

Original article

Taxonomic studies of *Phlebotomus sergenti* (parrot) (dip- tera: psychodidae) and its evolutionary relationship with its closest allies

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In the survey, the work was done to develop taxonomic record of sand fly *Phlebotomus* (*paaphlebotomus*) *sergenti* (Parrot) collected for the first time from new epidemic localities of cutaneous leishmaniasis in Dera Ghazi Khan (Pakistan). In view of the published reports about the detection of *Leishmania major*, the causative agent of cutaneous leishmaniasis from this species in many 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. sergenti* (parrot) is studied in details with special reference to its mouth parts, male and female genitalia. A key is also given to *P. sergentii* (parrot) and its closest allies. In this light its relationships with its closest allies is also briefly discussed.

Keywords: Sandflies, Pakistan,**INTRODUCTION**

Only the female of the sand flies are blood feeders and are of medically importance as vectors of many zoonotic diseases (arboviruses, bartonellosis and especially leishmaniasis, and sand fly fever)^[1] and also of several pathogens constituting serious health problems^[2].

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^[3,28].

Leishmaniasis in their various forms appear to be emerging globally^[4,27].

The resurgence of cutaneous and visceral leishmaniasis in many countries has once again highlighted the importance of sand flies as vectors of human diseases. Pakistan has several endemic foci of leishmaniasis and the disease is spreading continuously and sand flies are being recorded from new localities.

Previous studies of the sand fly fauna of Pakistan have been

fragmentary. No comprehensive taxonomic work exists in facilitating the identification of Pakistani sand fly species.

The morphology and diagnostic characters of Pakistani *P. sergenti* (Parrot) has not been thoroughly investigated. *P. sergenti* (Parrot) was originally collected from Constantine locality in Algeria by Parrot^[5]. In Pak-Indian sub continent, this fly was first reported from Dera Ismail Khan by Newstead and Sinton^[6] and from Quetta by Sinton^[7].

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Alger, 18:310,

Phlebotomus (*Paraphlebotomus*) *sergenti* Parrot, Theodor, 1948, Bull. ent.

Res. 39: 97,

Lewis^[8] collected it from some parts of Pakistan and gave a brief note "the cibarium of both sexes is very like that of *P. papatasi* (Scopoli) but the teeth are smaller and less scattered. The labral crest of male is like that of *P. alexandri*." Aslamkhan and Rafiq^[9], Burney and Lari^[10], Aslamkhan et al^[11,12]. did not provide the diagnostic characters of this fly. Phylogenetic relationship of this fly with its other closest allies was also not discussed. Therefore, in view of insufficient information about its diagnostic characters, presently taxonomic characters of *P. sergenti* (Parrot) collected from Dera Ghazi Khan (Punjab) and especially its mouth parts, male and female genitalia are studied and is keyed out from its closest allies i. e. *P. alexandri*^[13] and *P. nuri*^[14]. The evolutionary relationship of this species is also briefly discussed.

MATERIALS AND METHODS

The present investigation was carried out on the materials (18 specimens of *Phlebotomus sergenti*) collected from human residences, colleges, schools, hotels buildings of Dera Ghazi Khan (Pakistan) during May, 2006 in day and night with sucking tubes and sticky traps. The collected materials was preserved, processed and dissected by conventional methods^[15,16]. Identification of specimen was carried out with the help of available literature^[8,14]. Morphometric 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 CIPA Group^[17]. Prepared permanent slides were deposited with the author's collection of sandflies, Department of Zoology, University of Balochistan, Quetta.

RESULTS

Phlebotomus (*Phlebotomus*) *sergenti* Parrot^[5,7,8]
Phlebotomus (*Phlebotomus*) *sergenti*, Parrot, 1940,
 Arch. Inst. Pasteur,

Material examined : ♀ 11.

Wing (X100) 2.50 long, length/ breadth 3.20. $\alpha/\beta = 1.1$, $\delta = 0.01$, $\Pi = 0.01$, $\gamma = 0.45$. Length of Palpomeres (X100): 0.95 long, relative length: 1,3,4,3,8 formula 1,2-4,3,5, segment p-V is quite longer than all the others, p-IV and p-II are same size, p-III greater than p-IV. Newstead's spines implanted closely on p-III. Lengths of antennal segments: AIII-0.30, AIV and AV each 0.13 long. Two antennal ascoids on AIII-AXV, on AIII, it is at 0.73, on AIV and AV, each at 0.38. Ascoid at A3 0.06 long and on A4 0.03 long. AIII > A-IV + A-V.

Mouth parts: (X100)

Proboscis 0.35 long, Labrum projecting beyond the clypeus is a strong chitinized structure, shaped like the blade of a dagger, 0.33 long, sides parallel, apex bluntly pointed and margins furnished with a series of long leaf like sensillae closely together and numbering about 9 on either side, Hypopharynx 0.33 long, tapers off much more towards the apex with marginal leaf like sensillae much shorter and placed so closely together as to present a finely serrated edge, its apical part is broadly concave and in its center a salivary ducts runs. Mandible (Fig. 1A) 0.34, long, 0.02

broad, blade like outer edges markedly serrated, tips of the mandibles extend further than those of maxillae, blades of mandibles are about as broad as hypopharynx, dental depth 0.09. Maxilla (Fig. 1B) 0.32 long, much narrower than mandibles, stout basally but narrows very much towards its apex, one edge is provided with six lateral teeth (2 large and prominent) widely separated and the opposite edge with minute 16 ventral teeth set closely together. Cibarium (Fig. 1C) a strong chitinized tube like structure, the inner cavity 0.06 broad and 0.04 in height, chitinous arch present, minute spicules present at sides. Pharynx, backward continuation of buccal cavity, 0.24 long, anterior breadth 0.04 and

posterior breadth 0.08, anterior edge of armature forms an almost straight line, whereas the posterior part composed of broad and leaves like spicules. Pharynx 0.20 long and is 2.5 times as long as broad, posterior end is twice as wide as anterior.

Height of armature 0.04, anterior edge of armature forms an almost straight line whereas posterior part of armature composed of several broad and long leaves like spicules (Fig. 1D) with basal part consisting of straight transverse edges with minute teeth on it.

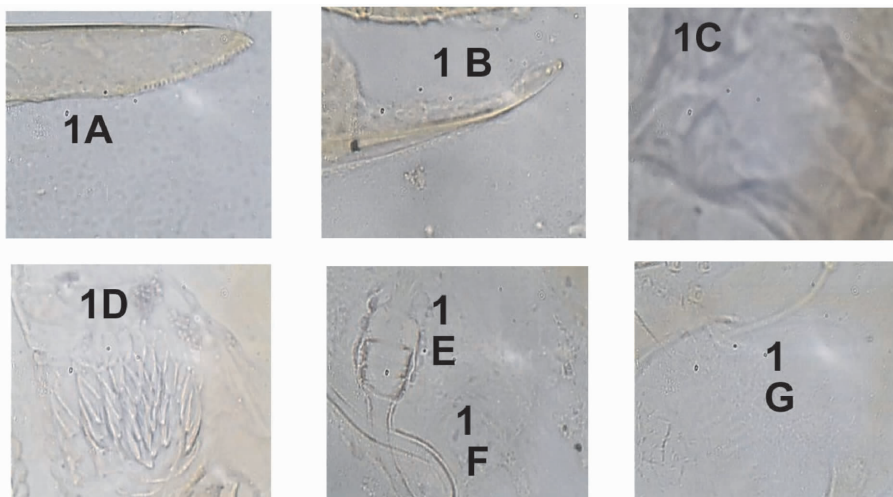


Fig. 1. ♀ *Phlebotomus sergenti* (Parrot): A, Mandible X400; B, Maxilla X400; C, Cibarium X400; D, Pharyngeal armature X400; E, Spermatheca X400; F, ducts with striations X200; G, armature in genital atrium X400.

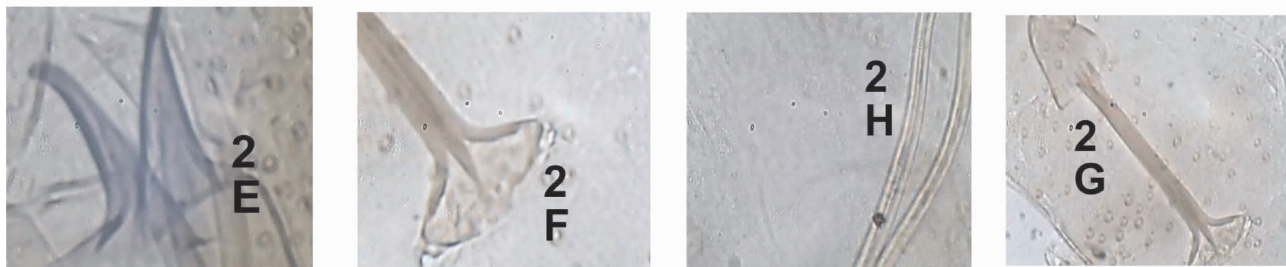
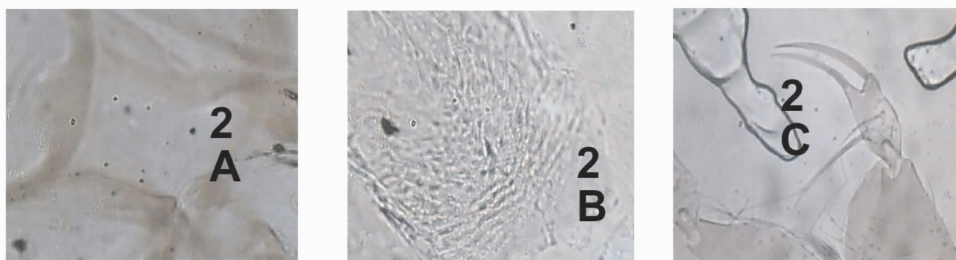


Fig. 2. ♂ *Phlebotomus sergenti* (Parrot): A, Cibarium X400; B, Pharyngeal armature X400; C, Style X100; D, Basal process with hair like brush, Pharynx X200; E, Aedeagus X400; F, plunger of the sperm-pump X400; G, body of the sperm-pump X200; H, genital filaments with weak striations X400.

Female genitalia X100

Spermatheca (Fig. 1E) 4-5 segmented, 0.03 long, 0.01 broad, apical segment global, hair-like tubules 0.01 long at the apical segment, spermathecal ducts 0.20 long, with transverse striations (Fig. 1F) and

with separate openings in to genital atrium which is 0.14 broad, distal portion of ducts becomes swollen like asymmetrical bell with shrunken edges while opening in to genital atrium which has a patch of armature in the center (Fig. 1G). Furca 0.15 long,

Material examined : ♂ 6

Wing 2.50 long, 0.78 broad, $\alpha/\beta = 1.33$, $\delta = 0.01$, $\gamma = 0.435$, $\Pi = 0.01$. Palps 0.65 long, relative length, 1, 2.5, 4, 2.5, 6.25, formula, 1, 2-4, 3, 5. Antennal segment 3, 0.30 long, A4 and A5 each, 0.14 long,
 - 7 - AIII > A-IV + A-V. Ascoid on A3 0.07 long, at 0.68 and on A4 0.06 long and at 0.23. Ascoid formula 2/3-15.

Mouth parts X100

Proboscis 0.26 long. Labrum 0.23 long. Hypopharynx 0.24 long, Mandibles absent. Maxilla 0.23 long, a sword like structure without teeth. Cibarial anterior cavity 0.04 broad, without teeth (Fig. 2A), chitinous arch developed. Pharynx 0.21 long, posterior breadth more than twice fore-breadth, height of armature 0.05, basal consisting of a series of transverse ridges with serration whereas anterior slightly elongated tapering to apex, lateral armature mostly directing towards center of pharynx (Fig. 2B).

Male genitalia X100

Coxite 0.25 long, a basal process (0.06 long) with small rounded head consisting of about 15 long thick-yellow pigmented hairs (0.07 long) directing below (Fig. 2D) is a characteristic feature of this species, a little difference in breadth of head of basal process and its neck. Style (Fig. 2C) short in size (0.1 long) and 0.05 broad, style with 2 apical spines (0.13 long) slightly curved and situated on small tubercles of equal length and thickness. Two spines present almost middle of the body of style, one of them short (0.08 long), thin and straight while the other one 0.12 long, situated near the basal border of style and slightly curved and thinner than the terminal spines, but relatively thicker than the short-thin median spine. Aedeagus (Fig. 2E) 0.05 long, darkly pigmented with slightly curved apex. Plunger of the sperm-pump (Fig. 2F) peculiar in shape, body of the sperm-pump (Fig. 2G) 0.15 long, genital filaments (F) with less conspicuous transverse striations (Fig. 2H) about 1.3 times this length. Surstyle 0.24 long.

Key to the species of the sub genus *Paraphlebotomus*

Female without cibarial armature or pigment patch, male with four or

five spines on style; all abdominal sclerites with erect hairs genus *Phlebotomus*

The sub basal process of coxite is medium sized to large and bears longhairs and style with 4 spines sub genus *Paraphlebotomus*

♂ flies with thickened basal lobe of the coxite thickened, antenna 3 short and thick, plunger of sperm pump not much wider than barrel, barrel not much longer than wide, basal coxite tuft short and broad,

♀ pharynx triangular with sides and posterior margin almost straight, armature with large scale like plates, A3 shorter than labrum ... alexandri

♂ flies has basal process of coxite long & thick, with very long hairs, terminalia longer than in other *Paraphlebotomus*, aedeagus long conical, straight,

♀ pharynx bottle shaped, spermatheca 7-8 segmented, with narrow apical segments

..... .. *P. nuri*

♀ pharynx slender, with sides straight or slightly concave after posterior bulge, hind margin convex, rows of minute teeth on posterior scales, A3 slightly shorter than labrum, spermatheca a single capsule,

♂ pharynx narrowing after posterior bulge, A3 longer than labrum, paramere long and slender, barrel of sperm pump longer than wide, surstyle longer than coxite *P. kazeruni*

Distribution : Present study, new record, Dera Ghazi Khan (Punjab-Pakistan). *P. sergenti* (Parrot) in Pak-Indian subcontinent seemed to be confined to the plains of north, central and western region^[8,13,18-22].

DISCUSSION

Diagnostic structures of *P. sergenti* (Parrot) were compared with the published data of this species from other territories (Table 1 & 2). Wing length of ♀ flies of the present study were found shorter from central Asian flies^[23], larger from Balochistan specimens^[20] and similar from African flies^[24]. Antenna

3 was observed larger from Balochistan flies^[20] and similar from central Asian^[23] and African flies^[21]. Egyptian flies have 6-7 spermathecal segments more

than of present specimens, however, spermatheca of Punjab flies have 4-5 segments as compared with 3 segments in central Asian flies^[23].

Table 1. Taxonomic characters of ♀ *Phlebotomus sergenti* (Parrot)

Characters	Present Study (mm)	Balochistan flies (Kakar- sulemankhel, 2004) (mm)	Transcaucasia & Central Asia Perfiliev, 1968 (mm)	Egypt (Lane, 1986) (mm)	Africa (Kirk & Lewis, 1951) (mm)
Wing Length	2.50	2.28	2.5-2.7	-	2.5
Wing length / breadth	3.20	3.32	3.1	-	3.1
α/β	1.1	1.15-1.29	-	-	1.0
A3 Length	0.30	0.28	0.27-0.31	-	0.27-0.30
Ascoid formula	2/3-15	2/3-15	2/3--15	-	2/3-15
A3/ A4 + A5	AIII > A-IV + A-V.	AIII > A-IV + A-V.	AIII > A-IV + A-V.	-	-
A3/ Labrum	0.90	1.0	-	-	-
Labrum	0.33	0.28	0.32-0.45	-	-
Maxillary teeth	6 lateral 16 ventral	7 lateral 16 ventral	- -	- -	- -
Cibarium	6 spicules near side walls, 4 minute chi- tinous dots in cen- ter, chitinous arch well developed chi- tinous arch	spicules at side walls & with minute dots, chitinous arch devel- oped,	-	-	-
Pharynx length / breadth	2.5	1.31-1.42	-	triangular, with sides & posterior margins straight	-
Spermatheca	4-5 segmented globe- like apical segment,	4-5 segmented head globe like,	3 segmented, apical segment large,	6-7 segments	4-6 segments
ducts	with separate open- ings & striations	with separate open- ings & striations	-	-	-
genital atrium	with armature	-	-	-	-

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Table 2. Taxonomic characters of ♂ *Phlebotomus sergenti* (Parrot)

Characters	Present Study (mm)	Balochistan flies (Kakar-sulemankhel, 2004) (mm)	Transcaucasia & Central Asia (Perfiliev, 1968) (mm)	Egypt (Lane, 1986) (mm)	Africa (Kirk & Lewis, 1951) (mm)
Wing Length	2.50	1.84-2.7	1.8-2.2	-	1.9
Wing length/breadth	3.20	3.3-3.6	3.4-4.0	-	3.58
α/β	1.33	1.0	-	-	1.23
A3 Length	0.30	0.25-0.31	0.22-0.36	long & slender	0.25
Ascoïd formula	2/ 3-15	2/ 3-15	2/3-15	2 /3-15	2/3-15
A3/ Labrum	1.30	1.23	-	longer than labrum	-
Labrum	0.23	0.20-0.25	0.24-0.32	-	-
Pharynx length /breadth	2.62	3.14-3.3	-	-	-
Coxite length	0.25	0.22-0.29	0.23-0.25	slender & longer	0.25
Coxite / Style	2.5	2.4-2.9	2.09-2.2	-	2.27
Coxite / A3	0.83	0.88-0.93	0.80-0.92	-	1.0
Coxite / labrum	1.08	1.1	-	-	-
Style	with 2 terminal & 2 median Spines	with 2 apical & 2 central spines	2 apical & 2 median spines	2 terminal & 2 central spines	2 apical & 2 sub-median spines
Style length	0.1	0.09-0.10	0.11	less than half length of coxite	0.11
Aedeagus length 0.05	0.063	0.08	-	-	0.06
F/ P	1.3	1.07	-	-	-
Surstyle length	0.24	0.25	0.26-0.28	-	0.13

Likewise, ♂ flies of D. G. Khan were observed having shorter wing length than of African^[24] and central Asian flies^[23]. Similarly, labrum, coxite, aedeagus and surstyle were measured shorter than of central Asian flies^[23].

The present work is in conformity with the findings of Lewis^[14], Lane^[20] and Kakarsulemankhel^[25]. However, some minor variations in the measurements of taxonomic characters were noted which were due to certain climatic factors (mainly humidity)^[25].

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

in the country. Further study on this fly is only possible with its correct identification among its allies, which was the main object of the present study.

Keeping in view of its wide distribution throughout the Old world, especially its presence in human residences in the areas of cutaneous leishmaniasis and its established vectorial ability, clearly demands to initiate a comprehensive program for the control of sand flies and leishmaniasis.

Phylogenetic relationship

Sand flies placed in the sub genus *Paraphlebotomus*

(Theodor) have their separate clades of evolution. Sergenti, alexandri, nuri groups appears to be sister groups for sharing their synapomorphies of cylindrical spermatheca with 4-9 segments. Similarly, caucasicus and sergenti shares the synapomorphies of 4-5 segmented spermatheca with global apical segment. Likewise, alexandri and nuri obviously are sister groups for both share the apomorphies of 6-9 segmented spermatheca with rather narrow apical segment. P. kazeruni, P. sergenti, P. alexandri, P. nuri appears unique with aut-apomorphy of nearly rounded spermatheca with 2 segments, pharynx with uniform blunt teeth, directed obliquely down, to the center, pharyngeal armature occupies only base of the pharynx and it is almost rectangular in shape, and pharyngeal armature occupies most of the broad part of the pharynx respectively. These four species, however, are clearly separated on the basis of their clear cut autapomorphies as mentioned above.

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