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CLADISTIC ANALYSIS OF SANDFLIES (DIPTERA: PHLEBOTOMIDAE) WITH SPECIAL REFERENCE TO EXTERNAL GENITALIA

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ABSTRACT

Phlebotomidae is a hematophagous dipteran fly and vector of various types of leishmaniasis in India and other parts of the world. The disease has been endemic in the various parts of the country such as Bihar, Jharkhand,West Bengal, Eastern Uttar Pradesh, some part of Andhra Pradesh, Telangana and TamilNadu.There are several reports regarding the development of several new foci of the disease in the high elevation of the Himalaya, especially in the Jammu and Kashmir and Northwest Himalaya. Characters of the external male and female genitalia are considered as the most reliable tool in the systematic work (Neglected in the most previous works) and more than 26 characters have been identified for cladistic analysis. An attempt has been made for weighing of the characters and cladistic analysis based on genetalia of sandfly.

Figures : 02 References : 11 Tables : 02

KEY WORDS : Cladistic analysis, External genitalia, Sand flies.

Introduction

The true flies order Diptera includes more than 1, 50,000 species from the world. Despite of easy availability and extreme abundance, the phylogeny of the group is very poorly known. A group of hematophagous flies called Phlebotomidae (Nematocera) widely distributed from tropical to temperate region, currently has a little information related to phylogenetic relationships. The systematic of the group is not very well supported at the molecular level. Entomologists have isolated *Leishmania* species from the field collected sandfly specimens and identified its DNA sequence. Current molecular chronograms estimate the origin of the family during the Cretaceous period about 95 million years ago[2]. Most of the information available on the evolutionary history of the group is based on the non-phlebotomine subfamily Psychodidae *i.e.* generally found in decomposing biological material.

Phlebotomine sandflies includes significant vector species of *Leishmania* in India and other countries of the world. More than 1.2 million cases of cutaneous Leishmaniasis and 0.4 billion cases of visceral Leishmaniasis are estimated at global level. India has the several foci for Leishmaniasis with numerous mammalian hosts and reservoirs but no consistent work has been done on the external and internal genitalia of the fly. More than 26 characters of the external genitalia have been considered in the present study. The study will provide a concrete base for the future workers for further advanced studies.

Material and Method

For collection, preservation and dissection standard method is followed[3]. To obtain the specimens, an extensive survey of endemic areas of India was made. The specimens of various species were also studied in the collection of

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School of Entomology, St. John's College, Agra. For cladistic analysis, Out Group Comparison method was followed[10]. The characters used in the cladistic analysis and their different states are being summarized (Table-1).

Cladistic Analysis

In present study phylogenetic analysis is based on the cladistics methodology[5,11]. A list of 26 morphological characters of male and female genitalia was made on the basis of study on sand flies (Table-1). Evolution of morphological character of male and female genitalia are based on the following terms - synapomorphy, symplesiomorphy, convergence, parallelism, underlying synapomorphy, apomorphy and plesiomorphy.

The term "apomorphy" used for possession of derived condition of any characters and term "plesiomorphy" for possession of ancestral condition of any characters. The term synapomorphy is common possession of the apomorphic condition and symplesiomorphy possession of plesiomorphic condition.

The term convergence can be defined as the development of the similar characters independently in two or more lineage groups without the common ancestry and therefore convergence is the result of the parallel selection influencing the evolution of analogous structure and organs.

During the present investigation the polarity of the character (plesiomorph – apomorph) has been determined by the Out Group Comparison Method[9]. According to this rule, "Out of the two characters that are homologous and found within a single monophyletic group, one character that is also found in the sister group is the plesiomorphic character, whereas the character found only within a single monophyletic group is termed as the apomorphiccharacter".

The structural variation and morphological changes in the reproductive system of various animal taxa have been used to deduce their phylogeny. The structural diversity of male and female genitalia in sandflies represents a potential mean to study the phylogeny of sandflies.

In the present investigation we have studied 26 morphological characters of male and female genitalia of 42 Indian species for cladistics analysis. For cladistics analysis morphological characters from male and female genitalia were chosen, used by taxonomist in the identification and classification of sandflies.

The character are being used in cladistics analysis and their different states (Table-1).

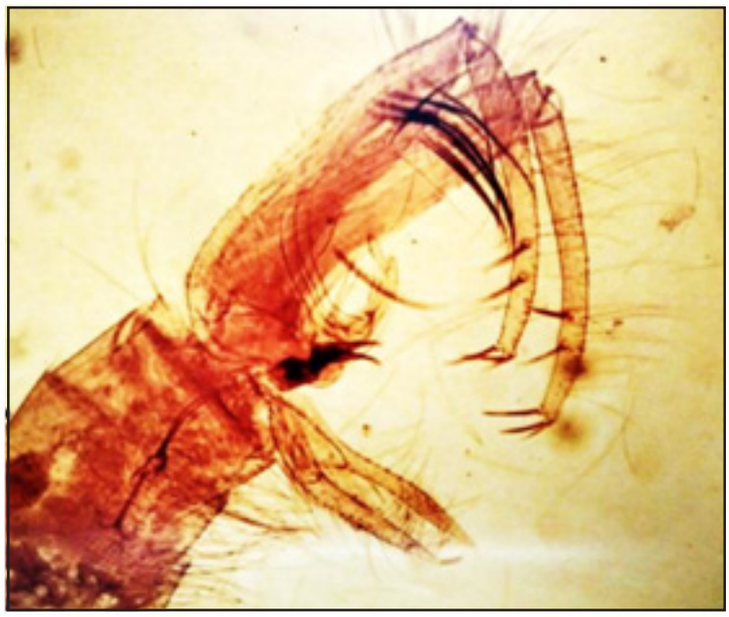

Fig.1 :*Phlebotomus (Phlebotomus) papatasi* Male genitalia

TABLE – 1 : Characters and their status for cladistic analysis

A.	Presence of a basal lobe on the coxite	No (P) [0]/ yes [1]
B.	Presence of a tuft of bristles on the coxite	No (P) [0]/ yes [1]
C.	Position of tuft of bristles on coxite	Subapical [1]/ median [2]
D.	Morphology of style	Ovoid and short [1]/ Medium length, narrow [2]/ cylindrical, narrow, very long [3].
E.	Ratio length/Width of style	Around 3 [1]/ around 4 [2]/5-7 [3]/8-10 [4]/>12[5]
F.	Ratio length of coxite/length of style	Less than 1.5 [1]/1.5-2[2]/ greater than 2[3]
G.	Number of spines on style	Four [4]/ five [5]
H.	At least one non-deciduous seta on style	Absent [1] / present [2]
I.	Spines on style	Short [1] / long [2]
J.	If five, distribution of spines on style	Three terminal, two median [1] / two terminal, three median [2]
K.	Position of terminal spines on style if two terminal and two median or submedian	Both apical [1] / One apical one Subapical [2]
L.	Morphology of parameres	Simple [1] /bilobed [2] /trilobed [3]
M.	Shape of apex of parameres	Rounded or pointed [1] / truncate, squared or hooked [2] / flat and elliptical (spatulate) [3]
N.	Presence of spines in paramere	No (P) [0] / yes [1]
O.	Presence of spines on surstyle	No (P) [0] / yes [1]
P.	No. of spines on surstyle	Two [1] / more than three [2]

Q.	Ratio length of surstyle / length of coxite	Distinctly less than 1 [1] / around 1 [2] / distinctly greater than 1 [3]
R.	Shape of aedeagus	Conical [1] / digitiform [2] / very short, rudimentary [3] / specific shape, bifurcate [4]
S.	Shape of apex of aedeagus	Rounded, blunt [1] / pointed [2] / with terminal bulb [3] / transparent [4]
T.	Ratio length of genital filament / genital pump	Less than 3 [1] / 3-5 [2] / 6.5-9 [3] / equal or > 9.5 [4]
U.	Modification of tip of genital filament	No (P) [0] / yes [1]
V.	Wall of spermatheca	Smooth [0] / ornamented [1]
W.	Smooth spermatheca with capsule or demarcation from spermathecal duct	Absent [0] / present [1]
X.	Spermathecal ornamentation	Segmented [1] / striated [2] / with row of spicules [3]
Y.	Opening of spermathecal duct	Separate [1] / by a common duct [2]
Z.	Spermathecal ducts	Uniform diameter (P) [0] / with a dilation other than at the base [1]

Data coding :

Plesiomorphic states are coded as zero while more apomorphic states are coded as 1. If there are more than two character states, these were coded as ordered multistate transformation series 0-n (Table-2).

Observations

A total of 350 specimens were studied, comprising 42 species, belonging to two genera, *Sergentomyia* and *Phlebotomus*re presented by 29 and 13 species respectively Phylogenetic investigation of the sand flies had many important characters such as morphology of style (character D), number of spines on style (character- G), morphology of paramere (character- L), wall of spermatheca (character-V) *etc.*

It is interesting to know that male genital armature of phlebotomidae has occupied a right place in the studies of phlebotomidae taxonomy but female genitalia neglected even today. Worker[8] analyzed the utility of all the structure of female genitalia in phlebotomidae taxonomy. The internal genital structure of female helps in identifying different species[7].

In case of male genital armature, basal process and hairy tuft of coxite play an important role in separating the different genera and subgenera of Phlebotomidae. Genus *Phlebotomus* can easily be separated from genus *Sergentomyia* by the presence of basal lobe on coxite. However in case of same genera, *Phlebotomus* (*Adlerius*,

TABLE – 2 : Character and their state in different species of sandfly

	Name of Species	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
1.	*Phlebotomus* (*Idiophlebotomus*) *tubifer*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1	-	1	0
2.	*Phlebotomus* (*Idiophlebotomus*) *teshi*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1	-	1	0
3.	*Phlebotomus* (*Phlebotomus*) *salehi*	1	1	1	3	5	1	5	1	1	1	-	3	1	0	1	2	1	1	1	1	0	1	-	1	1	0
4.	*Phlebotomus* (*Phlebotomus*) *papatasi*	1	1	1	3	5	1	5	1	1	1	-	3	1	0	1	1	1	1	1	1	0	1	-	1	1	0
5.	*Phlebotomus* (*Synphlebotomus) eleanorae*	1	0	-	2	-	1	5	1	2	2	-	1	3	0	0	-	2	1	1	1	0	1	-	1	1	0
6.	*Phlebotomus* (*Paraphlebotomus) sergenti*	1	0	-	1	1	3	4	1	2	-	1	1	3	0	0	-	3	1	1	1	0	1	-	1	1	0
7.	*Phlebotomus* (*Paraphlebotomus) alexandri*	1	0	-	1	2	2	4	1	2	-	2	1	3	0	0	-	2	1	1	1	0	1	-	1	1	0
8.	*Phlebotomus* (*Anaphlebotomus) colabaensis*	0	0	-	1	2	3	4	1	2	-	2	2	1	0	0	-	-	1	2	-	0	1	-	1	2	0

	Name of Species	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
9.	*Phlebotomus* (*Anaphlebotomus) stantoni*	0	1	2	1	1	3	4	1	2	-	2	3	1	1	0	-	2	1	2	1	0	1	-	1	2	0
10.	*Phlebotomus* (*Euphlebotomus) argentipes*	0	0	-	2	3	2	5	1	2	2	-	3	1	0	0	-	2	1	1	1	0	1	-	1	2	0
11.	*Phlebotomus* (*Euphlebotomus) newsteadi*	0	0	-	2	3	2	5	1	2	1	-	2	2	0	0	-	2	1	1	1	0	1	-	1	2	0
12.	*Phlebotomus* (*Adlerius) longiductus*	0	1	2	2	3	2	5	1	2	2	-	2	1	0	0	-	3	2	1	4	0	1	-	1	1	0
13.	*Phlebotomus* (*Larroussius) major major*	0	1	2	2	3	2	5	1	2	2	-	2	1	0	0	-	2	2	3	2	0	1	-	1	2	0
14.	*Sergentomyia* (*Sergentomyia*) *punjabensis*	0	0	-	2	2	3	4	2	2	-	-	1	2	0	0	-	1	2	1	2	0	0	0	-	2	0
15.	*Sergentomyia* (*Sergentomyia) theodri*	0	0	-	2	2	3	4	2	2	-	1	1	2	0	0	-	1	2	1	1	0	0	0	-	2	0
16.	*Sergentomyia* (*Grassomyia) indica*	0	0	-	2	2	3	4	2	2	-	1	1	2	0	0	-	1	1	1	2	1	1	-	3	2	0
17.	*Sergentomyia* (*Sintonius) christophersi*	0	0	-	2	2	2	4	2	2	-	1	1	2	0	0	-	1	1	2	2	0	1	-	1	1	0

	Name of Species	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
18.	*Sergentomyia* (*Sintonius) sirohi*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	2	-	0	1	-	1	1	0
19.	*Sergentomyia* (*Sintonius) orissa*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	2	-	0	1	-	1	1	0
20.	*Sergentomyia(Sintonius) clydei*	0	0	-	2	-	-	4	2	2	-	1	1	2	0	0	-	-	1	2	-	0	1	-	1	1	0
21.	*Sergentomyia* (*Sintonius) eadithae*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	2	-	0	1	-	1	1	0
22.	*Sergentomyia* (*Sintonius) hospitii*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	2	-	0	1	-	1	1	0
23.	*Sergentomyia* (*Neophlebotomus) purii*	0	1	-	2	-	-	4	2	2	-	1	1	2	0	0	-	-	1	1	-	0	1	-	2	2	0
24.	*Sergentomyia* (*Neophlebotomus) perturbans*	0	1	-	2	-	-	4	2	2	-	1	1	2	0	0	-	-	1	1	-	0	1	-	2	2	0
25.	*Sergentomyia* (*Neophlebotomus) zeylanica*	0	1	-	2	-	-	4	2	2	-	1	1	2	0	0	-	-	1	1	-	0	1	-	2	2	0
26.	*Sergentomyia* (*Neophlebotomus) arboris*	0	1	-	2	-	-	4	2	2	-	1	1	2	0	0	-	-	1	1	-	0	1	-	2	2	0

	Name of Species	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
27.	*Sergentomyia* (*Neophlebotomus) malabarica*	0	0	-	2	2	3	4	2	2	-	1	1	2	0	0	-	1	1	4	1	0	1	-	2	2	0
28.	*Sergentomyia* (*Neophlebotomus) hodgsonihodgsoni*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	1	-	0	1	-	2	2	0
29.	*Sergentomyia* (*Neophlebotomus) linearis*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	1	-	0	1	-	2	2	0
30.	*Sergentomyia* (*Neophlebotomus) iyengari*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	1	-	0	1	-	2	2	0
31.	*Sergentomyia* (*Neophlebotomus) dhandai*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	1	-	0	1	-	2	2	0
32.	*Sergentomyia* (*Neophlebotomus) chakravarti*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	2	0
33.	*Sergentomyia* (*Parrotomyia) africana magna*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	2	-	0	0	1	-	2	0
34.	*Sergentomyia* (*Parrotomyia) himalayaensis*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	2	-	0	0	1	-	2	0
35.	*Sergentomyia* (*Parrotomyia) babubabu*	0	0	-	2	2	3	4	2	-	-	1	1	2	0	0	-	1	1	4	2	0	0	1	-	2	0

	Name of Species	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
36.	*Sergentomyia* (*Parrotomyia*) *Baghdadis*	0	0	-	2	3	2	4	2	-	-	1	1	2	0	0	-	1	1	4	1	0	0	1	-	2	0
37.	*Sergentomyia* (*Parrotomyia*) *shortii*	0	0	-	-	-	-	4	2	-	-	-	1	2	0	0	-	-	1	2	-	0	0	1	-	2	0
38.	*Sergentomyia* (*Parrotomyia*) *barroudi*	0	0	-	2	-	-	4	2	2	-	-	1	2	0	0	-	-	1	2	-	0	0	1	-	2	0
39.	*Sergentomyia* (*Parrotomyia*) *modii*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1	-	2	0
40.	*Sergentomyia* (*Parrotomyia*) *kauli*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1	-	2	0
41.	*Sergentomyia bailyi*	0	0	-	2	-	-	4	2	2	-	1	1	2	0	0	-	-	1	2	-	0	0	1	-	-	0
42.	*Sergentomyia montana*	0	0	-	2	-	-	4	2	2	-	1	1	2	0	0	-	-	1	2	-	0	0	1	-	-	0

(-) No code applied to this taxon.

Larroussius,*Euphlebotomus* and *Anaphlebotomus*) coxite have no basal process.Similarly number of spines on style and shape and structure of style play a very important taxonomic tool for separating genus *Phlebotomus* and *Sergentomyia*.

In female genitalia the gonocoxite VIII is easily visible external structure which serves as a reliable character for distinguishing the species. It may be oval or triangular in shape.Similarly the shape and the number of segments in spermatheca also helpful in separating the different species. Genus *Phlebotomus* can be distinguishing from genus *Sergentomyia* due to bearing segmentation in spermatheca.

The Phlebotomine sandflies of the new world and Old world to be sister taxa with a monophyletic origin[5]. Among the non Phlebotomine Psychodids only the members of subfamily Sycoracinae and Trichomyiinae have paired spermathecae in female and paired structure like gonocoxite and gonostyle, bifurcate aedeagal filament in the genital of male. Sycoracinae and Horaiellinae much closer to Phlebotominae than Trichomyiinae[4]. The genitalic relationship of old world phlebotomines with the members of subfamilies Sycoracinae[6].

a. Genitalic character of male

The different parts of the male genitalia in Phlebotomidae are complex and bear diversity among subfamilies.In the ground plan of Nematocera the male external genitalia consist of following parts: IX tergum and IX sternum, each separated from each other; a pair of separated two segmented arms of an outer forceps, the basal segment of outer forceps are called as coxite or gonocoxite and the distal part is called as stylus or gonostylus; the copulatory organ aedeagus and a pair of un-segmented inner forceps between the copulatory organ and the arms of outer forceps.In addition to these there are more or less rudimentary terga and sterna of the X and XI segments and the cerci and anal orifice of XII segment.

In almost all Psychodidae including Phlebotomines, the male genitalia is rotated through 180 degree as a result the morphologically ventral structures, the hypandrium (IX sternites) and its derivatives are shifted to dorsal side and the dorsal structures such as lateral lobes,the derivative of epandrium (IX tergite) and cerci are shifted to ventral side. The hypopygium is not rotated in *Sycorax* spp. It is a plesiomorphic state.

The epandrium of *Phlebotomus* spp. is divided medially and formed the lateral lobes or

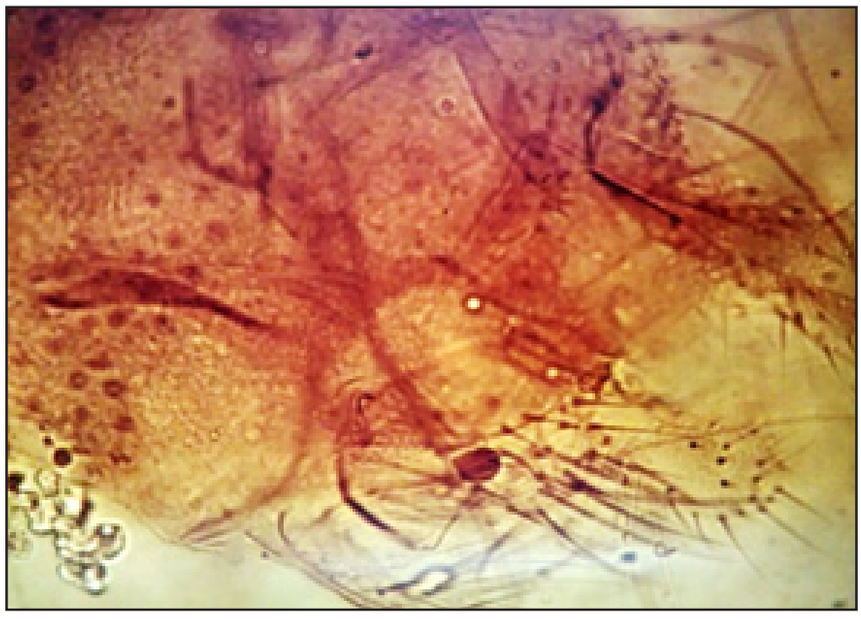

Fig.2 : *Phlebotomus (Phlebotomus) papatasi* Female genitalia

surstyle. These lobes are absent in Sycoracinae.These lobes are apomorphically long and equal to the length of gonocoxite and gonostyle in all Phlebotomines.The cerci of *Phlebotomus* spp. are membranous structure and situated in between the lateral lobes and much shorter than it. In *Sycorax* spp.cerci are a pair of short and fleshy lobes.The lobes of gonopods are coxite.In *Idiophlebotomus, Splaeophlebotomus, Chinius* and Sycoracinae the gonocoxite is shorter and broader than or as long as the gonostylus.In *Sergentomyia* and *Phlebotomus* of old world it is derived into a long gonocoxite.In *Lutzomyia* and *Adlerius* the gonocoxite has a tuft of hairs. In the subgenera *Phlebotomus, Paraphlebotomus, Synphlebotomus* it has a lobe with long hairs.The style is the basal part of gonopods and plesiomorphically it is thin, narrow and as long as coxite found in *Idiophlebotomus, Splaeophlebotomus* and Sycoracinae. Synapomorphically it is shorter and apically broader than the coxite of Phlebotomines.

Paramere are articulated anteriorly with the aedeagal sheath.It is a simple structure in *Idiophlebotomus* and *Sergentomyia.* In *Synphlebotomus* and *Paraphlebotomus* it has flat upper surface. It is trifurcate in *Phlebotomus, Euphlebotomus* and *Anaphlebotomus*.The aedeagus is simple in genus *Sergentomyia* but it is derived among all *Phlebotomus* spp.It is bifurcate in *Larroussius* and *Adlerius* and trifurcate or modified into a pair of lateral spines in *Euphlebotomus* spp. and *Anaphlebotomus* spp.

The base of genital pump plesiomorphically simple and broader distally among the species of *Idiophlebotomus* and *Splaeophlebotomus.* In all other taxa of Phlebotomines it is derived as T-shaped structure. The genital filament is extremely long in *Sergentomyia (Sintonius*) and *Lutzomyia*.In *Grassomyia* the tip of genital filament swollen. The rod like structure of gonocoxapodeme is also present in *Idiophlebotomus* and *Splaeophlebotomus.* These rods are also present in *Sycorax*spp. and considered as plesiomorphic feature.

b. Genitalic character of female

The female genitalia consist of gonostyli and gonapophyses borne on gonocoxite of abdominal segment of VIII and IX. In the basic pattern of female pterygote insect genitalia, the gonapophyses IX is rotated 180 degree laterally along their longitudinal axis and fused with each other along their dorsal margin to form a notum and interlocked on its ventral margin with a pair of gonapophyses VIII.Family Phlebotomidae has numerous potential synapomorphies including the fused plate like GC VIII, reduction of GP IX and fusion of GC IX with TX.

A large, membranous, un-segmented, tubular spermatheca is plesiomorphic character. The interseptal folding of spermatheca are finger like inwardly projection, providing shelter for spermatozoans which is a synapomorphy found in *Brumptomyia, Phlebotomus* and *Lutzomyia* spp. The absence of segmentation of spermatheca occurs in outgroup, the sycoracinae and the lower group of Phlebotomines like *Idiophlebotomus* and most of the subgenera of *Sergentomyia.*

The common spermathecal duct is a plesiomorphic character among the taxa with membranous, un-segmented spermatheca.In the subgenera *Phlebotomus, Paraphlebotomus* and S*ynphlebotomus* spermathecal duct is separated. Plesiomorphically the large, membranous spermatheca has a short spermathecal duct. In all subgenera of *Phlebotomus* the individual duct length is apomorphically medium to long.In *Euphlebotomus* the common duct is as long as the individual duct. In *Sintonius* spp. the common duct is longer than the individual duct. In *Anaphlebotomus* spp. the duct is extremely long.

The genital fork or furca is an inverted Y-shaped structure and derived from IX sternite. The Y-shaped structure has a median anterior arm and a pair of long lateroposterior arms.In *Sycorax* spp. the fork appears as triangular structure and the posterior arms are plesiomorphically much reduced.Synamorphically well-developed anterior and posterior arms are present in *Sergentomyia*, *Phlebotomus* and *Lutzomyia*spp.

c. Phylogenetic inference

During the present investigation a complete morphological study of male and female genitalia of sandflies was made. This study reveals that Sycoracinae is the out-group for Phlebotominae. The Phlebotominae is a monophyletic taxon relative to out-group i.e. Sycoracinae. Synapomorphies are: In female genitalia capsular spermatheca is segmented, duct length is medium to long and genital fork has well developed anterior and posterior arms. In male, genitalia has 180 degree hypopygial inversion, presence of surstyle and style have more than three spines.

Within Phlebotomidae *Idiophlebotomus* spp. and *Splaeophlebotomus* spp. are basal lineage showing sister group relationship. They share the synapomorphic membranous, tubular unsegmented spermatheca together with male genitalia features. The genus *Sergentomyia* is polyphyletic due to repeatedly evolved homoplastic spermathecal and male genital features. The genus *Phlebotomus* is a monophyletic taxon and its subgenera form sister group relationship.It has synapomorphies of true segmented spermatheca and several male genital characters. The genus *Anaphlebotomus* appears to be paraphyletic among the subgenera of *Phlebotomus.*

The present study also provides ample evidence to treat phlebotomidae and Psychodidae as independent families rather than keeping them at subfamily levels.The phylogenetic relationship of different taxa of family Phlebotomidae has also been discussed in the present study. The conclusion from this investigation is that Phlebotomidae shows the monophyletic origin. The genus *Sergentomyia* is a polyphyletic taxon while genus *Phlebotomus* is monophyletic taxon.

This analysis grouped together the species data in the generic clusters namely *Phlebotomus* and *Sergentomyia*.The taxa are well defined within subgenera *Phlebotomus*, *Adlerius*, *Paraphlebotomus*, *Larroussius* and *Synphlebotomus* of genus *Phlebotomus*. Similarly genus *Sergentomyia* differentiated taxa in five subgenera *Sergentomyia*, *Parrotomyia*, *Sintonius*, *Randonomyia* and *Grassomyia*.

In conclusion it is clear that not only the male genitalia but also the female genetalia structure can provide stable and important taxonomic character for the proper placement of different taxa in family Phlebotomidae.The phylogenetic relationship of different genus and subgenus of family Phlebotomidae is cladistically analysed by Out Group Comparison Method and homologies of male and female genital structure is also given among the different taxa.For cladogenesis of Phlebotomidae 26 characters have been selected from both male and female genital characters. This study concludes that old world phlebotomidae seems to be monophyletic origin.

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