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"Morphological Variation between *Phlebotomus argentipes*,Annandale and Brunette and *Phlebotomus papatasi* (Scopoli), Vector of Leishmaniasis, from India"

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Abstract

In India Phlebotomine sandflies of genus Phlebotomus are primarily responsible for the transmission of Leishmaniasis, a parasitic disease. Besides this, Phlebotomus species are also vector for bartonellosis and papatasi fever.Phlebotomus argentipes, Annandale and Brunette, 1908 and Phlebotomus papatasi (Scopoli) are considered as main vectors for the transmission of various forms of Leishmaniasis in many countries including India. The correct identification of these two species is very important for eradicating leishmaniasis from India. The objective of this study was to determine the morphological variation between these two species of Phlebotomus in their distributional area. Here we also studied the intraspecific variation of these two species complexes in India. A key is also constructed for the identification of male and female of these two Indian Phlebotomine species.

Key words: *Phlebotomus,Leishmaniasis,Morphological variationy.*

Introduction

Female sandflies of genus *Phlebotomus* are considered as a probable vector of leishmaniasis in India.Leishmaniasis is caused by infection of *Leishmania* parasites,which are spread by the bite of phlebotomine sandflies. There are several different forms of leishmaniasis, the most common forms are cutaneous leishmaniasis which causes skin sores and visceral leishmaniasis which also affects several internal organs usually spleen, liver and bone marrow. **Singh (2016)** has isolated *Leishmania* from the blood of rodents and other animals in India and confirmed the finding at molecular level. *Phlebotomus argentipes* **Annandale and Brunette**, 1908 and *Phlebotomus papatasi* (Scopoli) are the two predominant phlebotomine sandflies .

Phlebotomus (*Euphlebotomus*) *argentipes* is the main vector for the transmission of the parasites *Leishmania*

fig 1 sandfly

donovani, main causative agent of the visceral leishmaniasis (Kala Azar) in India. While *Phlebotomus* (*Phlebotomus*) *papatasi* is a proven vector of *Leishmaniamajor* in many places in India (Cutaneous leishmaniasis). *Phlebotomus argentipes* & *Phlebotomus papatasi* are widely distributed in various part of the country such as Bihar, Jharkhand, West Bengal, Eastern UP, Andhra Pradesh, Telangana & Jammu & Kashmir. At present Kala-Azar is endemic in 54 district of the country in which 33 district of Bihar, 4 district of Jharkhand, 11 district of WB and 6 district of Eastern UP. Leishmaniasis is prevalent in 98 countries, the annual incidence of the reported global Kala- Azar cases is 52200 of which 42619 is contributed from the Indian subcontinent (KA- road map-nvdbcp-Nov. 2014).

In India Leishmaniasis and Cutaneous Leishmaniasis are reported from endemic to non- endemic area **(Sharma et al. 2005)**.Considering the present condition of leishmaniasis in India information on morphological variation is important to understand the disease transmission potential of sandflies, vector species. Here we described morphological variation in *Phlebotomus* (*Phlebotomus*) *papatasi* and *Phlebotomus* (*Euphlebotomus*) *argentipes* populations collected from different regions of India.

Phlebotomus argentipes and *Phlebotomus papatasi* are considered as major control targets to eradicate leishmaniasis from India. This study is helpful to identify these two species of sandfly.

Fig. Sandfly

2. Materials & Methods

The phlebotomine sandflies were collected from various parts of the country during the year 2008-2014, particularly from the state of UP, Rajasthan, Bihar, Jharkhand, Himachal Pradesh and Jammu & Kashmir. The present study was done from the different regions of the country by making several field trips especially in the Kala Azar endemic zones.

The adult sand flies were collected with the help of aspirators, sticky traps and CDC light trap method. Sometimes collection can be easily made directly by alcohol rinsed brush from the cracks and the crevices of the buildings. The adult were preserved in 70% ethanol or pampel's fluid in collection tube. The collected insect were dissected and mounted on microslides by using methodology of saether (2002). Head, wings and genitalia were dissected and mounted on the slide for theidentification. Identification of sandflies (*Phlebotomus argentipes* and *Phlebotomus papatasi*) was done with the help of keys of The odor (1958), Artemiev (1978) and Lewis (1982).

3. Result

There are more than 40 species are recorded from India. The important ones are *Phlebotomusargentipes* and *Phlebotomu spapatasi* as they are the main causative vector of leishmaniasis in India and other countries. In this present work, various morphological characters of Phlebotomine sandflies were studied. About 300 sandflies of different species were examined out of which only 26 sandflies, belongs to *Phlebotomus argentipes* and *Phlebotomus papatasi*. A total of 28 morphological characters of Indian phlebotomine sandflies were studied to discriminate these two *Phlebotomus* species.

a. General morphological characters of Phlebotomine sandflies

Sandfly are hematophagus insect of great medical and veterinary importance. The body of sandfly is shaped as a mosquito but they are smaller than mosquito. The Phlebotomine sandflies are small, delicate, hairy flies with long slender legs and almost erect hairy wings. Sandfly's body is composed of a Head, a Thorax and an Abdomen.

i. Head:

The head is round with widely separated black compound eyes, Cibarium, Pharynx, antenna and mouth parts.

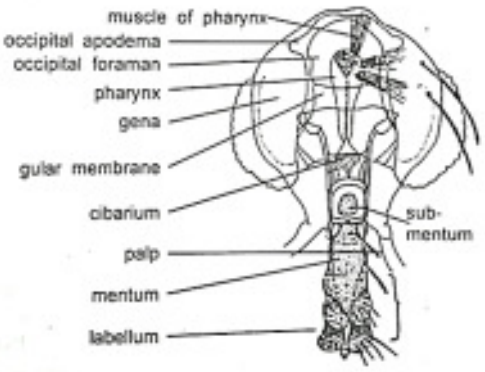


fig 2 Head

female proboscis is composed of a pair of mandibles, a pair of maxillae and a hypopharynx. All of these appendages lie between a ventral labium and a dorsal labrum. In male sandflies mouth parts are much reduced and lacks mandibles.

Each mandible is shaped like a broad pointed blade and contains small teeth on the inner edge, towards the apex. Hypopharynx is a blade shaped structure is perforated by narrow salivary duct.It bears leaf shaped serration near the apex in Genus *Phlebotomus*. Each maxilla is stout based but narrow towards its apex. The maxilla has two rows of teeth, lateral teeth on the outer edge and ventral teeth are on the inner edge. According to the shape of maxillae, Lewis (1978) divided all Sandflies into ridge tip and hook tip group. The hook-tip sandflies (genus *Phlebotomus*) feed mostly on mammals while the ridge-tip sandflies feed on reptiles. Labrum is sword shaped structure bears 4-8 sensilla at its tip and thick lateral hairs near the tip. Labium is long and thick.

fig 3 Antenna

The antennae are 16 segmented, paired and hairy structures in sandflies. There is no difference between antennae of male and female in sandflies.

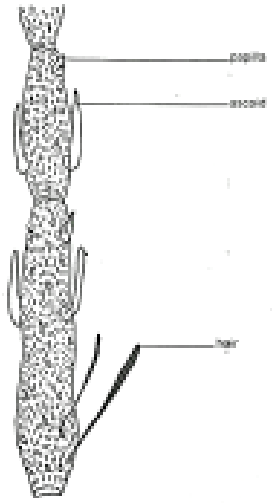


fig 4 Sensilla chaetica (Ascoids) & Papilla

The first antennal segment (A1, Scape)is ring shaped and is attached to the dorsal surface of head, between the eyes. The second antennal segment (A2, pedicel) is globe shaped. Third segment (A3) is the longest one. Fourth (A4) and Fifth (A5) segment are of equal length. Segment from sixth (A6) to sixteenth (A16) become gradually shorter and A16 is the shortest antennal segment. Each antennal segment from 3 to 15 has one or more sensilla chaetica (Previously known as Ascoids) and segments A3, A4 and A5 have one or more papilla. Ascoids are thin walled finger like sensory structures that are present on most antennal segments. Papillae are other sensory structure present on antennal segment 3-5.

The palps are 5 segmented appendages, attached to the base of labrum. First segment is the shortest one and third segment bear a tuft of short hairs on its

ventral surface. These hairs are chemoreceptors and termed as Newstead's organ.

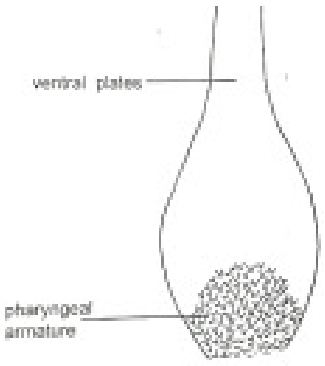


fig 5Ciabarium with hypopharynx

The cibarium is an internal head structure that is situated between the pharynx and the proboscis. The cibarium has two series of teeth. The anterior or vertical teeth project down in the cavity and posterior or horizontal teeth project backwards towards the pharynx.

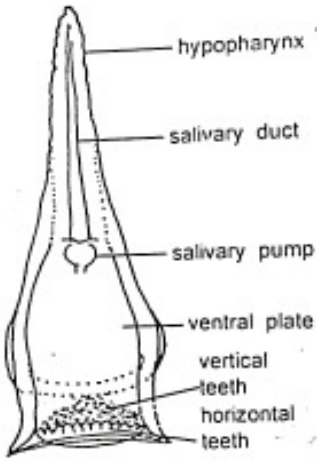


fig 6 pharynx

Lateral teeth also present in some *Lutzomyia* species.The pigment patch is present on the dorsal wall of the cibarium. The chitinous arch crosses the ventral wall of the cibarium in some species.The Pharynx is present posterior to the cibarium and consist two dorsal and one ventral plate. Base of pharynx bear pharyngeal armature (spicules and teeth).

ii. Thorax

The Thorax consists of 3 segments: Prothorax, Mesothorax and Metathorax. Each segment has one pair of legs. Mesothorax has a pair of wings and the Metathorax has a pair of halters, reduced wings. Sclerites also present in all the three segments i.e.Prothorax, Mesothorax and Metathorax. Some species of sandflies have pleural hairs on the sclerites of thorax(genus*Grassomyia*). Pigmentation of different sclerites and presence or absence of pleural hairs is important taxonomic characters in the identification of sandflies.

iii. Wing

Wings in sandflies species are rhomboidal in shape, narrow at both ends, hairy and held vertically up over abdomen. The basic plan of wing venation is constant in the Phlebotomine sandflies .The veins of wings of sandflies are Costa, Subcosta, Radius, Median, Cubitus and Radio-medial veins. The length of veins, the places where they end at the periphery of the wing and the points where they branch are the important taxonomic features of sandfly'swings. The distance between these branching points are used to obtain the

measures ALPHA, BETA, GAMMA DELTA and EPSILON.

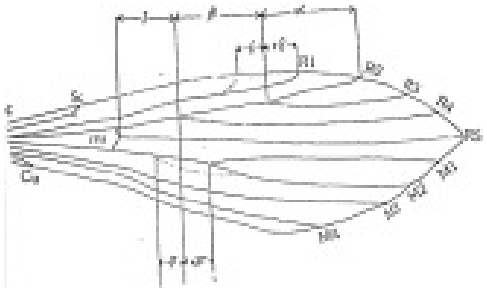


fig 7 Wing

· Alpha is the length of R2 from its junction with R3 to Costa

· Beta is the length of R2+3 from its junction with R4

· Gamma is the length of R2+3+4 from origin of R5

· Delta is the length of R1 from the junction of R2+3

· Epsilon is the length of R3 from its junction with R2 to Costa

iv. Abdomen

The sandfly abdomen is cylindrical and consists of 10 segments. The first abdominal segment has only tergite whereas segments 2-7 have tergite on dorsal side and sternite on ventral side. All tergite have erect or recumbent hairs on tergite.The last three segment of abdomen are transformed into genital segment.

v. Male Genitalia

The terminalia of male sandflies is rotated through 180 degree so that tergites are on the ventral side and sternites are on the dorsal side. The appendages of male genitalia are Coxite, Surstyle, Parameres, Aedeagus, Cerci, Styles and Genital pump. Coxite is the largest and paired appendages of male genitalia. Sometimes it has basal processes or group of hairs on its inner sides.

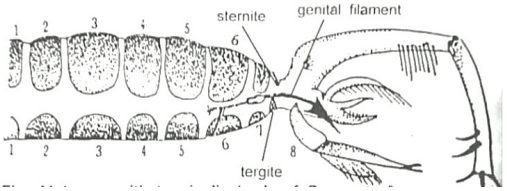


fig 8 Abdomen with terminalia (male of P. papatasi)

Surstyle are paired, long and cylindrical appendages on ventral side. Some species have short spines at the tip of surstyle. Parameres are paired appendages with variable shape. Some species have 1-2 dorsal or ventral process on the parameres. Aedeagus is adark hard appendages of different length and shape. Cerci are paired soft and hairy appendages that are present between surstyles.The styles are paired appendages with several spines .The styles are attached to the distal ends of coxites.Genital pump is present in abdomen and attached with a pair of genital filaments.

vi. Female Genitalia

Fig 9 Abdominal tergits stermites and appendages (female)

In the female genitalia, the morphology of internal organs is used for specific identification. The internal structures of female genitalia are genital fork, spermatheca and spermathecal duct.Genital fork arises from genital orifice and support paired spermatheca. Spermatheca are paired capsule with long and short ducts. Spermatheca may be smooth or ornamented (segmented). Paired cerci are attached to the segment 10 of female sandflies.

***b. Phlebotomus (Euphlebotomus) argentipes* Annandale&Brunette:-**

Phlebotomus argentipes is the well-known vector of visceral leishmaniasis (VL) in India and other countries. *Phlebotomus argentipes* are now considered as a species complex comprising three species (previously reported as a species complex of two species).It shows geographic variation that is linked with various morphological characteristics. One of the most important character is the difference in the length of sensilla chaetica (previously known as antennal ascoids (Ilango, 2000). There is no morphological variation in the head and thorax of male and female sandflies except the genital segments of its.

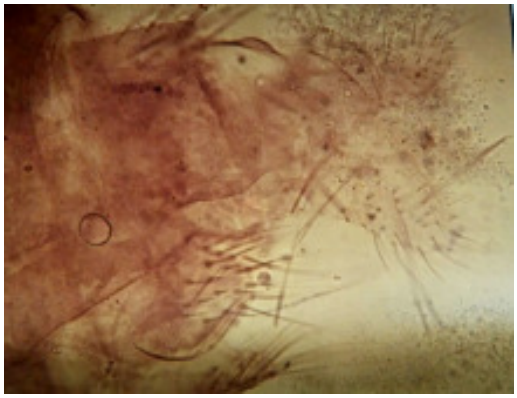
i. **Male:**

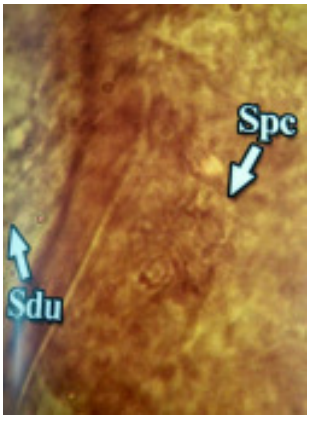
Middle sized sandfly coxite broad with no basal lobe and bristles.Style narrow,medium length and with five long spines,two terminals and three median.Paramere with two long ventral processes and long distal part. Aedeagus long-conical, with pointed end and slightly curved and have lateral

Fig. *Phlebotomus (Euphlebotomus) argentipes* Male genitalia

spine. Surstyle with no spine.

ii. Female:





Pharynx with several convex rows of pale teeth and toothed lines.Cibarium with 4-5 pale horizontal teeth.Spermatheca with about 15 narrow segments. The apical segment enlarged and separated from others by deep furrow.Seminal capsule is 0.85 mm long and its duct is short and partly fused with a common opening. Cerci elongated and post genital plate is nearly triangular in shape with two spines.

***c. Phlebotomus (Phlebotomus) papatasi* (Scopoli)**

Phlebotomus papatasi is the principal vector for anthroponotic Cutaneous Leishmaniasis (CL) in the Old world. It is reported from Southwest and Central Asia, North Africa and Indian subcontinent. It is

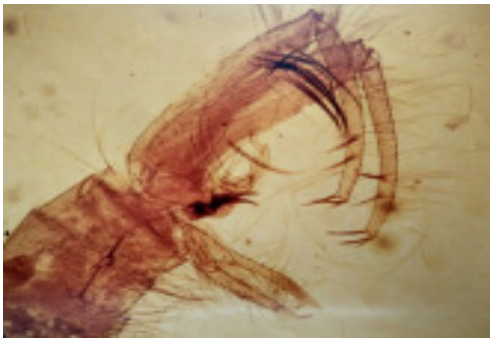
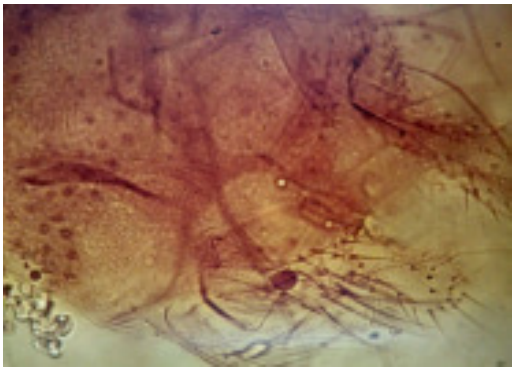
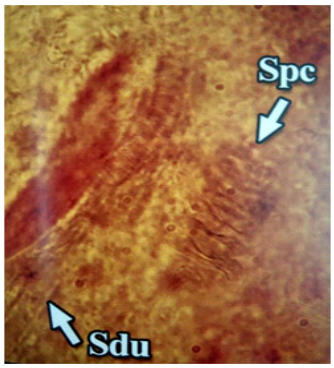


Fig. *Phlebotomus (Phlebotomus) papatasi* Male genitalia

also responsible for the transmission of sandfly fever in all its distributional area of India.

i. **Male-** A large pigmented sandfly. Antennal papillary formula is 1/3-5. Cibarial teeth are notpresent or rudimentary. Terminalia is very long. Coxite with very small basal process and long hairs distend.Style is long, cylindrical with 5 spines,three terminal and two basal on ventral side. The basal spines are much closer to one another than to the terminal spines. Paramere short with two dorsal processes. The second process is long and narrow, with hairs on its ventral side.Aedeagus is short and conical. A Surstyle has two short spines at the end.





ii. Female-Cibarium with well-developed chitinous arch, one central and two lateral groups of ventral denticles. Pharynx is bottled shaped with armature on the basal part. Spermatheca 8-10 segmented and cylindrical in shape. The head is as wide as spermatheca and spermathecal duct opening separate. Cerci long and oval.Postgenital plate is square in shape and bare.

Morphological characters of Sandflies	***Phlebotomus argentipes***	***Phlebotomus papatasi***
1. Last antennal segment with two ascoids.(Male)	IX, X or XI segment	XV segment
2. Antennal papillary formula (both sexes)	1/3-4	1/3-5
3. Presence of cibarial armature (female)	Strong teeth present but not arranged in a palisade	Not present (rudimentary)
4. Female Pharyngeal armature	Small teeth or spines	Well developed, large teeth or scales
5. Anterior expension of the Pharyngeal armature	Slightly expanded	Not seen
6. Presence of basal lobe on the coxite	Not present	Yes
7. Morphology of basal lobe	No	Short with few bristles
8. Number of bristles on basal lobe	No	Fewer or equal to 20
9. Presence of tuft of bristles on the coxite	No	Yes
10. Position of tuft of bristles on the coxite	Not present	Subapical
11. Average number of bristles on the coxal tuft	Not present	20 or fewer
12. Morphology of style	Cylindrical, narrow and have medium length	Cylindrical, narrow and very long
13. Ratio length/width of Style	5-7	>12
14. Spines on style	Five and long	Five and short
15. Distribution of spines on style	Two terminal and three median	Three terminal and two median
16. Morphology and shape of apex of paramere	Trilobed (with two ventral process), rounded or pointed	Trilobed (with two dorsal process), rounded or pointed
17. Presence of spines on Surstyle	Not seen	Yes
18. Number of spines on surstyle	Not seen	Two
19. Ratio length of surstyle/ length of coxite	Around 1	Less than 1
20. Shape of aedeagus	Long and Conical with pointed or slightly curved end	Short and Conical with pointed end
21. Lateral spine on Aedeagus tip	Present	Absent
22. shape and Wall of spermatheca	Carrot shaped structure with small head and small neck, wall of spermatheca is Ornamented (segmented)	Sausage shaped structure, with broad head. Neck is absent. Wall of spermatheca is Ornamented (segmented)
23. Number of spermathecal segment	13-20	Less than 13
24. Spermathecal duct	Uniform diameter	Uniform diameter
25. Opening of spermathecal duct	Open by a common duct	Separate opening
26. The genital fork	Limbs of the fork are narrow and ribbon like appearance on the edge, The base has a number of shallow serration	Limbs of the Fork are broad and flat. The base has a fringed like structure and wider than the fork
27. Post genital plate	It is triangular and has two spines on the ridge and less widely separated	No large spines are present on the ridge and composed of two portions, square in shape
28. Cerci	Elongated	Oval

e. Key for the identification of *Phlebotomus argentipes* and *Phlebotomus papatasi*

1. Teeth absent or if present always in the form of spicules and not arranged in a definite row,pigment patch always absent, hind end of abdominal tergites 2-6 with many erect hairs, style of male with 4 or 5 spines ……..Genus *Phlebotomus* -2

2. Male……3

Female……-4

3. Terminalia is very long. Paramere with 2 long dorsal processes. Distal part of paramere straight or slightly curved up. Second dorsal process long and thin. Style long with 5 short spines. Coxite with long and thick setae in distal part and very small basal process. Surstyle with short 2-3spines. Aedeagus conical and without spines……*Phlebotomus(Phlebotomus) papatasi*

Terminalia short or middle sized. Paramere with 2 long ventral processesand long distal parts. Style with 5 long spines. Coxite wide and without basal process.Surstyleswithout spines.Aedeagus short, with long lateral spines……*Phlebotomus (Euphlebotomus)argentipes*

4. Cibarium with spicules or unarmed. Pharynx armed. Palp extending further than antenna 3. Spermatheca segmented and without neck. Spermatheca bears 2-9 segments and a wide head. Apical segment is usual and not separated from other segments. …….. *Phlebotomus(Phlebotomus) papatasi*

Cibarium with spicules or unarmed. Pharynx armed. Palp extending further than antenna 3. Spermatheca segmented and without neck. Number of segments is more than 15 and apical segment of spermatheca enlarged or separated from others by deep furrow……*Phlebotomus (Euphlebotomus) argentipes*

f. Species Complexes

More than 40 species of Kala-Azar's vector are known from India. Many of them play a major role in the transmission of various human diseases such as various forms of Leishmaniasis. Taxonomist considered all these species as a single species and formed a species complex. The term 'Species complex' is applied in the cladistics sense i.e. to group closely related taxa, presumably recently evolved and united phylogenetically in that they share one or more synapomorphic features. The taxonomy of species complex of *p. argentipes* and *P. papatasi*, their ecological distributionwith adaptation are being summarized and their biology in relation to Leishmaniasis is also discussed.

i. The*Argentipes* Complex

Phlebotomusargentipes Annandale &Brunetti is the main vector of Kala-Azar in India. This species shows geographical and morphological variations. The geographical distribution of *P. argentipes* is from Iran and Afghanistan in west to Malaysia and Indonesia in south east.

Visceral Leishmaniasisis confined to north eastern and southern India and in Nepal and Bangladesh. So it is clear that *P. argentipes* exists in the form of two different population A and B, vector and non-vector species, based on the length ratio of s.chaetica and antennal flagellomere (Ilango,2000).Recently The *P. argentipes* complex was reassessed and reported to composed of three species namely *P.argentipessensustricto* Annandale & Brunette 1908, *P. glaucus* Mitra & Roy1953 and *P. annandalei* Sinton 1923 (Ilango, 2010). These species complex is formed after considering the relative length of s. chaetica compared to that of antennal flagellomere in the second antennal flagellomere, wing index, wing overlap and the length of common spermathecal duct for females and the gonocoxite and gonostyle ratio for males.

ii. The *Papatasi* Complex

In the previous study *P. papatasi* is not considered as a species complex. However some genetic variation and morphometric variation exist in the population of these species Here We have studied two closely related species of genus *Phlebotomus, P. papatasi* and *P. salehi* and considered them as a species complex.

This complex is widely distributed in India especially in dry hot regions. These two species are the main vector of cutaneous leishmaniasis. The species of this complex have long terminalia. Coxite bears a lobe and style with 5 spines. Paramere bear two dorsal process. Pharynx of female bear scales or ridges and spermathecal segments are equal. The differences between these two species are the surstyle of *P. papatasi* bear 2-3 spines while *P. salehi* bear 7 spines. In the male of *P. salehi* second dorsal process is short and wide. Pharyngeal armature of females of *P. salehi* consist of small short squamae, those in the centre are more hard and pigmented whereas in *P. papatasi* pharynx have coarse rounded squamae in basal part.

4. Discussion

In this present study, 20 species of sandflies were studied, including two species of the genus *Phlebotomus*. These two species of genus *Phlebotomus* are *Phlebotomus* (*Phlebotomus*) *argentipes* and *Phlebotomus* (*Euphlebotomus*) *papatasi*, have great medical significanceas they are the well-known vector of Visceral Leishmaniasis and Cutaneous Leishmaniasis in India and other countries.

As we know the correct specific identification of the sand flies vector is essential for application of the control strategies to prevent and eradicate Leishmaniasis, vector born disease from the country. In this paper we reviewed and gave new diagnostic characters for the separation of these two medically important sandflies of genus *Phlebotomus*. The comparative study was also done on the morphological characters of these two species of sandflies, main vector of Kala-Azar (Leishmaniasis) in India and worldwide.

The principle morphological characters used to distinguish the male of *P. papatasi* and *P. argentipes* are sensilla chaetica (ascoid) of antenna, Papillary formula, Cibarial armature, Basal lobe of coxite, Spines of style and shape of Aedeagus etc.

In the previous studies it was found difficult to distinguish the female of these two species due to their morphological similarities. In this study we described the morphological characters used in the previous literature and also added some new characters to identify these species. In this research work we have studied in detail some external as well as internal morphological characters of genitalia of female sandflies of genus *Phlebotomus*. The morphological characters are antennal s. chaetica (ascoid), pharyngeal armature and genital characters like cerci, postgenital plate,shape of spermatheca and opening of spermathecal duct etc. These species specific characters will be helpful to distinguish these species from the population of sandflies.

A identification key is also formulated based on these morphological characters of *P.argentipes* and *P. papatasi* species of genus *Phlebotomus*.In Future more studies are required to identify these two species accurately.

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