INT. J. BIOL. BIOTECH., 5 (3-4): 179-192, 2008.

TAXONOMIC STUDIES OF *PHLEBOTOMUS PAPATASI* (SCOPOLI) (DIPTERA: PSYCHODIDAE) IN SINDH, PUNJAB AND N.W. F. P. AND ITS PHYLOGENETIC RELATIONSHIP WITH ITS CLOSEST ALLIES

Juma Khan Kakarsulemankhel

Investigator of Sand flies, Leishmaniasis, Helminths, Mosquitoes, & Ticks, Center for Advanced Studies in Vaccinology & Biotechnology, Brewery Road, Quetta, Pakistan.
dr.jumakhankakarsulemankhel@yahoo.com; Cell. # 0333-7841004

ABSTRACT

In the survey, the work was done to develop taxonomic record of sand fly *Phlebotomus* (*Phlebotomus*)) *papatas i* (Scopoli) collected for the first time from new epidemic localities of cutaneous leishmaniasis in Sindh, North West Frontier Province (NWFP) and Punjab provinces. In view of the published reports about the detection of *Leishmania major* in these flies in the neighboring countries, the correct identification of this species becomes of significant value in the study of epidemiology of leishmaniasis. Therefore, in order to facilitate Zoologists and Medical researchers in its correct identification, taxonomic characters of *P. papatasi* (Scopoli) are studied in details with special reference to its mouth parts, male and female genitalia and findings are presented in this paper. A key is also given to *P. papatasi* (Scopoli) and its closest allies and its relationship with them is also briefly discussed.

Key words: Sandflies, *Phlebotomus papatasi*, Sindh, Punjab, N.W.F. P.

INTRODUCTION

Only the female of the sand flies are blood feeders and are of medical importance as vectors of many zoonotic diseases (arboviruses, bartonelloses and especially leishmaniasis, and sand fly fever) (Alexander and Maroli, 2003) and also of several pathogens constituting serious health problems (Secundino *et al.*,2005).

Leishmania cycle occurs in 88 countries in biotopes ranging from a primary forest to xerophytic biotopes, from sylvatic to domestic environment, from low land to high land (Maroli and Khorey,2004; Dujardin, *et al.*,2007).

Leishmaniases in their various forms appear to be emerging globally (Ashford, 2000; Desjeux, 2001). The resurgence of cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) in many countries has once again highlighted the importance of sand flies as vectors of human diseases. Sand flies and the diseases associated with them are major concern in the country. *Leishmaniases* appears to be an emerging infectious disease in parts of north-east Afghanistan and in North West Frontier Province (Rowland *et al.*,1999; Kolaczinski *et al.*,2004; Khan, 2005; Bari & Rahman,2008), in Balochistan (Kakarsulemankhel,2004a; Ahmad *et al.*,2008), in Sindh (Hashiguchi *et al.*,2005; Kolachi *et al.*,2005; Wakil *et al.*, 2006).

In Pakistan, sand flies have not received the due attention they deserve. Sand flies are constantly being reported from the new localities in the country. Since the publication of Phlebotominae of west Pakistan by Lewis (1967), published literature on the taxonomy and morphology of Pakistani sand flies is meager However a few localized surveys have been carried out by Qutubuddin (1951), Nasir (1958), Barnett and McDonald (1964), Aslamkhan and Barnett (1966,1967), Aslamkhan and Rafiq (1980), Burney and Lari (1986), Rab *et al.* (1986), Safi (1993), Aslamkhan (1996), Aslamkhan *et al.* (1997,1998). They have mentioned only the names of the species they collected. Lewis (1967) too while re-describing *P. papatasi* did not furnish information about the proboscis, mandible, hypopharynx, maxilla, pharynx spermatheca, style, coxite and aedeagus sheath nor supplied their figures. The sketches furnished of cibarium and of labrum are quite varied. Similarly, Lewis (1978) did not discuss mandible, pharynx and male genitalia nor supplied their figures but furnished four figures of spermathecae quite different with one another in morphology. Phylogenetic relationship of this fly with its other closest allies was also not discussed.

The correct identification of the species of sand fly from endemic places of leishmaniases has become of significant value in the study of epidemiology of the disease. In addition, the modern interest in zoonoses, animal reservoirs of leishmaniases and the role of sand flies as vectors, all have greatly focused the significance of the correct identification of sand fly species. *P. papatasi* (Scopoli) is the proven vector of CL in many parts of the world including in neighboring country Iran (Yaghoobi-Ershadi, *et al.*, 1995; Parvizi *et al*.,2005, Parvizi and Ready, 2006). Therefore, in view of vectorial capacity of this species, insufficient information about its taxonomic characters and to facilitate medical doctors and Zoologists in correct identification of this species, diagnostic characters of *P. papatasi* (Scopoli) collected from three provinces *e. g.*, NWFP, Punjab and Sindh are studied in

detail especially its mouth parts, male and female genitalia and is keyed out from its closest allies i.e. *P. bergeroti* Parrot and *P. salehi* Mesghali. The evolutionary relationship of this species within the genus is also briefly discussed.

MATERIALS AND METHODS

The present investigation was carried out on the materials (185 specimens of *Phlebotomus papatasi*) collected from Sindh, NWFP and Punjab Provinces (Pakistan) during May,2006 with sucking tubes and sticky traps. The collected materials was preserved, processed and dissected by conventional methods (Young and Duncan,1994; Aslamkhan and Aslamkhan,2000). Identification of specimen was carried out with the help of available literature (Lewis,1967,1978; Artemiev,1978). Morph metric measurements and photographs were taken from camera mounted Olympus microscope (BX41). All of the structures were measured with a low magnification (X100). All given measurements are in mm. The data of specimens critically examined for the description and measurements are designated under "Material examined". Measured taxonomic characters are those suggested by Dedet *et al.* (1991). Prepared permanent slides were deposited with the author's collection of sandflies, Department of Zoology, University of Balochistan, Quetta.

RESULTS

Phlebotomus* (*Phlebotomus*) *papatasi (Scopoli) 1786,
(Figures 1A-1J, 2A-2J)

Bibio papatasi Scopoli 1786. Deliciae faunae et florae insubricate 1: 55
Flebotomus papatasi (Scopoli) Rondani, 1840. Memoria prima per servirealla Ditterologiia, Italiana, no. 1, 13.
Phlebotomus papatasi (Scopoli), Loew, 1847, Stetin. ent. Zig. 8, 15; Howlett,1915. Bull ent Res., 6, 294; Sinton, 1924. Indian J Med. Res., 11, 814
Phlebotomus (*Phlebotomus*) *papatasi* (Scopoli). Parrot, 1940. Arch. Inst. Pasteur Alger, 18, 310; Theodor, 1948. Bull. Ent. Res., 39, 106.
(see Lewis (1978, 1982) for complex nomenclature history)

Material examined **:** ♀ 103.

Wing (X100) 2.10-2.20 long, length/ breadth 3.08-3.14. α/β=1.60, δ=0.14, Π=0.03, gamma 0.40. Length of Palpomeres (X100):1.1 long, relative length: 1, 4, 5, 3.6, 8.6, formula 1,4,2,3,5, segment p-V is quite longer than all the others, p-IV are smaller than p-II, p-III greater than p-IV. Newstead's spines implanted closely on p-III. Lengths of antennal segments: AIII-0.22, AIV and AV each 0.11 long. Two antennal ascoids on AIII–AXV, on AIII, it is at 0.72, on AIV and AV, each at 0.33. Pre-apical single papilla present on AIII, AIV, and AV. On AIII it is implanted usually near tip of the ascoid and is at 0.94, on AIV and AV, each 0.88. AIII is almost equal to the joint length of A-IV and A-V.

Mouth parts**:** (X100) (Figs. 1A-1G)

Proboscis 0.42 long. Labrum (Fig.1A) 0.39 long, a hard sword like structure, composed of 2 stout thick apical median sensila, 4 sub apical, and about 9 adorals along the shaft, apical breadth of labrum 0.04, sensilla depth 0.05. Lewis (1978) suggested that pattern of sensilla varies between many individuals and species. Inside the body of labrum, 7-8 dot like teeth present: 2 arranged at each side and remaining implanted irregularly. Mandibles broad pointed blades (Fig.1B) 0.38 long, maximum apical breadth 0.01, minute teeth implanted on the apex, on the inner margin and on the edges of the blade, dental depth is 0.1. Maxillary blades hook-shaped (Fig.1C) 0.37 long, stout at base but narrows towards its apex, outer edge of the apex composed of a row of about 6 lateral teeth: 4 large dagger shaped more prominent, remaining two dot like and less obvious, 17 ventral teeth with broad based but apex hook shaped implanted on inner edge at some distance from the apex, dental depth 0.14. Hook shaped maxillary blades are the features of genus *Phlebotomus* (Lewis,1975,1978). Hypopharynx symmetrical blade shaped structure (Fig.1D) 0.4 long, maximum apical breadth 0.04, dental depth 0.06, hypopharynx perforated by a salivary duct, with two apical and 14 leaf shaped lateral serrations (teeth) at both apical sides, a characteristics of genus *Phlebotomus* or smooth in genus *Sergentomyia* (Artemiev,1978; Lewis,1978). Cibarium structure (Fig.1E) formed

by the continuation of hypopharynx while its dorsal plates are formed by the continuation of labrum, inside breadth 0.04 and height 0.03 excluding side walls, cibarial-ventral plate is without any kind of dentition some times with merely scattered minute spicules, a characteristic of genus *Phlebotomus* (Lewis,1978) and dorsal plate lacking a pigment patch and anterior process. Pharynx (Fig.1F) is the posterior continuation of the cibarium and is bottle shaped, 0.25 long, anterior breadth 0.03, basal breadth 0.08, pharynx is 2.6 times as wide posteriorly as anteriorly, length of pharynx is thrice its breadth, height of armature is 0.32 of the length of pharynx. Pharyngeal armature in female flies (Fig.1G) is almost like male's armature but here it is highly developed and heavily pigmented with numerous strong scales pointing backwards. Base of the pharynx has armature: anterior edge of armature almost straight having triangular or quadrate scales whereas posterior basal armature composed of many short transverse ridges implanted with many dot like teeth.

***Female genitalia*:** (X100) (Figs.1H-1J)

Spermathecae (Fig.1H) conical shaped, 0.03 long, with 7-8 segments, top of the head of spermatheca with few hair like tubules structure 0.01 long, (intracellular ducts of secretary cells surrounding the spermatheca in distal part) (Artemiev,1978), anterior segments breadth 0.02, middle and posterior segments 0.03. Furca length 0.11. Spermathecal ducts (individual ducts length 0.23) with obvious transverse striations, base of the ducts like asymmetrical bell, ducts separately end (Fig.1I) in genital atrium 0.06 breadth, a row of armature (Fig.1J) present in almost center of the atrium. Presence of genital armature is a good taxonomic character to distinguish species of phlebotomine sand flies (Pesson *et al.*,1994; Killick-Kendrick *et al.*,1994; Valenta *et al.*,1999). This character was first of all reported in Pakistani sand flies by Kakarsulemankhel (2004b).

***Material examined* :** ♂ 75

Wing length (X100): 1.93-1.98, length/width ratio 3.5-3.6, α/β=2.0, δ=0.1, Π=0.08, gamma 0.38.Length of palpomeres (X100): relative length 1, 2.75,3.5,2.75,6, formula 1,2-4,3,5, p-II is equal in length to p-IV. Newstead's spines implanted closely on p-III. Lengths of antennal segments: AIII 0.25, AIV and AV each 0.15, 2 antennal ascoids on AIII-AXV, on AIII it is at on 0.68, on AIV & AV, at 0.25.1 papilla on AIII-AV, on AIII at 0.86, on AIV & AV, at 0.83.

***Mouth parts*:** (X100) (Figs.2A-2C)

Proboscis 0.3 long. Labrum 0.25 long (Fig.2A): apical maximum breadth 0.02, with 16 lateral sensilla and depth 0.04. Hypopharynx length 0.26 (Fig.2B): apical maximum breadth 0.02, with 12 lateral teeth on each sides, and dental depth 0.04. Mandibles absent. Maxilla: 0.23 long with blunt ends, teeth invisible. Cibarium (Fig.2C): inside height 0.02 and breadth 0.04 excluding side walls, absence of any kind of armature. Pharynx: length 0.20, anterior portion breadth 0.03, posterior maximum breadth 0.07, height of armature 0.07, length of pharynx is 2.86 of the breadth of pharynx as well as height of armature, anterior edge of armature forms a convex line, basal armature with straight punctiform ridges, whereas the anterior edge is composed of weak scales not pointing backwards.

Male genitalia (X100) (2D-2J)

Coxite 0.55 long, anterior breadth 0.14, there is a patch of about 8-9 densely packed, ventrally directed yellowish pigmented long hairs at 0.727 of the ventro-latertal surface of coxite towards its distal part, whereas towards its proximal part there is a small basal process situated at a tubercle having 5-6 short hairs. Style (Fig.2D) 0.4 long, 0.05 breadth, bearing 5 short, yellow pigmented thick spatulate spines: 3 terminal and 2 basal, the last distal basal spine is situated at 0.7, 2^{nd} distal basal spine is at 0.8 and 1^{st} apical spine is at 0.95 of the style, basal spines are much closer to one another (distance 0.04) than to apical spine (distance 0.05). Paramere tri-lobed (Fig.2E)): thin sickle shaped process 0.22 long (Fig.2F) with long hairs throughout its length at ventral side, there is a middle thin straight process 0.01 long and an outer broader also sickle shaped process 0.015 long. Surstyle (Fig.2G) 0.25 long, with 2 stout apical bristles. Aedeagus (Fig.2H) 0.1 long, conical, darkly pigmented with ventrally curved apex. Genital pump (P) along with other structures (Fig.2I) (semicircular funnel) (Fig.2J) 0.18 long, length of filament (F) arising from funnel reaching to aedeagus 0.32, filament with smooth transverse striations, F/P=1.77

Comparative note

This species is most closely related to *P. bergeroti* Parrot and *P. salehi* Mesghali so far been reported from Pakistan out of four species of the sub genus *Phlebotomus* Rondani and Berte of the Old World and also related with *P. duboscqi* Neveu-Lemaire. The morphology of the spermatheca, pharyngeal armature, presence of genital

armature, positions of the basal and middle spines of the style, upper parameral lobe much longer than the paramere, surstyle with two or some times 3 stout but similar apical bristles at once differentiates *P. paptasi (*Scopoli) from its other closest allies.

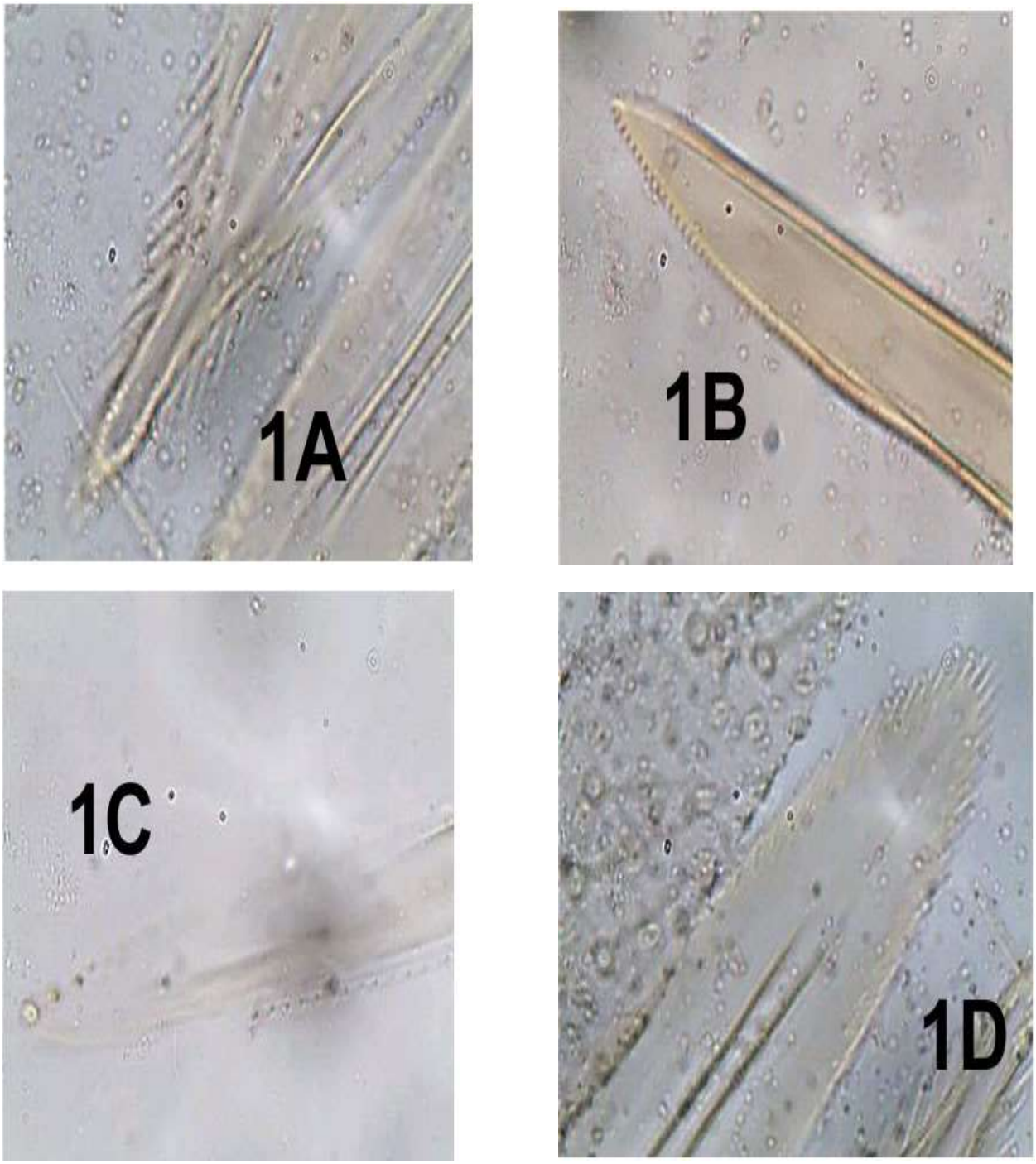
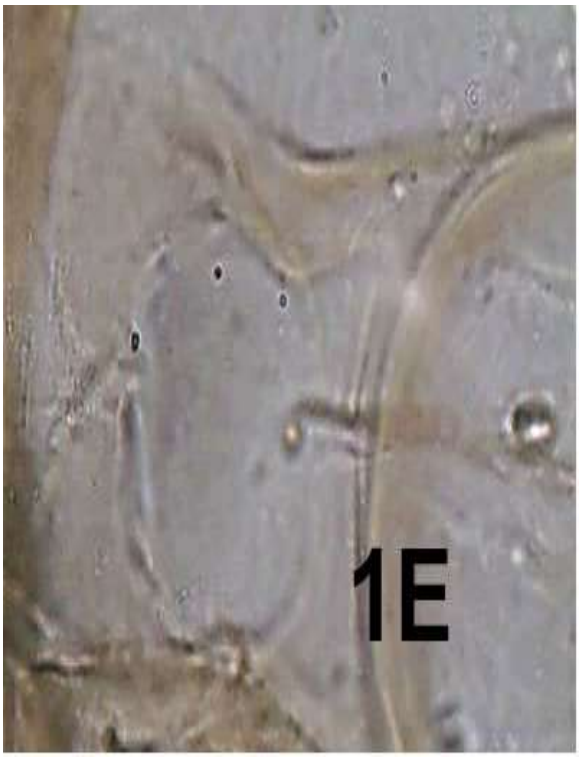


Fig.1. ♀ *Phlebotomus papatasi* (Scopoli): A, Labrum X400; B, Mandible X400; C, Maxilla X400; D, Hypopharynx X400.



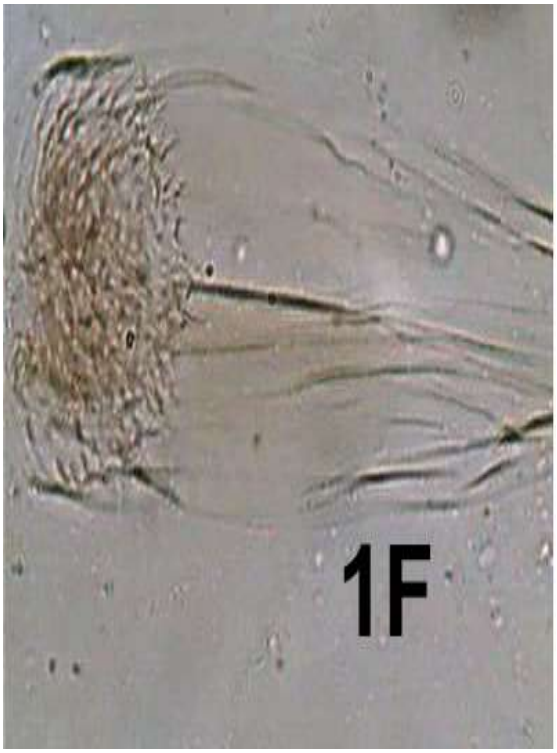


Fig.1. ♀ *Phlebotomus papatasi* (Scopoli): E, Cibarium X400; F, Pharynx X200; G, Pharnygeal armature X400; H, Spermatheca X400.

Fig.1. ♀ *Phlebotomus papatasi* (Scopoli): I, Spermathecal ducts with separate openings X400; J, Genital armature X1000.

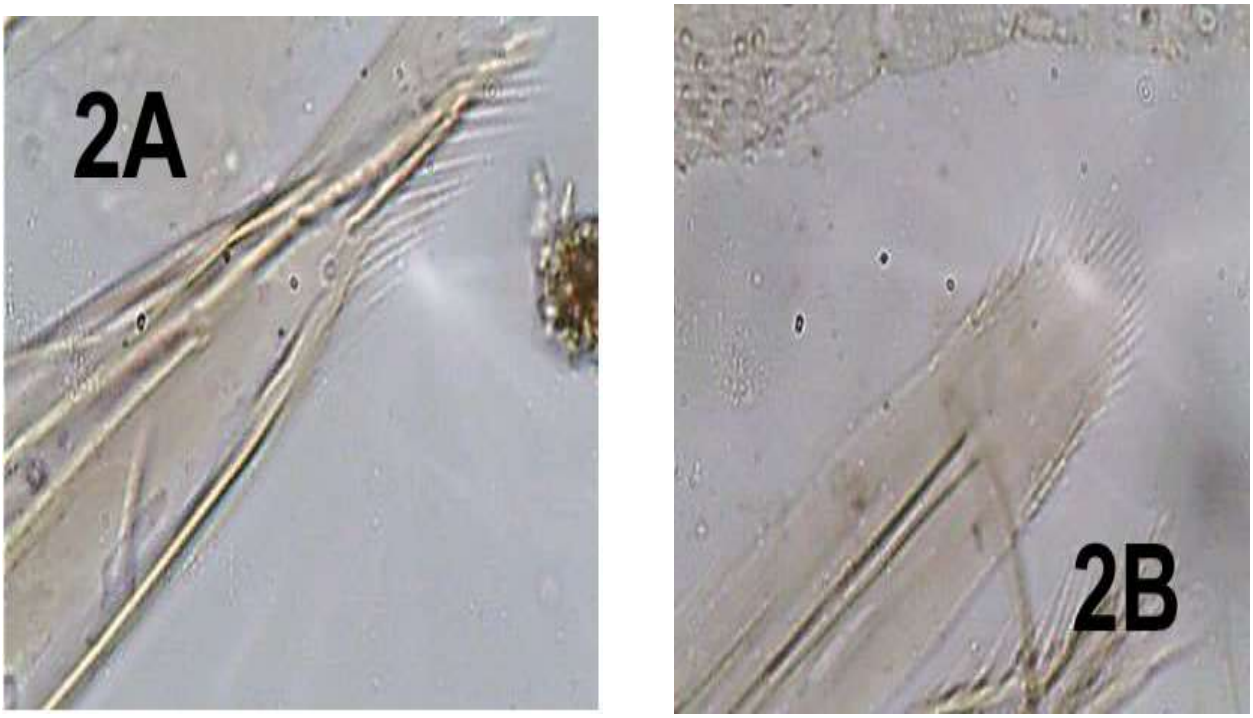


Fig.2. ♂ *Phlebotomus papatasi* (Scopoli): A, Labrum X400; B, Hypopharynx X400; C, Cibarium X400; D, Pharynx X400; E, Style, X100; F, Paramere tri-lobed X100; G, Surstyle X100; H, Aedeagus X200; I, Genital pump along with other structures, X100; J, Head of the Semi-circular funnel) of Spermathecal pump, X400.

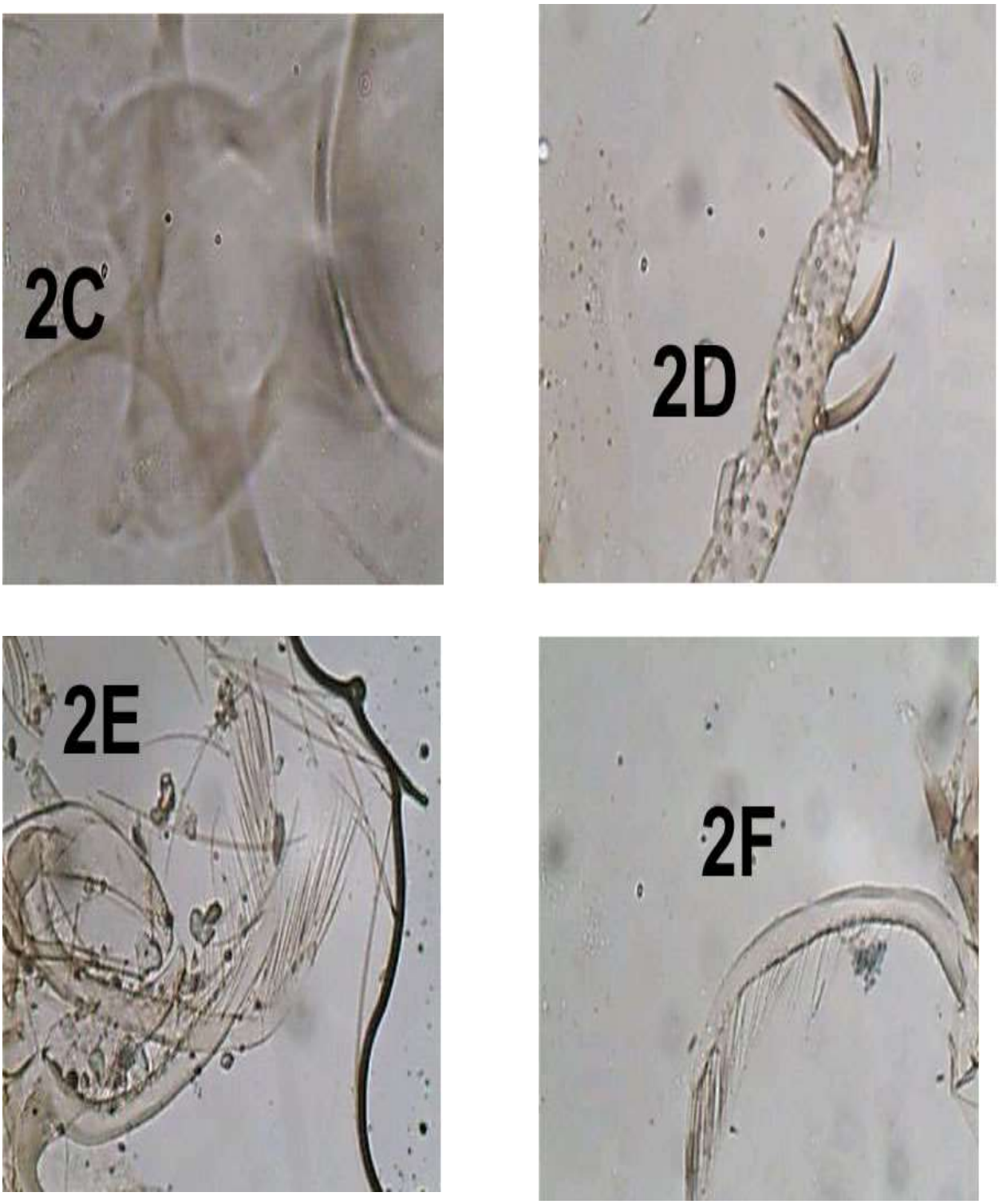


Fig.2. ♂ *Phlebotomus papatasi* (Scopoli): C, Cibarium X400; D, Pharynx X400; E, Style, X100; F, Paramere tri-lobed X100.

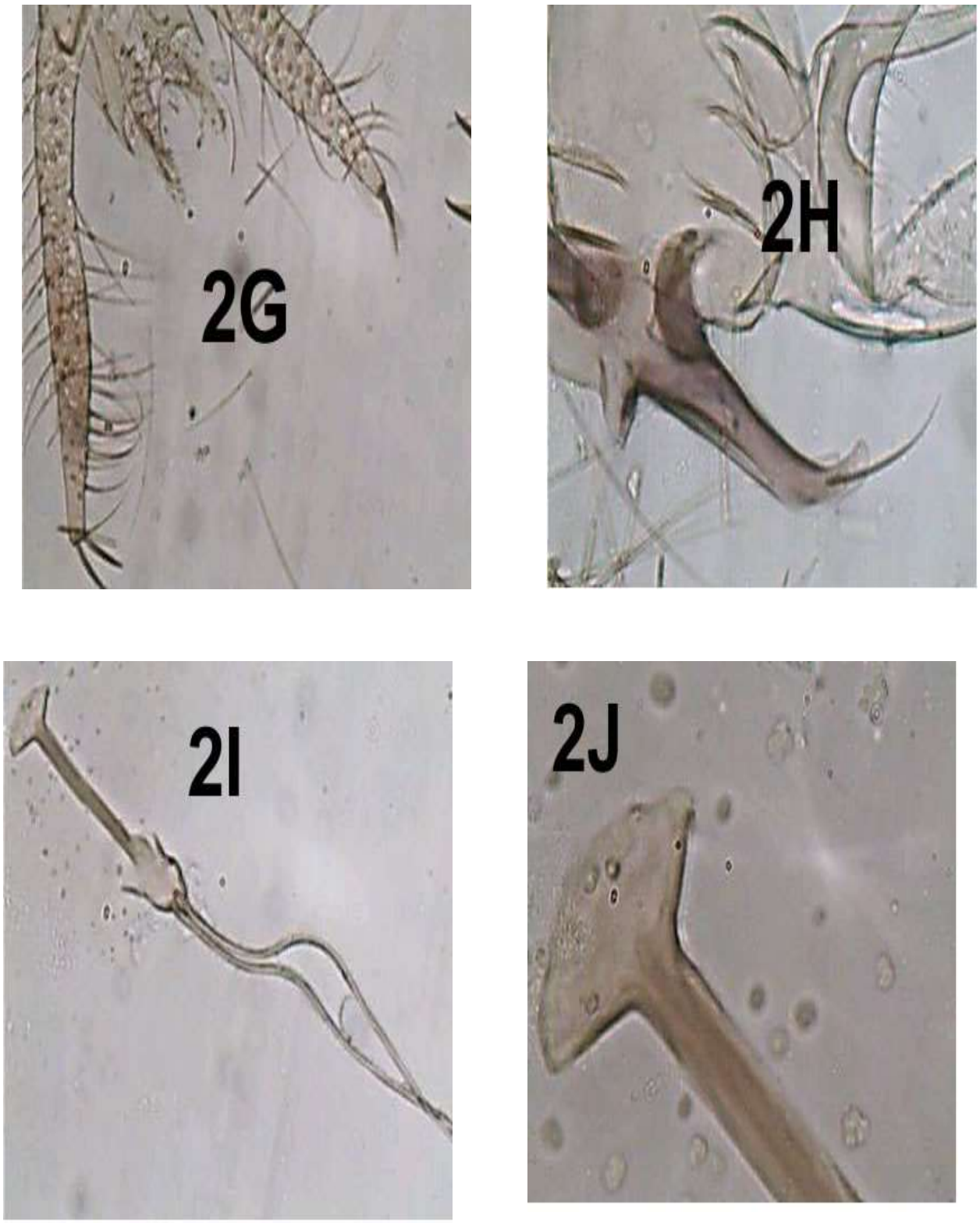


Fig.2. ♂ *Phlebotomus papatasi* (Scopoli): G, Surstyle X100; H, Aedeagus X200; I, Genital pump along with other structures, X100; J, Head of the Semi-circular funnel) of Spermathecal pump, X400.

Table 1. Taxonomic characters of ♀ *Phlebotomus papatasi* (Scopoli).

Characters	Present Study (mm)	Balochistan flies (Kakar-sulemankhel, 2004) (mm)	Lewis (1967) (mm)	S, Indian flies (Ilamgo et al., 1994)(mm)	Afghan flies Artemiev (1978) (µm)	Sudan flies (Kirk & Lewis, 1951) (mm)
Wing Length	2.10-2.20	2.0	2.01-2.57	2.10	-	2.0-2.40
Wing length / breadth	3.08-3.14	3.40-3.44	3.72-3.79	-	-	-
α/ β	1.60	0.46-0.70	1.30-1.60	1.36	-	1.30-1.80
A3 Length	0.22	0.24	0.24-0.30	0.25	-	0.24-0.30
Ascoid formula	2/3-15	2/3-15	2/3-15	2/3-15	-	2 / 3-15
A3/ Labrum	0.56	0.88	0.60-0.80	0.72	-	-
Labrum	0.39	0.27	0.30-0.40	0.33	-	-
Maxillary teeth	6 lateral 17 ventral	7 lateral 19 ventral	-	-	-	-
Cibarium	few spicules, well developed chitinous arch	few spicules, well developed chitinous arch	-	few spicules well developed chitinous arch	2 groups of vertical denticles, well developed chitinous arch	-
Pharynx length / Breadth	3.0	2.48-3.33	-	-	-	-
Spermatheca	head large & broad no distinct neck, segments decrease in size towards the duct, which open separately in to genital atrium	0.03 long, head large, segments decrease towards ducts	-	-	8-10 segmented with separate ducts	10 segments, decrease towards ducts

Table 2. Taxonomic characters of ♂ *Phlebotomus papatasi* (Scopoli).

Characters	Present Study (mm)	Balochistan flies (Kakar-sulemankhel, 2004) (mm)	Lewis (1967) (mm)	S, Indian flies (Ilamgo et al., 1994) (mm)	Afghan flies Artemiev (1978) (µm)	Sudan flies (Kirk & Lewis, 1951) (mm)
Wing Length	1.93-1.98	1.56-2.2	1.78-2.40	1.90	-	1.80-2.10
Wing length / breadth	3.5-3.6	3.81-4.0	3.80-3.95	-	-	3.75-3.96
α/ β	2.0	1.40-1.50	1.20-1.50	1.2	-	1.20-1.40
A3 Length	0.25	0.22-0.31	0.26-0.33	0.27	-	0.27-0.34
Ascoid formula	2/ 3-15	2/ 3-15	2/3-15	2 /3-15	-	2/ 3-15
A3/ Labrum	1.0	1.0	1.0-1.3	1.12	-	-
Labrum	0.25	0.20-0.24	0.22-0.28	0.24	-	-
Pharynx length / Breadth	2.86	2.55-3.63	-	-	-	0.55-0.60
Coxite length	0.55	0.53	-	0.50	-	-
Coxite / Style	1.375	1.20-1.76	-	1.20	-	-
Coxite / A3	2.2	1.70-2.40	-	1.85	-	-
Coxite / labrum	2.2	2.20-2.65	-	2.0	-	-
Style	with 5 short, stout spines	with 5 short stout spines	-	with 5 short stout spines	with 5 spines	with 5 spines
Style length	0.40	0.30-0.44	-	-	-	0.37-0.41
Aedeagus length	0.10	0.11	-	-	-	-
Paramere length	0.22	-	-	-	-	0.28-0.37
F/ P	1.77	1.70	1.6	-	1.70-2.3	-
Surstyle	each with 2 terminal spines	with 2 terminal spines	with 2 terminal spines	-	-	-

Key to the species of *Phlebotomus* (*Phlebotomus*) from Indo-Pakistan subcontinent

1. Female cibarium without teeth or pigment patch, spermatheca segmented or incompletely segmented with long ducts, male style with 3-5major spines …….....genus *Phlebotomus*

Male terminalia very long with five short spines of which 3 in apical position, coxite with long and thick setae in distal part and very small basal process, paramere with 2 long dorsal processes, surstyle with short and thick spines……..……. ...subgenus *Phlebotomus*

- Female pharyngeal armature (scaly) more hard, pigmented ant pointing backward, spermatheca narrowing to the base, distal part of paramere strongly curved up, 2nd dorsal process of paramere relatively small and widened at the end end……...*P. salehi*

- Female scaly pharyngeal armature directed obliquely down and pointing backward, one of the basal spines of the style is much closer to apical spines than to another basal spine……. *P. bergeroti*

Distribution

Present survey new record: Sindh: Jacobabad, Dilmurad, Thull, Tangwani, Zorgarh, Bakhshapur, Mirdost Ali, Garhi Khairo, Shikarpur, Garhi Yasin, Madeji, Shahdad kot, Chukhi, Quba Saeed Khan, Qambar Ali Khan, Sono Khan Chandio, Ghaibi Dero, Wagan, Warah, Junani, Tharri, Hajira, Gul Mohammad Tunio, Lalu Raunk, Lalo Lakhani, Rato Dero, Dokri, Nasirabad, Mehar, Shah Gudro, Faridabad, Khairpur Nathan Shah, Dadu, Juhi, Bhan Saeed abad, Shewan Sharif, Kotri, Thano Bhullo Khan, Hyderabad, Tandojam, Mirpurkhas, Badin, Naukot, Mithi, Islaamkot, Chachro, Umarkot, Sufi Faqir, Sindhri, Sangarh, Khadro, Nawab Shah, Moro, Nao Shehro Ferorze, Bhiria city, Kandiaro, Theri, Gambat, Kot Deji, Khairpur, Sukkur, Rohri, Ghotki, Mirpur Mathelo, Obaaro. Kandhkot, Kashmore, Shikarpur, Khairpur, Larkana, Mirpurkhas, Panu Aqil, TandoBago (Lewis,1967; present survey). Punjab: Bhawalpur, Dera Ghazi Khan, Multan, Rajanpur, Sadiqabad, Tonsa-shareef (New record, present survey). NWFP: Dera Ismail Khan (New record, present survey).

DISCUSSION

The basic taxonomic characters like most pharyngeal scaly armature not pointing backward or pointing backward, upper process of paramere not longer than paramere, upper process of paramere clothed with hairs on all sides or clothed with hairs on hind part observed in the present study are in accordance with the basic plan of *P. papatasi* (Scopoli) but with minor variations. Data of the present study are compared with the published data of this species from other territories (see Table 1 & 2). The present *P. papatasi* ♀ wing length was observed shorter than in flies from Rawalpindi (Lewis,1967) and Sudan (Kirk and Lewis,1951). Similarly, alar index was found shorter than in flies from Sudan (Kirk and Lewis, 1951), antenna 3 was noted shorter than in flies from Balochistan (Kakarsulemankhel,2004c), Rawalpindi (Lewis,1967), Southern India (Ilango *et al.*,1994), Sudan (Kirk and Lewis,1951). Labrum was found bigger than in flies from Southern India (Ilango *et al.*,1994) and Balochistan (Kakarsulemankhel, 2004c). Likewise, wing and antenna 3 in ♂ flies were found shorter than in the flies from Sudan (Kirk and Lewis (1951), Rawalpindi (Lewis,1967) and Balochistan. Labrum was observed shorter than in the flies from Rawalpindi (Lewis,1967) but larger than in the flies from Balochistan (Kakarsulemankhel,2004c) and Southern India (Ilango *et al.*,1994). Genital filaments /spermathecal pump ratio was noted shorter than in the flies from Afghanistan (Artemiev,1978) but greater than in the flies from Rawalpindi (Lewis,1967). However, other characters like ascoid formula, cibarium, spermathecae, style with 5 short and stout spines and surstyle with 2 apical spines were same.

The present work is in conformity with the findings of Lewis (1967, 1978,1982), Artemiev (1978) and Kakarsulemankhel (2004c) with some minor variations which is due to certain climatic factors (mainly humidity) (Belazzoug *et. al.*,1982).

It is hoped that present findings would provide the basis for further research on sand flies taxonomy in the country.

The wide distribution of *Phlebotomus papatasi* throughout the Old world, specially its presence in human residences in the areas of leishmaniasis and its established vectorial ability, clearly demand to initiate a comprehensive program for the control of sand flies and leishmaniasis in the country.

Evolutionary relationship

The species of sub genus *Phlebotomus* Rondani and Berte forms different sub-clades comprising sub-clades *papatasi* (Scopoli), *bergeroti* Parrot, *salehi* Mesghali and *duboscqi* Neveu-Lamaire. Typical synapomorphy, only the four species of the sub genus *Phlebotomus* have small spines. The apomorphic state only concerns the apical spatulate spines of *Phlebotomus.* The short spines on the styles and the spines on the lateral lobes are distinctive and probably apomorphic characters (Rispail and Leger,1998). The *papatasi* sub-clade is neatly held together by the synapomorphies characters like long termanilia, paramere with 2 long dorsal processes, style with 5 short spines and coxite with long and thick setae in the distal part. In the sub-clades of *papatasi* and *salehi* both are intact in having synapomorphic characters like having pharyngeal scaly teeth not pointing backward. Similarly, in their sub-clades *bergeroti* and *salehi* also tightly held together in having above mentioned characters. However, sub-clade of *bergeroti* is of particular importance and appears entirely isolated with autapomorphies in having characters like pharyngeal scaly teeth arranged obliquely and pointing backward and one of the basal spines of style is much closer to apical spines than to another basal spine. Similarly, sub-clade *salehi* is separated with its autapomorphies like upper process of paramere clothed with hairs on hind part only. *P. papatasi* sub-clade is still completely isolated within its sub genus *Sergentomyia* in its autapomorphic characters of male terminalia by having two basal spines of style much closer to each other, than to apical spines.

In its sub-clade *dubosqi* (so far not reported from this region) is probably more primitive than *bergeroti* or *salehi* (Lewis, 1982) and is isolated due to its apomorphics like upper process of paramere clother with hairs on all sides, of most pharyngeal teeth not pointing backward a character shared with *papatasi* and *salehi,* upper process of paramere covered with hairs on all sides but sharing with sub-clades *papatasi* and *salehi* in its synapomorphic characters like having most pharyngeal scaly teeth not pointing backward.

REFERENCES

Ahmad, I., Z. Humayun and M. Ahmed (2008). Patterns of cutaneous Leishmaniasis cases among troops and their families in Sibi. *Pak. Armed Forces Med. J.,* 58: 209-212.

Alexander, B. and M. Maroli (2003). Control of phlebotomine sand flies. *Med. Vet. Entomol.,* 17:1-18.

Artemiev, M. M. (1978). *Sand flies (Diptera, Psychodidae, Phlebotominae) in Afghanistan*, Kabul, 87 pp.

Ashford, R. W. (2000)**.** The Leishmaniases as emerging and re-emerging globally. *Int. J. Parasitol*.,30 :1269-1281.

Aslamkhan, K. (1996). Biodiversity of sand flies (Phlebotominae) of Pakistan. M. Sc. Thesis. Department of Zoology, Government College, Lahore, Punjab University, Pakistan, 144 pp.

Aslamkhan, K. and M. Aslamkhan (2000). Improvement in techniques of mounting adult sand flies (Phlebotominae) for identification. *Pak. J. Zool.,* 32: 181-182.

Aslamkhan, K., Aslamkhan, M. and Azizullah (1997). The distributional records of sand flies (Phlebotominae) of Pakistan and Kashmir from 1908 to 1996. *Pak. J. Zool.,* 29**:** 351-360.

Aslamkhan, K., Aslamkhan, M. and Azizullah (1998). Biodiversity of sand flies of Pakistan and Kashmir. *Pak J Zool,,* 30: 13-21.

Aslamkhan, M. and H. C. Barnett (1966). Studies on the bionomics of sandflies. *Ann. Rept. Uni. Md. Sch. Med. ICMR.* 54-65.

Aslamkhan, M. and H. C. Barnett (1967**)** Studies on sand fly fever and on the bionomics of sandflies. *Ann. Rept. Uni. Md. Sch. Med. ICMR.* 129-157.

Aslamkhan, M. and S. Rafique (1980). Studies on cutaneous leishmaniasis and sand flies of Balochistan. *Ann. Rept. Uni. Md. Sch. Med. ICMR*. 315-324.

Bari, A. U & S. B. Rahman (2008). Many faces of cutaneous leishmaniasis. *Ind.J. Dermatol. Venerol. Leprol.,* 74: 23-27.

Barnett, H. C. and W. A. Mcdonald (1964). Studies on sand fly and sandfly fever. *Ann. Rept. Uni. Md. Sch. Med. ICMR*, 54-56.

Belazzoug, S., Mahzoul, D., Addadi, K. and J. P. Dedet (1982). *Sergentomyia minuta parroti* (Adler & Theodor, 1927) en Algierie (Diptera: Psychodidae). *Annales de Parasit Hum. Comp.,* 57: 621-630

Burney, M. I. and F. A, Lari (1986)**.** Status of cutaneous leishmaniasis in Pakistan. *Pak. J. Med. Res.,* 25: 101-108.

Dedet, J.P., E.L. Gomes, R. Ferreira-Rangel, A.L. Falcao, E.A.B. Galati, H. Bermudez, M.V. Herrero, C. Ferro, D. Feliciangeli and D. Hervas (1991). Proposition of a standard description for Phlebotomine sand flies. *Parassitologia,* 33 (Suppl.): 127-135.

Desjeux, P. (2001).The increase of risk factors for Leishmaniasis world wide. *Trans. R. Soc. Trop. Med. Hyg.,* 95: 239-243.

Dujardin, J.C., S. De. Doncker, D. Jancquet, A. L. Banuls, M. Balavoine, D. Van. Bockstacle, M. Tibayrenc, J. Arevalo, and D. le Ray (2007). Clone propagation and the first generation of karyotype diversity: an in vitro *Leishmania* model. *Parasitology*, 134: 33-39.

Hashiguchi, Y., P. A. Barroso, D. J. Marco, A. M. Bhutto, F. R. Soomro, J. K. Kakarsulemankhel, *et al.* (2005). Cutaneous leishmaniasis in Sindh and Balochistan, Pakistan, with special reference to vector sand flies and causative agents. Proc. 5th. Int. Symp. on Phlebotomine sand flies. *Arch. de L' Ins.t Pasteur de Tunis* Jan-Jun, 82: 81.

Ilango, K., V. Dhanda, R. Srinivasan, A. B. Sadannand and R. P. Lane (1994). Phlebotomine sand flies (Diptera: Psychodidae) of Tamil Nadu, and Pondicherry, Southern India, in relation to visceral leishmaniasis. *Ann. Trop. Med. Parasit*., 88: 413-431.

Kakarsulemankhel, J. K. (2004a). Present situation of cutaneous leishmaniasis in Balochistan, Pakistan. *Pak. J. Bio. Sci.*,7: 698-702.

Kakarsulemankhel, J. K. (2004b). New Record of Armature in the Genital atria of female Sand flies of Pakistan to discriminate species of phlebotomine sand flies (Diptera: Psychodidae). *Pak. J. Biol. Sci.*, 7: 912-915.

Kakarsulemankhel, J.K. (2004c)**.** Re-description of species of sandflies of the subgenus *Phlebotomus* (Diptera, Psychodidae) of Balochistan, Pakistan. *Pak. J. Zool.*, 36: 143-164.

Killick-Kendrick, R., Y. Tang and M. Killick-Kendrick (1994). Phlebotomine Sand flies of Kenya (Diptera: Psychodidae). IV. The armature in the genital atrium of female *Larroussius* as a means of identification. *Ann. Trop. Med. Parasitol.* 88: 433-437.

Kirk, R. and D. J. Lewis (1951)**.** The Phlebotomineae of the Ethiopian region. *Tran. Roy. Ent. Soc. Lond.* Part 8, 383-510.

Khan, Z. (2005). Cutaneous leishmaniasis in N. W.F.P. *J. Postgrad. Med. Inst.,* 19: 226-228.

Kolachi H. B., M. Y. Dahar, S. L. Rathi and A. Khaskheli (2005). Epidemic of cutaneous leishmaniasis in Taluka Johi, District Dadu, Sindh. *Infect. Dis. J. Pak*., Apr-Jun: 37-40.

Kolaczinski, J., S. Brooker, H. Reyburn and M. Rowland (2004). Epidemiology of anthroponotic cutaneous leishmaniasis in Afghan refugee camps in north west Pakistan. *Trans. R. Soc. Trop. Med. Hyg.*, 98: 373-378.

Lewis, D. J. **(**1967). The Phlebotomine sand flies of west Pakistan (Diptera, Psychodidae). *Bull. Brit. Mus. Nat. Hist.* (Ent.): 19: 1-57.

Lewis, D. J. (1975). Functional morphology of mouth the parts in the New World phlebotomine sand flies (Diptera: Psychodidae). *Trans. R. Ent. Soc. Lond.*126: 497-532.

Lewis, D. J. (1978). The phlebotomine sand flies (Diptera: Psychodidae) of the Oriental Region. *Bull. Brit. Mus. Nat. Hist.* (Ent), 37: 217-343.

Lewis, D. J. (1982). A taxonomic review of the genus *Phlebotomus* (Diptera, Psychodidae). *Bull. Brit. Mus. Nat. Hist.* (Ent.), 45: 121-209.

Maroli, M. and C. Khoury (2004). Prevention and control of leishmaniasis vectors: current approaches. *Parassitologia*, 46: 211-215.

Nasir, A.S**.** (1958). Sandfly fauna in west Pakistan. *Pak. J. Hlth*. 8: 21-22.

Parvizi, P., I, Mauricio, A. M. Aransay, M. A. Miles, and P. D. Ready (2005). First detection of *Leishmania major* in peridomestic sandflies: comparison of nested PCR of nuclear its ribosomal DNA and semi-nested PCR of minicircle kinetoplast DNA. *Acta Trop.,* 93: 75-83.

Parvizi, P. and P. D. Ready (2006). Molecular investigation of the population differentiation of *Phlebotomus papatasi*, important vector of *Leishmania majo* in different habitats and regions of Iran. *Iranian Biomed. J.*, 10: 69-77.

Pesson, B., G. Mandulo-Leblond, M. Killick-Kendrick, Y. Tang and R. killick-Kendrick (1994). The armature in the genital atrium as a new taxonomic character distinguishing females of *Phlebotomus papatasi* and *P. duboscqi* (Diptera: Psychodidae). *Ann. Trop. Med. Parasitol.* 88: 539-542.

Qutubbudin, M. (1951). The sand fly fauna of Kohat valley, NWFP, Pakistan. *Pak J. Helth.*1: 34-36.

Rab, M. A., F. H. Azmi, J. Iqbal, J. Hamid, A. Ghafoor, M. I. Burney, and M. A. S. Rashti (1986). Cutaneous leishmaniasis in Balochistan: Reservoir hosts and sand fly vectors in Uthal-Lasbella. *J. Pak. Med. Assoc.,* 36: 134-138.

Rispail, P. and N. Leger (1998). Numerical Taxonomy of old World Phlebotominae (Diptera: Psychodidae). 1. Consideration of Morphological characters in the genus *Phlebotomus* Rondani & Berte 1840. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro*, 93: 773-785.

Rowland, M., A. Munir, N. Durrani, H. Noyes and H. Reyburn (1999). An out break of cutaneous leishmaniasis in an Afghan refugee settlement in north-west Pakistan. *Trans. R. Soc. Trop. Med. Hyg.*, 93: 133-136.

Safi, F. S. (1993). A contribution to the sand fly fauna of Peshawar. M.Sc. Thesis, Government College, Lahore, Punjab University, Pakistan, 61 pp.

Secundino, N. F. C., I. Eger-Mangrich, E. M. Braga, M. M. Santoro and P.F.P. Pimenta (2005). *Lutzomyia longipalpis* Peritrophic Matrix: Formation, structure and chemical composition. *J. Med. Entomol.,* 42: 928-938.

Valenta, D. T., N. Anez, Y. Tang and R. Killick-Kendrick (1999). The genital atrium as a good taxonomic character to distinguish between species of phlebotomine sand flies (Diptera: Psychodidae) from Venezuela. *Ann. Trop. Med. Parasitol.* 93: 389-399.

Wakil, A., F. M. Bilqees, and A. Salim (2006). Cutaneous leishmaniasis in Dadu district during 2001- 2004. *Proc. Parasitol*., 41: 19-39.

Yagoobi-Ershhadi, M. R., E. Javadian, and G. H. Tahvildare-Bidruni (1995). *Leishmania major* MON-26 isolated from naturally infected *Phlebotomus papatasi* (Diptera: Psychodidae) in Isfahan Province, Iran. *Acta Trop.*, 93: 75-83.

Young, D. G. and M. A. Duncan (1994). Guide to the Identification and Geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). *Mem. Am. Entomol. Inst.,* 54. Associated Publishers, 881 pp.

(*Accepted for publication September 2008*)