



Full Length Article

New Records of Sand Fly *Sergentomyia punjabiensis* with Special Reference to Mouth Parts, Male and Female Genitalia and its Phylogenetic Relationship with Closest Allies in Sindh, Pakistan

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ABSTRACT

In this survey, sand fly *Sergentomyia* (*Sergentomyia*) *punjabiensis* (Sinton) was recorded first time from the epidemic localities of cutaneous leishmaniasis in Sindh Province (Pakistan). 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 the *Sergentomyia* flies 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 its correct identification, taxonomic characters of *S. punjabiensis* (Sinton) are studied in detail with special reference to its mouth parts, male and female genitalia. A key is also given to *S. punjabiensis* (Sinton) and its closest allies. In this light its relationships with its closest allies is also briefly discussed.

Key Words: Sandflies; Sindh; Pakistan

INTRODUCTION

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

There are two genera *Phlebotomus* and *Sergentomyia* in the Old World and genus *Lutzomyia* in the New World. The representatives of the genus *Sergentomyia* Franca and Parrot mostly prefer to feed on reptiles and can be separated from the genus *Phlebotomus* Rondani and Berte in Rondani, 1840, their maxillae have ridge-tip and hypopharynx almost smooth (Lewis, 1975 & 78; Artemiev, 1978).

Pakistan has several endemic foci of leishmaniasis, 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.

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.*, 2005), the correct identification of the species becomes of significant value in the study of epidemiology of leishmaniasis and other viral diseases. We report here the prevalence of *S. punjabiensis* (Sinton) from new endemic places of cutaneous leishmaniasis (CL) in Sindh Province. This fly was collected from wild as well as in houses. Presently, *S. punjabiensis* (Sinton) is not only studied in detail with reference to its mouth parts and genitalia thus facilitating its identification, but keyed out from other known species of the sub genus *Sergentomyia* Franca and Parrot and relationship of these taxa are also briefly discussed.

MATERIALS AND METHODS

The present investigation was carried out on the materials (49 specimens of *S. punjabiensis*) collected from the field, human residences, colleges, schools and hostels by means of sucking tubes and sticky papers in day and night times in Sindh Province during May, 2006. Collected flies were transferred in vials containing 70% alcohol and shifted to Zoology Department, University of Balochistan, Quetta, where descriptive observational study was carried out. Each fly was processed and dissected following the conventional techniques (Young & Duncan, 1994; Aslamkhan & Aslamkhan, 2000). Characteristics structures were studied, measured, taxonomic notes were prepared and their

photographs were taken through camera mounted Olympus microscope (BX41). Most structures were measured with magnification of 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 Bermudes *et al.* (1991). Identification of specimen was carried out with the help of available literature (Lewis, 1967 & 78; Artemiev, 1978). Prepared permanent slides were deposited with the author's collection of sandflies, Department of Zoology, University of Balochistan, Quetta.

RESULTS

***Sergentomyia (Sergentomyia) punjabiensis* (Sinton).**
Phlebotomus minutus var. *antennatus* Newstead; Sinton 1924.

Phlebotomus antennatus Newstead; Sinton 1927.

Phlebotomus punjabiensis Sinton 1933.

Sergentomyia (Sergentomyia) punjabiensis (Sinton) Theodor 1948.

Phlebotomus antennatus var. *decacanensis* Qutubuddin 1952.

Sergentomyia (Sergentomyia) punjabiensis (Sinton), Lewis 1978.

Material examined ♀ **25.** Wings (X100): 1.30-1.35 long, length to breadth ratio 4.09-4.19, α β alar index 0.6, δ =+0.03, γ =0.25, Π =0.15. Palps length 0.55, palpal ratio 1, 3, 5, 6, 12.5, formula 1, 2, 3, 4, 5. P3 has 10-14 spatulate Newstead's sensillae at its basal third of the segment. Antennal segment (X100) A3 very short (0.08 long), A4 and A5 each 0.05 long, $A3 < A4 + A5$, ascoid short and at 0.78 of A3, ascoid at A4 at 0.05 of the segment, ascoid formula 2/3-15.

Mouth parts (X100). Proboscis 0.20 mm long. Labrum (Fig. 1A) 0.14 mm long, 0.02 mm broad, a strong chitinized structure, relatively narrow, sides parallel, apex bluntly pointed with 3 apical sensillae, margins furnished with a series of leaf like sensillae closely together and numbering about 6-8 on either side. Hypopharynx (Fig. 1B) 0.15 mm long, 0.02 mm broad, a bilaterally symmetrical blade shaped structure, tapers off much more gradually towards the apex and marginal minute leaf like serration as to present a finely serrated edge, its terminal portion broadly concave and in its center, salivary duct runs. Hypopharynx with minute serration is the characteristic feature of the genus *Sergentomyia* (Artemiev, 1978). Mandible (Fig. 1C) 0.15 mm long, 0.01 mm broad, broad blade like structure, outer edges markedly serrated, outer edge of dorsal surface strongly chitinized forming the actual blade whereas the ventral surface consists of soft membrane, tips of mandibles extend further than those of maxillae, dental depth of 0.07 mm.

Maxilla (Fig. 1D) 0.14 mm long, 0.02 mm broad in its

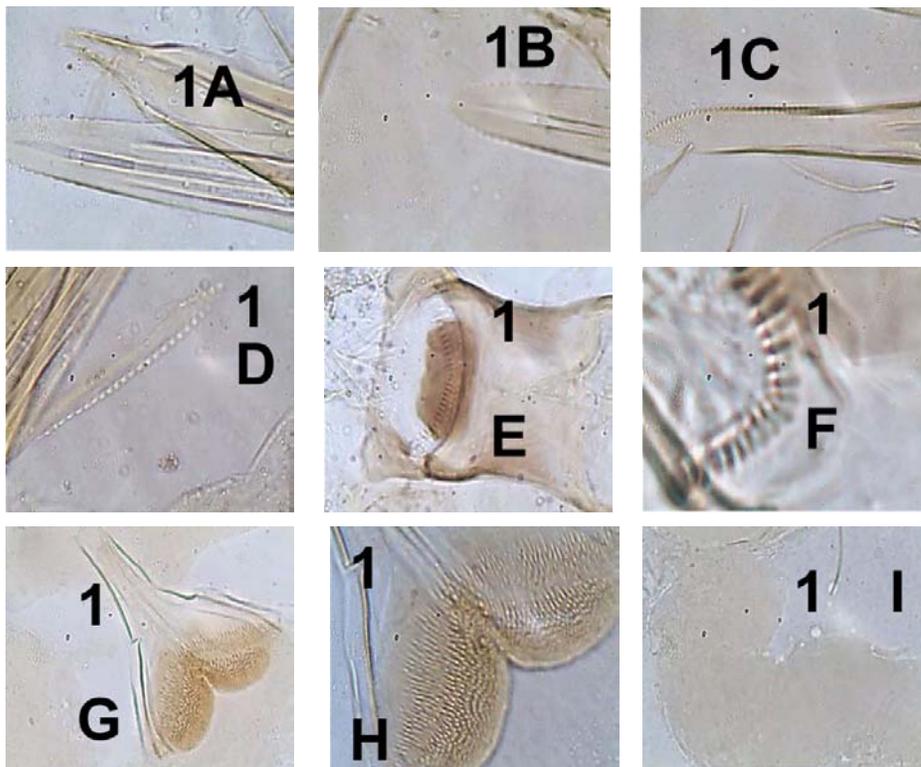
broadest part, a little broader than mandibles, at its distal end, one end (lateral) is provided with 7 chitinous dot-like rounded teeth while the other end (ventral) contains a row of about 27 tooth like processes pointing backwards and gradually diminishing in size from before backwards and a ventral depth of 0.08 mm. Cibarial cavity (Fig. 1E) 0.05 mm broad, chitinous arch absent or weakly developed, a pigment patch (0.03 mm long, 0.01 mm broad) of dark brown color present attached with chitinous arch, a concave row of about 26-28 teeth nearly uniform (Fig. 1F), arranged on buccal plate, outer ones are somewhat pointed. Pharynx (Fig. 1G) is the backward continuation of the buccal cavity and is 0.13 mm long, basal part is 5.5 times broader than its anterior part, basal part with a deep median notch, pharyngeal armature (0.05 mm in height) highly developed, dark-yellow pigmented occupying most of the posterior portion of the pharynx and consists of at least three different kinds of teeth (Fig. 1H): basal and most posterior armature in the form of rounded dot-like denticles whereas median-apical and latero-apical armature composed of very long spicules obliquely down to the base.

Female genitalia. Spermathecae tubular (Fig. 1I): 0.025 mm long, tubular, more wide anteriorly than posteriorly, spermathecal ducts delicate and relatively broader (0.028 mm broad) arising from each spermatheca and combine with one another after some distance, thereafter converts in to a common duct falling into genital atrium (breadth 0.08 mm). Furca 0.06 mm long.

Material examined ♂ **22.** Wings (X100) 1.22-1.28 mm long, length/bread 4.06-4.26, α β =0.48, δ = zero, γ =0.20, Π =0.18. Palpas (X100): 0.54 mm long, ratio 1, 2, 3.3, 8.3, formula 1, 2, 3, 4, 5. Antennal segment 3, 0.1 mm long, A4 and A5 each 0.06 mm long, ascoid at A3 is 0.43 of the segment, ascoid at A4 is 0.45 of the segment, ascoid very small (0.02 mm long) single ascoid on A3-A15.

Mouth parts (100X). Proboscis 0.16 mm long. Labrum 0.13 mm long. Hypopharynx 0.12 mm long. The hypopharynx of male resembles that in the female in general plan but the serration at the point are much more delicate being fli-form, very thin and flexible instead of leaf like of female. Mandibles absent. Maxilla 0.12 mm long. The maxillae of male and female are very similar but maxillary blade of the male is more narrower and its apical portion differs in respect of teeth installed on it. Cibarium (Fig. 2A) cibarial cavity sharply angular, its breadth and height excluding side walls are 0.04 and 0.03, respectively below the ventral plates a brown cylindrical median pigment patch slightly concave without anterior process present in which a row of about 30 dot like denticles and of about 22 uniform pointed teeth arranged on a concave line. Pharynx (Fig. 2B) flask shaped, 0.17 long, its broadest part is 2.5 times than its anterior part, armature very weak, faint and consists of short and curved lines confined at base of pharynx, height of armature is 0.2 of the length of pharynx.

Fig.1. Female *Sergentomyia (Sergentomyia) punjabiensis* (Sinton): A, Labrum X400; B, Hypopharynx X400; C, Mandible X400; D, Maxilla X400; E, Cibarium X400; F, Cibarial teeth X1000; G, Pharynx (w.m.) X200; H, Pharyngeal armature X400; I, Spermatheca X400



Male genitalia (X100). Coxite 0.23 mm long, 0.08 mm broad. Style (Fig. 2C) 0.1 mm long, 0.02 mm broad, with four apical spines almost the length of style, a small ventral seta at 0.7 of the style. Paramere (Fig. 2D) 0.13 mm long, with a broad posterior base (0.05 mm broad) and a long neck (0.04) terminating into a beaked end (or like a bird's head). Surstyle 0.17 mm long, apical ends of paramere a little shorter than surstyle terminal end. Genital filaments smooth (Fig. 2E), 0.20 mm long, pump 0.10 mm long F/pump=2.0. Plunger narrower than barrel (Fig. 2F), barrel as long as wide (Fig. 2G). Aedeagus sheath (Fig. 2H) 0.08 mm long, apical end almost flat and 0.01 mm broad and slightly curved ventrally.

Key to the species of *Sergentomyia (Sergentomyia)* from indo-Pakistan subcontinent

1. Buccal cavity with a definite row of teeth and a pigment patch, style of male fly with four spines and an accessory seta.....genus *Sergentomyia*.

Spermatheca tubular with smooth walls of uniform width along their length, A3 shorter than segment 4 and 5 together, usually shorter than labrum, aedeagus stout.....sub genus *Sergentomyia*.

. Spermatheca tubular with smooth sides, a wide spermathecal duct, thick aedeagus, paramere blunt.....*S. dentate*.

. Buccal cavity of female with 17-22 teeth arranged on a concave row, median teeth smaller, pharynx narrow,

armature of pharynx do not reach the lateral walls of pharynx, base of pharynx with a central notch, male buccal cavity consists of 15-18 teeth on a concave line, median teeth smaller, paramere with slightly hooked end, aedeagus slightly curved, style with 2 apical and two subapical spines.....*S. d. arpaklensis*.

. Female with wider pharynx, buccal cavity having 14-18 teeth with central teeth shorter than the laterals, paramere blunt.....*S. theodori*.

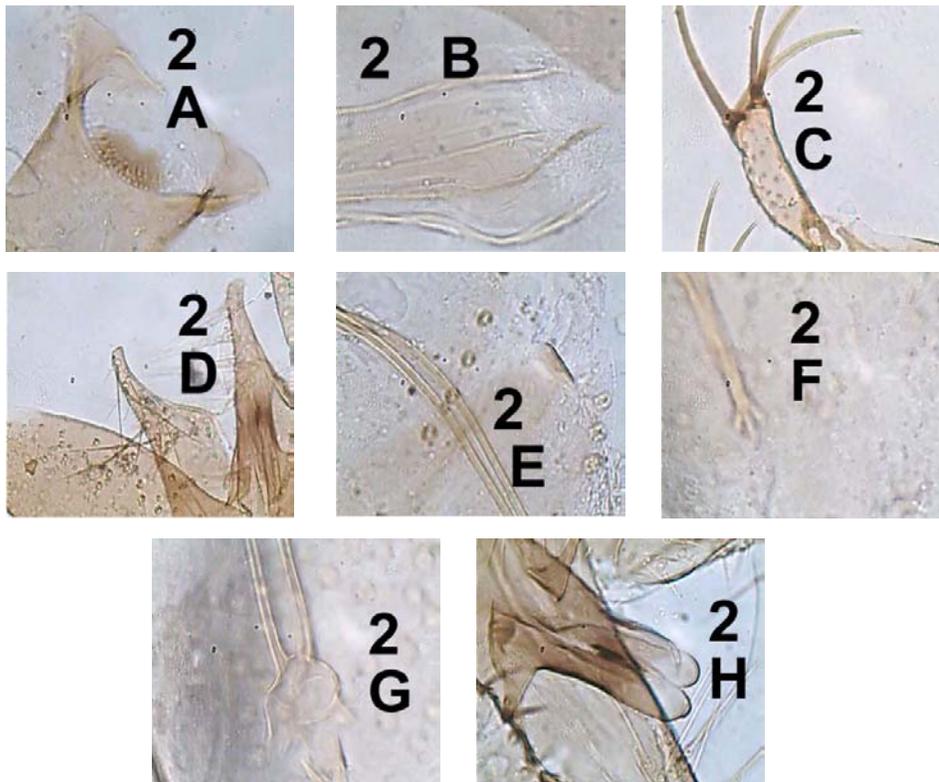
. Female buccal cavity with a concave row of 17-22 teeth, median teeth more shorter, pigment patch without anterior process, pharynx broad, base of pharynx with a slight median notch, comparatively bigger pharyngeal pigmented teeth, male cibarium with 15-22 teeth arranged on a concave row, median teeth shorter, style with 2 apical and two sub apical spines.....*S. t. pashtunica*.

Female with 16-20 uniform cibarial teeth, dark pigment patch present with a short forward extension spermatheca more rounded, spermathecal ducts less broader, paramere rounded ends, filaments smooth.....*S. murghabiensis*.

. Buccal cavity of female having about 20 teeth in a concave line, central teeth shorter than the lateral ones, pigment patch with long anterior process, pharynx with membranous base, paramere with rounded ends, aedeagus thick and straight.....*S. mervynae*.

. Cibarium with 18-22 teeth of uniform size on a

Fig. 2. Male *Sergentomyia (Sergentomyia) punjabiensis* (Sinton): A, Cibarium X400; B, Pharynx X400; C, Style X400; D, Paramere X400; E, genital filaments X400; F, Plunger, X400; G, Barrel X400; H, Aedeagus X400



concave row, pigment patch without anterior process, pharynx very broad and base with a deep median notch, paramere with rounded ends, buccal cavity with concave row of 16- 21 uniform teeth, 3-4 small teeth in the center arranged on a concave line.....*S. fallax afghanica*.

Comparative note. This species is related to other species of the subgenus *Sergentomyia* (Franca & Parrot) like *S. fallax afghanica* Artemiev, *S. murghabiensis* Perfiliev, 1939, *S. mervynae* Pringle (1953), *S. dentata* Sinton (1933) and *S. dentata arpaklensis* Perfiliev (1933), having short and bead like A3, buccal teeth arranged on a concave curve, uniform cibarial teeth (*S. murghabiensis*, *S. fallax afghanica*), buccal cavity with large lateral teeth and small central ones (*S. theodori*, *S. mervynae*, *S. dentata*), usually pigment patch present, pharynx very broad and with strong armature (*S. palestinensis*), pharynx very broad but with very small armature (*S. fallax afghanica*), base of the pharynx with a very deep median notch (*S. fallax afghanica*, *S. punjabiensis*), slight notch (*S. theodori*) to no notch (*S. murghabiensis*), pharynx with membranous base (*S. mervynae*) and without membranous base (*S. dentata*), tubular spermethecae with smooth sides and wide ducts open into a common duct, in male aedeagus sheath thick and broad, paramere with slightly hooked end (*S. dentata*, *S. punjabiensis*), paramere with rounded blunt ends (*S. murghabiensis*), cibarial teeth on a concave line, with uniform teeth (*S. murghabiensis*), central teeth shorter than

the laterals (*S. theodori*, *S. mervynae*), pigment patch usually present, genital filaments smooth, F./P. ratio=2.25-3.25 (*S. murghabiensis*), 3.71-3.75 (*S. mervynae*), 3.33-4.0 (*S. theodori pashtunica*). *S. punjabiensis* can easily be separated from the same: in female, pharynx very wide posteriorly with a deep hind-median notch and its armature very strong and cibarial teeth nearly equal in size, in male paramere hooked.

Distribution. Present study, new record: Bihiria city, Dadu, Dera Ghazi Khan, Jacobabad, Juhi, Khairpur Nathan Shah, Larkana, Madeji, Mehar, Moro, Nau Shehro Feroz, Qambar Ali Khan, Shahdad Kot, Shikarpur, Yasin Garhi. Dera Ismail Khan (Lewis, 1967). Belpat, Dhadar, Lehri, Uthal (Kakarsulemankhel, 2004).

DISCUSSION

Taxonomic structures of *S. punjabiensis* (Sinton) collected from Sindh Province were compared with the published data of this species from other territories (Table I & II). ♀ *S. punjabiensis* of Sindh province were observed with a shorter wing length than from Punjab and NWFP flies (Lewis, 1967) and larger from Balochistan flies (Kakarsulemankhel, 2004).

A3/labrum of Sindh flies were found shorter than of Punjab and NWFP flies (Lewis, 1967). Labrum in Sindh flies were found shorter than from Punjab and NWFP flies

Table I. Comparison of Taxonomic characters of *S. punjabiensis*

Taxonomic Characters		Balochistan (Kakarsulemankhel, 2004)	Punjab & NWFP (Lewis, 1967)
Wing	Length	1.16 (1.12-1.20)	1.40 (1.21-1.50)
	Breadth	0.28 (0.25-0.32)	0.31 (0.28-0.33)
	Alar index	0.61 (0.60-0.62)	0.60 (0.40-0.90)
Labrum	Length	0.11 (0.10-0.12)	0.14 (0.13-0.15)
	AIII	0.07 (0.07-0.08)	0.09 (0.08-0.09)
AIII/ Labrum		0.66 – 0.07	0.6 – 0.7
AIII/ A4+A5		A3 < A4 + A5	A3 < A4 + A5
Ascoid on A4		0.42	0.40
Palpal formula		1, 2, 3, 4, 5	1, 2 (3-4), 5 or 1, 2, 3, 4, 5
Palpal ratio		10:23:34:44:76	10:12: 13
Cibarium		With about 28 uniform, equal sized teeth arranged on a convex line, pigment patch broad and dark chitinous arc absent or ill developed	With about 30 nearly uniform teeth about 10 punctiform teeth present pigment patch broad and very dark chitinous arc absent
Pharynx		1.4 times as long as wide, with sharply defined deep median notch at base	1.3 to 1.8 times as long as broad with deep median notch
Spermatheca		Tubular with delicate ducts	Tubular with delicate ducts

Table II. Comparison of Taxonomic characters of *S. punjabiensis*

Taxonomic Characters		Balochistan (Kakarsulemankhel, 2004)	Punjab & NWFP (Lewis, 1967)
Wing	Length	0.96- 1.04	1.35 (1.28 – 1.47)
	Breadth	0.22 – 0.24	0.27 (0.25 – 0.29)
Labrum	Length	0.11	0.13 (0.12- 0.14)
	AIII	0.08	0.10 (0.09 – 0.11)
AIII/ Labrum		0.71	0.8 (0.7 – 0.8)
AIII / A4 + A5		0.78 – 0.80	A3 < A4 + A5
Ascoid on A4		0.36	0.30
Cibarium		With about 18 uniform, pointed teeth, and few dot like denticles scattered at the base of teeth, a slightly brown short triangular pigment patch, chitinous arc absent	With about 20 nearly equal pointed teeth and a few small punctiform teeth, pigment patch variable, usually short and broad chitinous arc absent
Pharynx		Armature consists of a series of Weak, faint, short, straight and curved lines	Pharynx with faint, scaly armature
Style		With 4 apical spines, seta at 0.75	With 4 apical spines, seta at 0.70
Paramere		With beak like apex	With beak like apex
Aedeagus		Thick with rounded end	Thick with rounded end
Genital filament / Pump		3.0 – 3.25	3.5

(Lewis, 1967), whereas larger in Balochistan flies. Similarly, antennal segment was found shorter in length than of Balochistani flies. Similarly, ♂ flies of the present study were found with shorter antennal segment A3 and also shorter F/P ratio as compared to the published data from Balochistani flies (Kakarsulemankhel, 2004) and flies from NWFP and Punjab (Lewis, 1967).

The present work is in conformity with the findings of Lewis (1967 & 1978) and Kakarsulemankhel (2004). However, some minor variations in the measurements of taxonomic characters were noted 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. 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 wide distribution of *S. punjabiensis* (Sinton) in Pak-Indian sub continent and Sri Lanka, its present prevalence in human residences in the areas of cutaneous leishmaniasis and also in the light of

observations made by Geevarghese *et al.* (2005) and Mukherjee *et al.* (1997) about the vectorial capacity of *Sergentomyia* flies, this all clearly demands that pathogens transmission capacity of this fly may be investigated and a comprehensive program for the control of sand flies and leishmaniasis may be initiated on war footings in the country.

Evolutionary relationship. Theodor (1948) divided the genus *Sergentomyia* Franca and Parrot in to five sub genera: *Sergentomyia* Franca and Parrot (subdivided in two groups *minuta & fallax*), *Sintonius* (Nitzulescu), *Parratomyia* (Theodor), *Rondanomyia* (Theodor) and *Grassomyia* (Theodor). The members of the genus *Sergentomyia* (Franca & Parrot) are distributed in the Triad zone of Oriental region and also in peripheral areas of west zone including Pakistan, Nepal, Burma, southern Chinese belt, Taiwan, Philippines, Indonesia and Sri Lanka, appears to form a distinct clade. The species of this clade have buccal teeth in transverse row and usually with a pigment patch, style of male with four major spines and an accessory seta. Its subgenus

Sergentomyia Franca and Theodor is entirely isolated in having tubular spermatheca with smooth sides and a wide duct and the aedeagus short and thick but still playing out group relationship with the rest of subclade subgenera in having all basic characteristics of genus *Sergentomyia*. The *dentata-theodor-mervynaegroup* maintains its distinct subclade by having apomorphies of shorter central buccal teeth than the lateral ones (in male as well as in female) and style with two apical and 2 sub apical spines. The *fallax-murghabiensis*-group maintain their separate sub clades and identity in having uniform buccal teeth arranged on a concave line but in *S. fallax-afghanica* autapomorphies include pharynx very broad, with a deep median notch at the base and with very small pharyngeal teeth, while pharynx not so broad, without a notch at the base and rather big teeth in *S. murghabiensis*. The species of *fallax-murghabiensis*-group play sister group relationship with each other and in their synapomorphies they appear to be closely related in having non-uniform buccal teeth.

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