIII narrow and usually hidden in the abdomen. Tergite IX and sternite IX together form the genital ring.
- Coxite broad and bear a group of 16-18 long, dark and thick setae near the distal end. A small basal process present near the dorsal side of coxite.
- Stylus long and wide but less than half as wide as coxite with 5 short and stout spines (3 apical and 2 submedian).
- Paramere trilobed with two dorsal processes. Ventral lobe small, dorsoventrally compressed with a small light spine at the tip and setae at its inner margin. One dorsal process is sickle shaped and curved while another dorsal process is long and digitiform.
- Aedeagus short, long and conical. Its apex is pointed and slightly curved ventrally
- Genital filament and Genital pump is long and their ratio is 2.18.

3.2.2 *Phlebotomus argentipes*

Phlebotomus argentipes

- Coxite long, broad and bare.
- Stylus long, cylindrical with 5 long spines (2 terminal and 3 median).

- Paramere trilobed with two ventral processes and a long distal part. The base of paramere is broad and gradually tapering to the apex.
- Aedeagus long and conical with pointed apex. It bears a pair of slightly curve lateral spines.
- Surstylus long and without spine.
- Genital pump and Genital filament are long and dark brown in colour. Their ratio is 2.55.

3.2.3 *Sergentomyia punjabensis*

Sergentomyia punjabensis

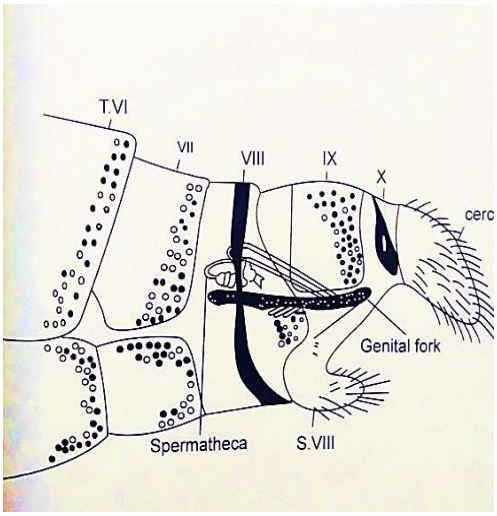
- Coxite elongated and tapering towards its anterior end with a large number of hairs. It bears no basal process
- Stylus elongated and slender with 4 terminal spines. Spines of style is as long as the length of style with pointed end. Aventral seta is also present near the apex of style
- Paramere long, beak shaped and rounded terminally with few spines at their margin.
- Aedeagus straight, thick and digitiform.
- Genital pump is long and the ratio of genital filament and pump is 3.18.

3.2.4 *Sergentomyia theodori*

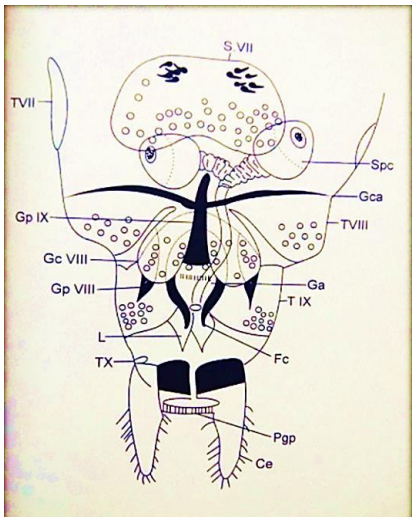
- Coxite long and wide with tapering apex and hairs.
- Stylus long and wide. It is cylindrical with two terminal and two subterminal spines. All spines of stylus of equal length and longer than style. Ventral seta subterminal to the apex of stylus.
- Paramere long, slender and hooked. Its Inner margin of paramere curved downward and form a hook shaped apex. It is gradually narrowing towards anterior end with large
- *Sergentomyia theodori* number of hairs at their distal part.
- Aedeagus long, slightly curve, thick and finger shaped with a small apical notch. Surstyle as long as coxite.
- Genital pump long and Genital filament is smooth and ratio of Genital filament and Genital pump =2.68.

3.3 Basic Structure of Female Genitalia of Sand Fly

Female genitalia of Sand fly is formed by VIII, IX, X and XI segment. Basic component of female genitalia are as follows: Epigynium and Hypogynium, Gonapophysis VIII and IX, Gonocoxite VIII and IX, Cerci & Post genital plate, Spermatheca and Seminal capsule, Genital atria and its Armature [2,14-20,10].



Female Genitalia-Lateral view



Female

3.3.1 Genitalia-ventral view

1. **Epigynium and Hypogynium:** The segment VIII of female genitalia forms the main shaft of female genitalia. Tergite VIII forms the epigynium and Sternite VIII forms the hypogynium. Epigynium and Hypogynium jointly known as subgenital plate or extra genital plate.

2. **Gonapophysis VIII and IX:** In basic structure of female genitalia of *Diptera* the reduced gonapophysis VIII enclose the inverted gonapophysis IX. In Sand fly gonapophysis IX is reduced and it consist mainly a thin Notum without distinguishable rami. A pair of lobe like membranous structure called labia is present at the caudal end of gonaphophysis IX. In *Phlebotomidae* gonapophysis VIII is represented as the caudalateral projection of gonocoxite VIII.

3. **Gonocoxite VIII and IX:** Gonocoxite XIII is fused, large plate like structure with anterior transverse Gonocoxapodeme and its caudal projection represent gonapophysis VIII. The fused gonocoxite VIII is known as subgenital plate and it bears many setae in their posterior half. Gonocoxite IX is fused with tergite IX and known as Gonotergite IX.

4. **Cerci and Post Genital Plate:** Segment X is represented by sternite only and tergite X is reduced to small oval plate. Segment XI of abdomen of sand fly is represented by Post Genital Plate and the appandages of segment XI constitutes the Cerci. The shape of Post genital plate is varied in different species.

5. **Spermatheca and Seminal Capsule:** It represent the internal reproductive system of *Phlebotomidae*. In the spermathecal spermatozoa are stored and released when the eggs are passed from the oviduct. Spermatheca includes the seminal capsule, head, the spermathecal duct and spermathecal gland. The spermatheca and their duct show enormous diversity of shape and pattern.

6. **Genital Atria and its armature:** Genital atria and its armature of female sand fly lies between the arms of the furca. The spines of the armature point towards the opening of the atrium. The shape and size of armature, the number, size and arrangement of spines are different between species.

3.4 Taxonomic Peculiarities of Female Genitalia of Sand Fly Species

3.4.1 *Phlebotomus argentipes*

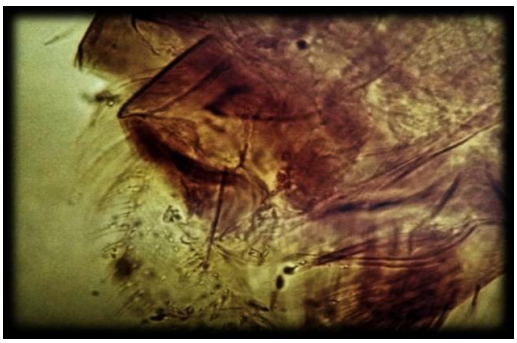
Phlebotomus argentipes

- Tergite VIII normal. Sternite VIII have curved Gonocoxapodeme and fused plate like Gonocoxite VIII.
- Each genital plate of sternite VIII triangular in shape and inner border of each genital plate at the point of bifurcation concave and at the lower portion it is convex. The inner border of both genital plate make the genital opening heart shaped. The outer border of each plate form an obtuse angle at the lower half.
- Gonapophysis VIII fused with gonocoxite VIII. Gonapophysis IX well developed and It is wide at its anterior end and tapering posteriorly
- Tergite IX rectangular in shape and hairy.
- Tergite X converted into two small sclerotized plates. Coxosternapodeme curve and surrounding the genital atrium.
- Cerci elongate and labia membranous.
- Post genital plate nearly triangular in shape with two spines.
- Spermatheca in contracted condition, 15 segmented and carrot shaped .Apical segment of spermathecal enlarge and separated from others by more deep furrow.
- The head small and neck absent. Seminal capsule 0.85 mm. long and its duct is short and partly fused with a common opening.

3.4.2 *Phlebotomus colabaensis*

- Tergite VIII and sternite VIII well developed.
- Each genital plate of sternite VIII oval in shape and have long setae in their posterior half region.
- Gonocoxapodeme well developed and slightly curved. Notum thick, long and tapering posteriorly.

- Gonapophysis VIII not separated from gonocoxite VIII.
- Tergite IX well developed and rectangular in shape with numerous hairs.



Phlebotomus colabaensis

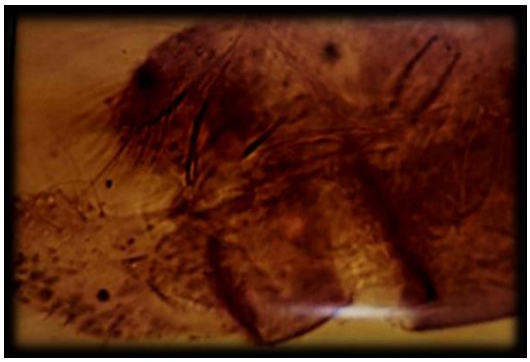
- Tergite X sclerotized with lobe cerci on each side. Coxosternapodeme is curved and S shaped.
- Post genital plate triangular in shape with five spines, cerci medium sized, long and pediform with evenly distributed hairs.
- Labia membranous.
- Spermatheca 15 segmented with short neck, carrot shaped with small end segment.
- Spermathecal duct long about four times length of spermatheca ,opening common.

3.4.3 *Sergentomyia babu babu*

Sergentomuia babu babu

- Tergite VIII large and setose. Sternite VIII constitutes broad & oval plate. Several long setae present on posterior half of sternites VIII.
- Gonocoxapodeme thick and straight.
- Caudolateral projections of gonocoxite VIll represent gonapophysis VIII. Both gonocoxite VIII fused and the junction between two halves of sternite VIII form an acute angle. Length and width of each genital plate.
- Notum distinct long.
- Tergite IX large with small jateral projection.
- Tergite X small and sclerotized. Coxosternapodeme distinct, curved and covers a dome shaped large genital chamber. Labia membranous and separate with some microtrichia.
- Post genital plate is rounded posteriorly each genital plate with few spines (5-7).
- Genital opening is distinct, large and cylindrical. Cerci long, thin, elongate pubescent. Seminal capsule two, large, oblong, unsegmented with small knob of hairs on distal end. Spermathecal duct broad and coiled with common opening into genital atrium.

3.4.4 *Sergentomyia kauli*



Sergentomuia kauli

- Tergite VIII normal. Sternite VIII medium, fused plate like bearing numerous setae in their posterior half region.
- Each genital plate of stemnite VIII oval long and wide. The inner border of each genital plate convex in the middle and concave in the upper and lower portion.
- Genital opening is distinct and dumb bell shaped.
- Gonocoxapodeme thin and not straight.
- Notum slightly curved and tapering posteriorly.
- Tergite IX hairy, large membranous and rectangular in shape.
- Tergite X reduced to sclerotized plate. Gonosternapodeme curved and inner margin of fork concave.
- Post genital plate is almost rounded bearing a small knob at distal end with three spines. Cerci long, oval having evenly distributed hairs and Labia membranous.
- Seminal capsule two, oval with terminal knob and few hairs. Spermathecal duct broad

and long. Spermathecal duct have common opening.

4. CONCLUSION

It has been revealed through this study that Male and Female genitalia is a significant morphological tool for the taxonomic study of Sand fly and it can also be used for the formulation of dichotomus key for identification of Sand fly. The female sand fly has a considerable importance in medical field as it transmit many fatal diseases so it's identification has a great significance in Leishmaniasis control. The role of Genitalia in the discrimination of genera and species of Sand fly can not be neglected as it bears many unique diagnostic features. Its study is also essential for Phylogenetic study of Sand fly.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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