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IDENTIFICATION AND NEW DISTRIBUTIONAL RECORDS OF *GRASSOMYIA DREYFUSSI TURKESTANICA* (THEODOR & MESGHALI,1964) AND ITS PHYLOGENETIC RELATIONSHIP WITH ITS CLOSEST ALLIES

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ABSTRACT

The work was done to develop taxonomic record of sand flies *Grassomyia dreyfussi turkestanica* (Theodor & Mesghali) collected for the first time from endemic places of Sindh and Punjab provinces. In view of the published reports about the detection of encephalitis viruses from the species of the genus *Sergentomyia* (Franca and Theodor) from the Indian localities and their possible role in kala-azar transmission, the correct identification of sand fly species becomes of significant value in the study of epidemiology of leishmaniasis and other viral diseases. Therefore, in order to facilitate Zoologists and Medical researchers in correct identification of sand fly, morphology of taxonomic characters especially wings, cibarium, mouth parts, male and female genitalia of *Grassomyia dreyfussi turkestanica* (Theodor & Mesghali) is studied and results are presented. A key is also given to this species and its closest allies of the genus *Grassomyia*. In this light, its phylogenetic relationship with its closest allies is also briefly discussed.

Key words: Sandflies, Pakistan, Sindh, Punjab provinces

INTRODUCTION

Leishmaniases in their various forms appear to be emerging globally (Ashford, 2000; Desjeux, 2001). Phlebotomine sand flies (Diptera: Psychodidae) transmit many zoonotic diseases (arboviruses, bartonelloses and especially leishmaniasis) of importance of human health in at least 80 countries (Alexandar and Maroli, 2003). Among diseases transmitted by sand flies, leishmaniasis is very important, caused by infection by protozoa of the genus *Leishmania* (Garcia-Almagro, 2005).

Pakistan has several endemic foci of leishmaniasis and the disease is spreading continuously and sand flies are being recorded from new localities. Sand flies of one of the important subgenus *Sergentomyia* are quite prevalent in the country.

Previously, several viruses have been found in sand flies (Lewis, 1978). In view of the recently published reports about the detection of encephalitis viruses from the species of the genus *Sergentomyia* (Franca and Theodor) from the Indian localities and their possible role in kala-azar transmission (Geevarghese *et al.*,2004), the correct identification of the species becomes of significant value in the study of epidemiology of leishmaniasis and other viral diseases. Identification of sand flies is difficult and bit confusing. 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 flies. 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.

Sergentomyia dreyfussi turkestanica was first described by Theodor and Mesghali (1964) from Turkestan and Iran Shaher (Iranian Balochistan). Perfiliev (1968) recorded *S. squamipleuris karkalensis* from

Turkmenistan and suggested that it was apparently identical to *S. dreyfussi turkestanica* (Theodor and Mesghali). Later this species was recorded from Afghanistan by Artemiev (1978). However, these authors did not describe nor sketch mouth parts, aedeagus, paramere, spermathecal pump, genital filament and genital furca.

Therefore, in view of insufficient study, *Grassomyia dreyfussi turkestanica* (Theodor & Mesghali) collected from Sindh and Punjab provinces, is not only studied in detail with reference to its mouth parts and genitalia but is keyed out from the other known species of genus *Grassomyia* (Theodor) from this region. Its evolutionary relationship within the genus is also briefly discussed.

MATERIALS AND METHODS

The present investigation was carried out on the materials (25 specimens of *Grassomyia dreyfussi turkestanica*) collected from Sindh and Punjab Provinces 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). 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 (1993). Prepared permanent slides were deposited with the author's collection of sandflies, Department of Zoology, University of Balochistan, Quetta.

RESULTS

Grassomyia dreyfussi turkestanica (Theodor & Mesghali)

Sergentomyia squamipleuris karkalensis, Perfiliev, 1968, Israeli Program for Translation;

S. (*Grassomyia*) *dreyfussi turkestanica,* Artemiev, 1978, Kabul, Afghanistan; Lewis, 1987, 12: 165 (raised to genus)

Material examined : ♀ 12

Anterior femora with spines (Fig.1A). Wings (X100) 1.28-1.50 long, length/breadth 3.7, α/β=0.89, δ=0.15, Π=0.22, gamma 0.35. Palps
(X100) 0.56 long, ratio 1,2,4,4,8 and formula 1,2, 3-4, 5. Antennal segment 3 (X100) 0.13 long, A4 and A5 each 0.08 long, ascoid at A4 0.33 of the segment. Ascoid formula 1/4-15.

Mouth parts

Labium long, thick and consists of mentum and 2 segmented labellum with basal and apical segment, the overall length of this composite structure also known as Proboscis 0.17 long. Labrum relatively narrow and the sides parallel, apex bluntly pointed with 2 small apical sensillae and margins furnished with a series of leaf-like sensillae closely together and numbering about 10 on either side, labrum 0.16 long. Each mandible
is broad, 0.14 long, blade like, outer edges of dorsal surface of mandible is strongly chitinized, forming the actual blade which is markedly serrated. Hypopharynx 0.16 long, tapers off much more gradually toward the apex and the lateral leaf like sensillae are much shorter and placed so closely together as to present a finally serrated edge, its apical part in broadly concave and in its center salivary duct runs. Maxilla 0.15 long, much narrower than mandibles, stout basally, but narrows very much towards its apex and has two rows of weak and hardly visible teeth. Cibarium (Fig.1A) a strong chitinized tube, 0.07 broad, weak chitinous arch present, but some time absent, lateral walls of cibarium with triangular inward projections, pigment patch dark, 0.06 long and 0.01 broad, slightly concave with a short anterior process, 35-45 horizontal teeth arranged in a convex row at the base of teeth, a row of about 30-32 rounded chitinous denticles present. Posterior to the cibarium, lies a large chitinous structure-Pharynx (Fig.1B), large flask-shaped, (Fig.1B) 0.16 long, length is 2.28 times its greatest breadth, a patch of coarse pigmented teeth present at the base, completely touching the side walls of pharynx (Fig.1C), patch 0.06 long and 0.05 broad.

Female genitalia

Spermatheca (Fig.1D) (X400) with a characteristic morphology, not-egg shaped, 0.80 long, fore width (0.60) and basal width (0.56) almost equal, central breadth of capsule 0.64, central and distal parts of capsule consist of irregular rows of minute chitinous scales, apical part of spermathecal capsule with short hair-like brush of tubules (0.2 broad, 0.16 long) Furca (X400) 1.4 long.

Material examined : ♂ 1 4

Femur 1 with short spines. Wings (X100) 1.25-1.28 long, length/ breadth 5.0-5.12. α/β=0.51, δ=0.05, Π=0.22, gamma 0.25. Palps (X100) 0.52 long, ratio 1,3,5,6,11, formula 1,2,3,4,5. Antennal segment 3, 0.12 long, A4 and A5 each 0.07 long, ascoid at A4, 0.33, ascoid formula 1/4-15.

Mouth parts (X100)

Proboscis 0.16 long. Labrum 0.14 long. Mandibles absent. Hypopharynx 0.014 long. Maxilla 0.13 long. Cibarium (Fig.2A) 0.05 broad, chitinous arch developed at lateral margins but ill developed at center, anterior
process absent, about 23 small teeth on an almost straight line and above teeth a small pigment patch is present. Pharynx (Fig.2B) 0.12 long, length/ breadth 3.0, weak armature at the base.

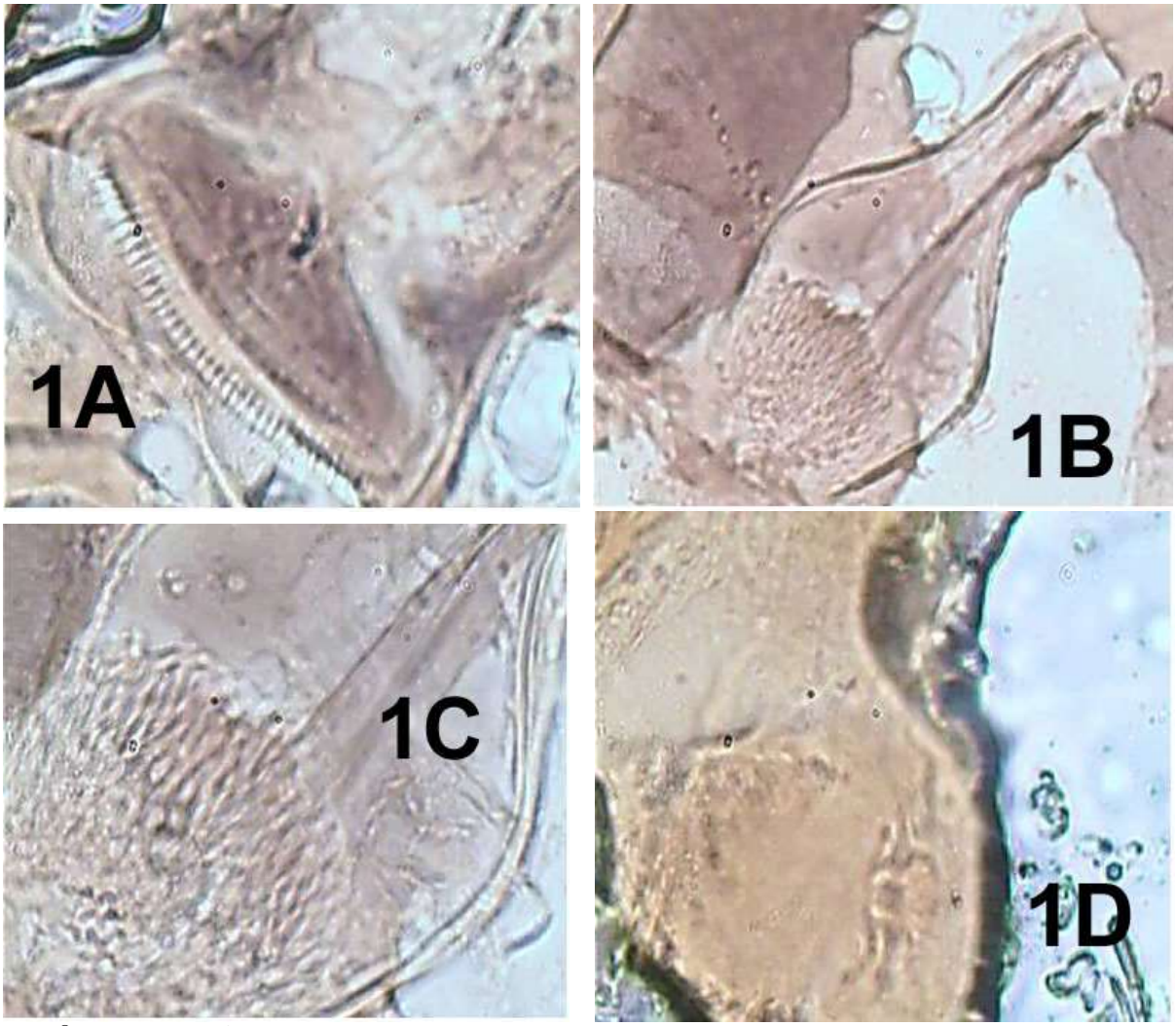

Fig.1. ♀ *Grassomyia dreyfussi turkestanica* (Theodor & Mesghali): A, Cibarium X400; B, Pharynx, whole mount X200; C, Pharyngeal armature X400; D, Spermatheca X400.

Table 1. Taxonomic characters of ♀ *Grassomyia dreyfussi turkestanica* (Theodor & Mesghali)

Characters	Present Study (mm)	Afghan flies Artemiev (1978) (μm)	Iranian flies Theodor & Mesghali (1964) (mm)
Wing Length	1.28-1.50	-	-
Wing length / breadth	3.7	-	-
α/ β	0.89	-	-
A3 Length	0.13	172-192	0.20-0.22
Ascoid formula	1/ 4-5	1/4-15	-
A3/ Labrum	0.81	1.1-1.4	-
Labrum	0.16	-	-
Maxillary teeth	hardly visible	-	-
Cibarial teeth,	35-45	37-44	40
Pharynx length / Breadth	2.0-2.28	-	-
Spermatheca	large, covered With spines	large, covered with spines	large, covered with spines

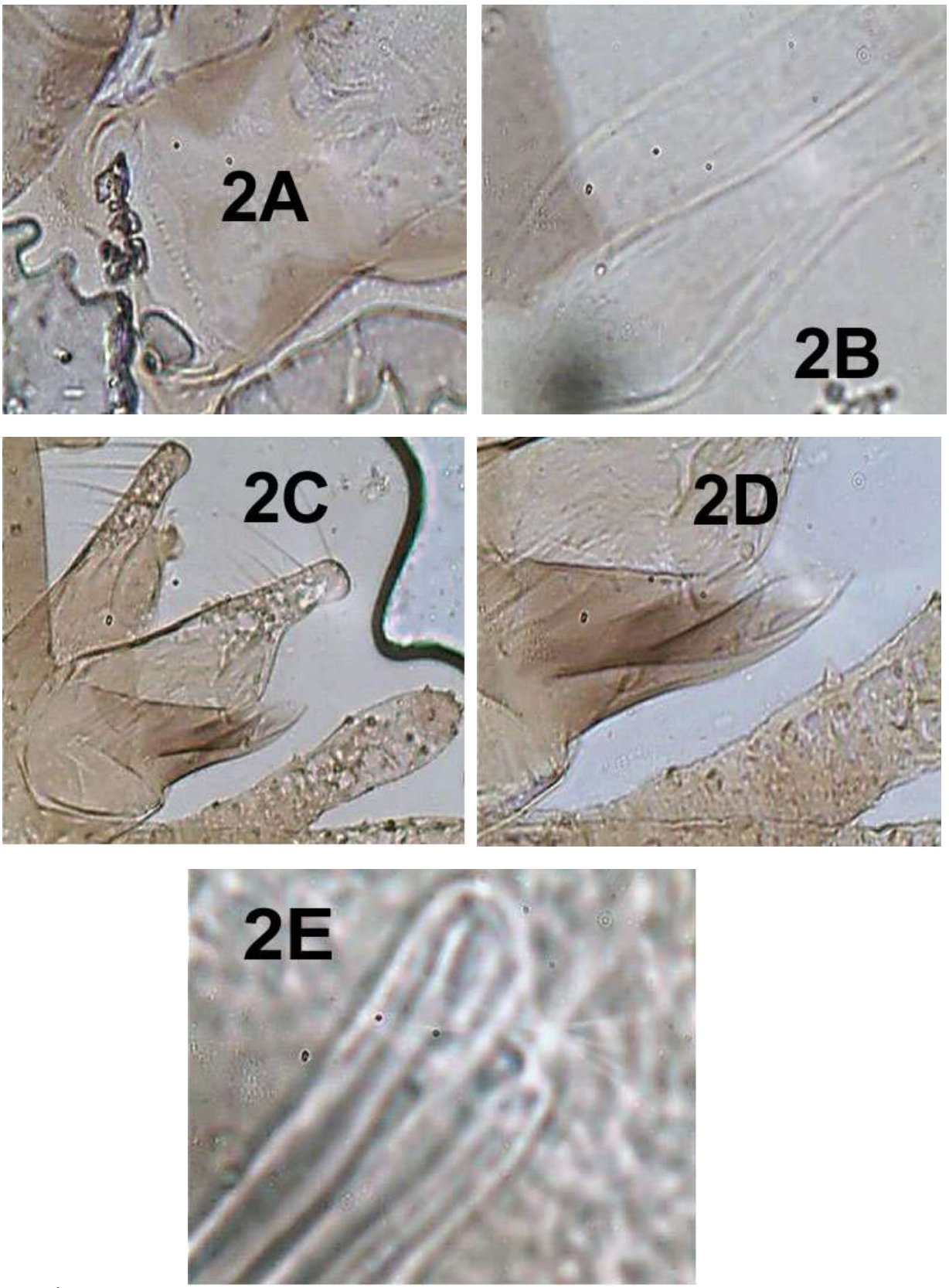

Fig.2. ♂ *Grassomyia dreyfussi turkestanica* (Theodor & Mesghali): A, Cibarium X400; B, Pharynx X400; C, Male Terminalia X200; D, Aedeagus, X400; E, Genital filaments with dilated ends X1000.

Male terminalia (X100) (Fig.2C)

Coxite 0.22 long, 0.06 broad. Style 0.08 long, 0.02 broad, with two terminal and 2 sub-terminal spines. Paramere 0.13 long, 0.04 broad, with thick blunt end. Surstyle 0.14 long and its head far projecting forward than of Paramere. Aedeagus (Fig.2D) 0.08 long, 0.01 broad, knife shaped with apex curved-up. Genital filaments with broad ends (Fig.2E), filaments with transverse striations. Spermathecal pump (P) 0.12 long. F/P=3.0.

Table 2. Taxonomic characters of ♂ *Grassomyia dreyfussy turkestanica* (Theodor & Mesghali)

Characters	Present Study (mm)	Afghanistan flies Artemiev 1978) (μm)
Anterior femora	with spines	with spines
Wing Length	1.25-1.28	-
Wing length / breadth	5.0-5.12	-
α/ β	0.51	-
A3 Length	0.12	176-230
Ascoid formula	1/ 4-15	1/ 4-15
A3/ Labrum	0.86	1.29-1.50
Labrum	0.14	-
Cibarial teeth,	23	18-27
Pharynx length / breadth	3.0	-
Coxite length	0.22	180-210
Coxite / A3	1.83	0.91-1.02
Coxite / labrum	1.57	-
Style length	0.08	85-100
Aedeagus	0.08	-
Paramere	0.13, blunt ends	blunt, hooked ends
F/ P	3.0	2.97-3.71
Filaments	with dilated ends	with dilated ends

Distribution : Present study, new record. Punjab: Dera Ghazi Khan, Multan. Sindh: Dadu, Sehwan Sharif.

Keys to the species of the genus Grassomyia

1. Convex cibarial tooth row and rounded spermatheca in the female and genital filaments with extended tips

- Anterior femora without spines and chitinous arch absent in both the sexes, spermatheca egg-shaped with transverse striations, paramere with rounded ends…… ……*S. indica*

- Anterior femora with short spines, chitinous arch well developed, spermatheca with short-cylindrical and covered with dense yellow spicules, paramere with hooked end …… *S. dreyfussi turkestanica*

DISCUSSION

These are small and moderately dark sandflies. Theodor (1958) placed them in the sub genus *Grassomyia.* Previously, *Grassomyia* (Theodor) was considered a subgenus of genus *Sergentomyia* but Lewis (1987) raised *Grassomyia* to a genus level. Present specimens show presence of a few but very weak maxillary ventral teeth and in some samples apparent absence of maxillary teeth. Probably, this feature as well a wide variability in the number of cibarial teeth, both has led to Lewis (1987) giving this taxon genus.

Previously specimens found in Turkestan and Iran have been considered as *S. indica* Theodor,1931 by Lewis *et al.* (1961), but Theodor and Mesghali (1964) considered them very close to *S. dreyfussi* Parrot,1933. The *turkestanica* specimens from Iran and Turkestan were having a relatively long 3rd antennal segment (0.20-0.22)

where as a short A3 (0.12-0.17) was present in *S. squamipleuris* Newstead, 1912 and *S. indica* Theodor, 1931. *S. dreyfussi* Parrot, 1933 was having 8-9 spines on the anterior femora but Iranian and Turkestan specimens have 3-4 such spines. However, ♀ *turkestanica* specimens have 40 teeth in cibarium as against 4-46 in *S. dreyfussi* Parrot,1933 and only 33-36 in *S. indica* Theodor, 1931. Spermathecae of *turkestanica* were large and covered with spines as in *S. dreyfussi* Parrot, 1933. In the light of these characters, Theodor and Mesghali (1964) observed that specimens from Iran and Turkestan resemble *S. dreyfussi* Parrot, 1933 more closely than any of the other species of the subgenus (*S. squamipleuris, S. dreyfussi,S. indica, S. inermis* Theodor,1938). Therefore, they considered specimens from Iran and Turkestan as a subspecies and the name *S. dreyfussi turkestanica* was proposed. *S. dreyfussi* was known only from N. Africa and Ethiopia and the subspecies *turkestanica* from Iranian Balochistan to Turkestan. However, this sub species has now been collected from Sindh & Punjab areas of Pakistan by the present author and taxonomic characters of the present samples are compared with the published data of this species from other territories in Table 1 & 2.

♀ A3 and A3/ labrum were observed shorter than Afghanistan and Iranian samples (Artemiev, 1978; Theodor and Mesghali, 1964). Ascoid formula and number of cibarial teeth were found similar with that of Afghanistan flies (Artemiev,1978), however, less number of cibarial teeth were reported in Iranian flies (Theodor and Mesghali,1964). ♂ A3 was found shorter than of Afghanistan flies (Artemiev,1978). Coxite, style and F/P were observed larger than of Afghanistan specimens (Artemiev, 1978).

Paramere, filaments with dilated ends and ascoid formula of Pakistani flies were found similar with that of flies from Afghanistan (Artemiev,1978). Since Theodor and Mesghali (1964) did not report the ♂ *S. dreyfussi turkestanica,* so present data could not be compared with them.

The present work is in conformity with the findings of Theodor and Mesghali (1964) and Artemiev (1978). However, some minor variations in the measurements of taxonomic characters and also in number of cibarial teeth have been noted in the present study, which may be due to certain climatic factors (mainly humidity). Belazzoug *et. al.*(1982) while working on sand flies of different ecological zones of Algeria observed that the number of cibarial teeth varies according to climatic factors mainly humidity.

It is hoped that present findings would provide the basis for further research on sand flies taxonomy in the country. Keeping in view of its restricted distribution and specially its presence in human residences in the areas of cutaneous leishmaniasis, vectorial ability of *Grassomyia dreyfussi turkestanica* clearly needs to be investigated.

Evolutionary relationship

Theodor (1948) divided the genus *Sergentomyia* (Franca and Parrot) in to five sub genera: *Sergentomyia* (Franca and Parrot) (which subdivided in two groups *minuta* and *fallax*), *Sintonius* (Nitzulescu), *Parratomyia* (Theodor), *Rondanomyia* (Theodor) and *Grassomyia* (Theodor). However, Lewis (1987) raised *Grassomyia* to a genus level. Genus *Grassomyia* appears to form a distinct major clade and unique with aut-apomorphy of convex cibarial tooth row and rounded spermatheca in the female and genital filaments with extended tips. *Indica* and *turkestanica*-groups appear to be sister group for their synapomorphies of cibarium of female with convex comb of pointed teeth, pharynx of female bulging near hind end, and genital filaments with rounded expanded tips. The two species, however, are clearly separated on the basis of their clear cut autapomorphies as shown in the keys.

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