# Mouth-parts, male and female genitalia as identification tools of *Sergentomyia* (*Sintonius*) *clydei* and phylogenetic relationships

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#### Abstract

Sergentomyia (Sintonius) clydei (Sinton) is for the first time recorded from the endemic localities of cutaneous leishmaniasis in Sindh, Punjab and N.W.F province (Pakistan). In view of the published reports about the detection of encephalitis viruses from the species of the genus Sergentomyia Franca & Theodor from the Indian localities and their possible role in kala-azar transmission, the correct identification of sand flies becomes of significant value in the study of epidemiology of leishmaniases and other viral diseases. In the present paper, species of the subgenus Sintonius Nitzulescu are not only keyed and their distributional ranges are given but in order to facilitate zoologists and medical researchers, the morphology of diagnostic characters of S. clydei, especially mouth parts and male and female genitalia are studied, and results presented. In this light, their phylogenetic relationships are also briefly discussed. Key words: sand flies, Pakistan, Sindh, North West Frontier province, Punjab

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# 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 leishmaniases) of importance of human health in at least 80 countries (Alexandar & Maroli, 2003). Among diseases transmitted by sand flies, leishmaniases 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 *Sintonius* Nitzulescu are quite prevalent in the country *viz. S. hospitii* 

(Sinton), S. christophersi (Sinton), S. tiberiadis pakistanica Artemiev & Safayanova (Lewis, 1967, 1978; Artemiev & Safayanova, 1974; Artemiev, 1978).

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 & Theodor from the Indian localities and their possible role in kala-azar transmission (Geevarghese *et al.*, 2005), the correct identification of sand flies becomes of significant value in the study of epidemiology of leishmaniases 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. Lewis (1967) recorded *S. clydei* from Karachi, Lahore, Mir Mohammad, Rawalpindi and Taxilla but measurements of taxonomic characters like mouth parts (hypopharynx, maxilla, mandible, cibarium and pharynx), genital structures (spermatheca, coxite, style, paramere, aedeagus and surstyle) were not furnished. Further, neither measurements, nor accounts nor drawings of diagnostic features were supplied by Rab *et al.* (1986) and Aslamkhan *et al.* (1997, 1998). Evolutionary relationship was not discussed. In view of the insufficient description especially of mouth parts, male and female genitalia, presently, these characters of *S. clydei* are not only studied but are keyed out from the other known species of subgenus *Sintonius* for its correct identification as additional information. This was the principal objective of the present study. Its evolutionary relationships with its closest allies are also briefly discussed.

## Materials and methods

The present investigation was carried out on the materials (44 specimens of *S. clydei*) collected from Sindh, North West Frontier province (N.W.F.P.) and Punjab province of Pakistan during May, 2006 using sucking tubes and sticky traps. The collected material was preserved, processed and dissected by conventional methods (Young & Duncan, 1994; Aslamkhan & 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). Most of the structures were measured with a low magnification (×100) whereas spermatheca, ducts and

furca were examined under high magnification ( $\times 400$  and  $\times 1000$ ). The entire 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 (1991). Prepared permanent slides were deposited with the author's collection of sand flies, Department of Zoology, University of Balochistan, Quetta.

# Results

The subgenus Sintonius is composed of clydei, christophersi, eadithae (Sinton), hospitii, orissa Kaul & Lewis, sirohi, Kaul, Dhanda & Modi, tiberiadis (Adler, Theodor & Lourie), tiberiadis pakistanica. It is defined on the basis of the following characters: the segmented spermatheca, usually small, aedeagus narrow and pointed.

### Key to the species of the subgenus Sintonius

## Male

1. Pale sand flies, cibarial teeth 3-18 long, pointed, standing on a straight line
- Dark sand flies, cibarial teeth 19-32, arranged on a slightly curved line 4
2. Cibarium with 3-5 separated teeth and several vertical denticles, small pigment patch drop
shaped, antenna 3 with 1 papilla christophersi*
- Cibarium with 10-18 teeth
3. Cibarium with 10-12 teeth, comparatively shorter than of female, rather wide pigment
patch (0.02 mm broad), A3 without papilla <i>tiberiadis pakistanica</i> **
- Cibarium with 10-18 teeth in comb formation, middle ones smaller than the laterals
4. Cibarium with 19-24 small teeth, in groups of 2-3, pigment patch small with a long process
- Cibarium with 30-32 teeth, arranged in convex line, anterior femur with 8-10 short spines

## Female

1. Cibarium with 3-35 teeth
- Cibarium with a convex row of 65-80 long teeth, black pigment patchhospitii

<sup>\*(</sup>India, Pakistan, Guinea, Chad, Ethiopia, Sudan, Egypt, north Yemen, Saudi Arabia, Oman)

<sup>\*\*\*(</sup>Pakistan, Southern Afghanistan, Turkministan, Eastern Iran)
\*\*\*\*(Northern and Central Africa, Yemen, Saudi Arabia, Kuwait, Iraq, Iran, Central Asia, Afghanistan, Pakistan, Northern India)

#### Sergentomyia (Sintonius) clydei (Sinton)

(Figures 1A-D, 2A-D)

Phlebotomus clydei Sinton, 1928, Indian J. Med. Res. 16: 312.

Phlebotomus (Prophlebotomus) clydei (Sinton), Parrot, 1940, Archs. Inst. Pasteur Algier. 30: 312. Sergentomyia (Sergentomyia) clydei (Sinton), Theodor, 1958, Fliegen palaearkt Reg. 9c: 51; Theodor & Mesghali, 1964, J. Med. Ent. 1: 297; Lewis, 1967, Bull. Brit. Mus. Nat. Hist. 19: 42; Perfiliev, 1968, Isr. Prog. Sci. Trans. 347; Artemiev, 1976, Med. Parazit. 45: 37; Lewis, 1978, Bull. Brit. Mus. Nat. Hist. 37: 308.

**Material examined** – 23  $\bigcirc$ , 21  $\Diamond$ , collected from foci of leishmaniases in Sindh, N.W.F. P., Punjab provinces, during May 2006.

**Female** – Wings (×100) 1.68-1.72 long, 0.40-0.44 broad,  $\alpha/\beta$  (alpha/beta) = 0.75,  $\delta$  =

0.18,  $\Pi = 0.0.12$ , gamma 0.32.

Antennae and palps – A3 (×100) 0.12-0.13 long, ascoids (×400) comparatively short (0.6 long), but little shorter to reach to the next segmental articulation, 2 ascoids from A3 to A15, ascoid on A3 at 0.72 of the segment, A4 and A5 (×100) each 0.07 long, ascoid at A4 and A5 at 0.33. Palps (×100) 0.7 long. Newstead sensilla present at palp 3 at 0.15 of its length, palpal ratio 1, 2, 3, 2, 6 and formula 1, 2-4, 3, 6.

Mouth parts, cibarium, pharynx – Labium long, thick and consists of mentum and 2segmented labellum with basal and apical segment, the overall length of this composite structure known as proboscis (×100) 0.22-0.24. Labrum (×100) 0.21-0.22 long, relatively narrow, sides parallel, apex bluntly pointed with three apical short and stout sensilla, lateral sensilla very short and almost smooth. Mandible (fig. 1A) (×100) 0.20-0.21 long, blade like structure, outer edges markedly serrated and composed of minute teeth, dental depth ( $\times 400$ ) 0.8, one side is gradually but sharply pointed. Hypopharynx is symmetrical blade shaped structure, the marginal leaf-like sensillae are much shorter as to present almost smooth margins, a characteristic feature of sand flies of the genus Sergentomyia, its apical part is broadly concave and in its centre a salivary duct runs. Hypopharynx (×100) 0.20 long. Maxillary blades (each 0.21 long) stout basally but narrows very much towards its apex, it has two rows of teeth, 3 apical hooked shaped lateral teeth and 27-29 small and sharp ventral teeth arranged in a line (fig. 1B), dental depth ( $\times 400$ ) 1.52, just from the mouth to a little proximal of the base of the clypeus and extending till the end of membranous junction with the pharynx, lies a strong chitinized tube known as buccal cavity (cibarium), its breadth ( $\times 100$ ) 0.05, an almost straight cibarial teeth arc contains about 11-13 sharply pointed needle-like but widely spaced, at the base of teeth there is a single row of rounded punctiform denticles (fig. 1C). Pigment patch is quite broad (0.04) and cone-shaped and gradually narrows towards upper side and transforms in to a long anterior process. Posterior continuation of the buccal cavity is the lamp-glass-shaped pharynx (×100) 0.17 long, posterior maximum breadth 0.07, basal breadth 0.05 which contains weak armatures in the form of numerous small spines carried on transverse ridges, posterior part of pharynx is 1.75 times greater than its narrowest anterior part.

Genitalia – Spermatheca (×1000) 3000 long, with 10-12 segments, gradually narrowing towards the base (fig. 1D). Furca (×400) 1.4 long, genital atrium (×400) 0.96 broad.

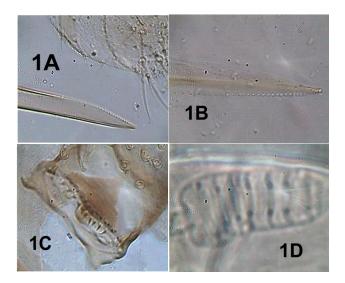
**Male** – Wings (×100) 1.63-165 long, 0.40-0.42 broad, α/β (alpha/Beta) = 0.83,  $\delta$  = 0.07, Π = 0.05, gamma 0.28.

Antennae and palps – A3 (×100) 0.16-0.17 long, ascoid (×400) 0.48 long, ascoid at 0.60, A4 and A5 (×100) each 0.1 long, ascoid at 0.37. Palps (×100) 0.67 long, ratio 1, 3.3, 5.3, 3.3, formula 1, 2-4, 3 ,5.

Mouth parts, cibarium, pharynx – Labium long, thick and consists of mentum and 2segmented labellum with basal and apical segment, the overall length of this composite structure (×100) 0.22. Labrum (×100) 0.20-0.21 long. Hypopharynx (×100) 0.20 long. Cibarium inside breadth (×100) 0.05, pigment patch small with a long anterior process, below to it a row of horizontal teeth (16-24) arranged on a slightly curved line, teeth are in groups of 2 or three (fig. 2A). Pharynx ( $\times$ 100) 0.16 long and lamp-glass-shaped, anterior breadth 0.03, not so marked dilated posteriorly, posterior bulge 0.05 broad.

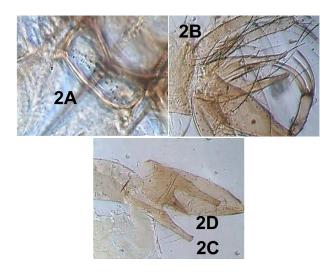
Genitalia (fig. 2B) – Coxite (×100) 0.23 long, 0.07 broad, style 0.10 long, 0.03 broad, a long ventral seta at 0.78, style with 2 terminal and two subterminal spines. Paramere (fig. 2C) quite narrow with beaked end, paramere (×100) 0.20 long, posterior maximum breadth 0.05, paramere becomes narrower at 0.60, aedeagus (×100) 0.13 long, less broader at base and with sharply pointed ends (fig. 2D) surstyle (×100) 0.23 long, its apical end projects out quite forward than to apex of paramere, genital pump (P) (×100) 0.12 long, filament (F) 0.45 long with faint striations, F/P = 3.75.

**Distribution** – North Africa, Central Africa, Yemen, Saudi Arabia, Kuwait, Iran, Iraq, Central Asia, Afghanistan, Pakistan, India. Present study, new record. Sindh: Dadu, Jacobabad, Qambar Ali khan, Larkana, Madeji, Shahdad Kot. N.W.F.P.: Dera Ismail Khan. Punjab: Multan, Dera Ghazi Khan. These localities are endemic foci of cutaneous leishmaniasis.



**Figure 1.** Female *Sergentomyia (Sintonius) clydei*: A, mandible (×400); B, maxilla (×400); C, cibarium (×400); D, spermatheca (×1000).

**Comparative note** – This species shares some of its characters such as usually small spermatheca but with regular segmentation. It is distinct however, in its group having about 13 sharply pointed needle like horizontal teeth, 12-30 vertical teeth in one or two rows, quite broad pigment patch with a long anterior process in female, and cibarium with a single row of vertical denticles, horizontal teeth usually small and in groups in males.



**Figure 2.** Male *Sergentomyia (Sintonius) clydei*: A, cibarium (×400); B, male terminalia (×100); C, paramere (×100); D, aedeagus (×100).

## Discussion

Small segmented spermathecae characterizes this subgenus which occurs largely in the drier parts of the Old World (Lewis, 1978). *S. clydei* is unusual in that it is one of several species of *Sergentomyia* which bite man (Quate, 1964). *S. clydei* has been used to transmit experimentally *Leishmania adleri* from lizards to man, causing a transient infection and giving immunity against one form of *L. donovani* (Garnham, 1971).

Previously, Lewis (1967) recorded *S. clydei* from Kandhkot and Karachi and distribution of this species in the country was thought to be very limited.

The morphology and measurements of various characters of *S. clydei* show considerable differences when compared with the published data of this species from other territories (table 1, table 2). Female *S. clydei* of the present study were observed having shorter wing and alar

index than published data of this species from Waziristan (Sinton, 1928), Rawalpindi (Lewis, 1967) and Ethiopian region (Kirk & Lewis, 1951). A3 was observed to be shorter than flies from South India (Ilango *et al.*, 1994), Afghanistan (Artemiev, 1978), Waziristan (Sinton, 1928), Rawalpindi (Lewis, 1967). Labrum of present flies was observed shorter than in the flies from Rawalpindi (Lewis, 1967) and south India (Ilango *et al.*, 1994). Cibarial teeth were fewer in numbers than in the flies from Egypt (Lane, 1986), Ethiopian region (Kirk & Lewis, 1951), Waziristan (Sinton, 1928) and Afghanistan (Artemiev, 1978). Specimens of male *S. clydei* were observed with shorter wings than in the flies from Ethiopian region (Kirk & Lewis, 1951), Rawalpindi (Lewis, 1967) and Waziristan (Sinton, 1928). A3 was found to be shorter than in the flies from Afghanistan (Artemiev, 1978) and Rawalpindi (Lewis, 1967). Filament/sperm pump ratio was also noted shorter than in the flies from south India (Ilango *et el.*, 1994), Afghanistan (Artemiev, 1978) and Rawalpindi (Lewis, 1967).

While examining,  $1 \\ \circ \\ \circ$  and  $5 \\ \circ \\ S$ . *clydei* from Sinai (Egypt), Lane (1986) observed that *S*. *clydei* seems to be very closely related to *S*. *adleri* (Theodor) and there has been much discussion on whether they are conspecific. Quate (1964) and Lewis & Buttiker (1982) have treated them as separate species. Main difference lies in the number of horizontal and vertical teeth of cibarium. In Saudi Arabian form, *S*. *clydei* was noted having 12-17 and 12-13 horizontal and 15-46 and 16-34 vertical teeth (Lewis & Buttiker, 1980, 1982) respectively.

Form of *S. adleri* has not so far been found in Pakistan. Another ally (*S. (Sintonius) tiberiadis pakistanica*) of *S. clydei*, has been described from Ahmed Khel (N.W.F.P.-Pakistan) on the basis of  $1 \, \bigcirc$  by Artemiev & Safayanova (1974). This subspecies according to Lewis (1978) differs from the Ethiopian and Palestine nominal subspecies in having a spermatheca narrowing toward the apex with more (9-12) segments, a common spermathecal duct and 10-17 cibarial teeth instead of 17-18. This subspecies has been reported from Afghanistan by Artemiev (1976).

Keeping in view of a fairly wide distribution in Asia as well as in Africa and its collection from human residences of cutaneous leishmaniasis areas, earlier workers have suspected over *S. clydei* of being a possible vector in the Sudan (Heisch & Guggisberg, 1953). In many African countries, *S. clydei*, essentially an out door species, may bite man viciously in large numbers (Kirk & Lewis, 1940) and one of 40 specimens fed on a patient with post-kala-azar dermal leishmaniasis subsequently showed flagellate development, though not of the anterior type. According to Heisch & Guggisberg (1953) these results are suggestive and thus *S. clydei* should be regarded as a possible vector of leishmaniasis in east Africa.

Characters	Present study (mm)	Waziristan (Sinton, 1928) (mm)	Rawalpindi (Lewis, 1967) (mm)	S. India (Ilango <i>et al.</i> , 1994) (mm)	Afghanistan (Artemiev, 1978) (μm)	Ethiopian Region (Kirk & Lewis, 1951) (mm)	Egypt (Lane, 1986) (mm)	S. Arabia (Lewis & Buttiker, 1982) (mm)
Wing length	1.68-1.72	1.6-1.7	1.55-1.74	1.6	-	1.54-1.82	-	-
Wing length/ breadth	3.9-4.2	3.8-4.1	4.2-4.4	Not given	Not given	4.13-4.27	Not given	Not given
α/β	0.75	0.48-0.74	-	0.9	-	0.55-0.97	-	-
A3 Length	0.12-0.13	0.15-0.16	0.15-0.18	0.16	148-180	0.153- 0.185	-	-
A3/A4+5	0.8-0.9	$\begin{array}{c} A3 < A4 \\ + 5 \end{array}$	0.9-1.0	0.90	-	A3 < A4 + 5	-	-
A3/Labrum	0.7-0.8	-	0.17-0.23	-	0.8-1.0	-		
Labrum	0.20-0.21	-	0.8-1.0	0.6	0.6-0.7	-		
Maxillary ventral teeth	27-29	-	0.17-0.23	0.19	-	-	-	-
Maxillary lateral teeth	3	15	16-26	12	16-26	12-26	13-16	12-13
Dental depth	0.1	2.7-2.9	-	-	2	-	-	-
Cibarial teeth, large sharply pointed	11-13	15	12	12	11-15	12-16	13-16	12-13
Pharynx length/breadth	2	2.7-2.9	-	-	-	2	-	-

Table 1. Taxonomic characters of female S. clydei.

## Phylogenetic relationships

Theodor (1948) divided the genus *Sergentomyia* into five subgenera: (1) *Sergentomyia* (which subdivided into 2 groups, *minuta* and *fallax*), (2) *Sintonius*, (3) *Parrotomyia* Theodor, (4) *Rondanomyia* Theodor and (5) *Grassomyia* Theodor. The subgenus *Sintonius* appears to form a distinct major clade. The *clydei* group is quite distinct in its subclade by having apomorphitic characters like 18 or fewer cibarial teeth as compared with *hospitii* group which is quite separated in its subclade by possessing 35 or more cibarial teeth. Within its separate subclade, *clydei* group is branched in to 2 minor-subclades. The *S. clydei* appears unique in this group with autapomorphy of mandibles with pointed apex at their lateral side, spermatheca narrowing towards the duct and male cibarium with teeth in groups of 2 or 4, while *S. tiberiadis pakistanica* has its own autapomorphic features of narrowing spermatheca towards its head and mandibles with median pointed ends. However, *christophersi*, *hospitii* 

and *pakistanica* share the synapomorphies of narrow aedeagus and beaked paramere. These 3 species, however, are clearly separated on the basis of their clear cut autapomorphies as mentioned in diagnostic characters.

Characters	Present study (mm)	Waziristan (Sinton, 1928) (mm)	Rawalpindi (Lewis, 1967) (mm)	S. India (Ilango <i>et al.</i> , 1994) (mm)	Afghanistan (Artemiev, 1978) (μm)	Ethiopian Region (Kirk & Lewis, 1951) (mm)	Egypt (Lane, 1986) (mm)	S. Arabia (Lewis & Buttiker, 1982) (mm)
Wing length	1.6	1.6-1.7	1.55-1.74	1.6	-	1.54-1.82	-	-
Wing length/ breadth	3.8-4.0	3.8-4.1	4.2-4.4	Not given	Not given	4.13-4.27	Not given	Not given
α/β	0.83	0.48-0.74	-	0.9	-	0.55-0.97	-	-
A3 Length	0.16-0.17	0.15-0.16	0.15-0.18	0.16	148-180	0.153- 0.185	-	-
A3/A4+5	0.8-0.9	A3 < A4 + 5	0.9-1.0	0.90	-	A3 < A4 + 5	-	-
Labrum length	0.20-0.21	-	0.17-0.23	-	0.8-1.0	-		
A3/Labrum	0.7-0.8	-	0.8-1.0	0.6	0.6-0.7	-		
Labrum length	0.20-0.21	-	0.17-0.23	0.19	-	-	-	-
Cibarial teeth	15-24	15	16-26	12	16-26	12-26	13-16	12-13
Pharynx length/breadth	2	2.7-2.9	-	-	2	-	-	-
Coxite length	0.23	0.25-0.27	-	-	228-271	0.213- 0.271	-	-
Style length	0.10	0.11	-	-	104-120	0.10-0.12	-	-
Aedeagus	0.13	0.06-0.07	-	-	-	0.045- 0.072	-	-
Paramere	0.20	0.21-0.22	-	-	-	0.168- 0.225	-	-
Genital filament	0.45-0.46	0.11-0.24	-	-	-	-	-	-
Genital filament/ Sperm pump	3.75	-	4.5	4.6	4.0-5.0	-	-	-
Surstyle length	0.23	0.24-0.27	-	-	-	0.27	-	-

 Table 2. Taxonomic characters of *S. clydei* (Sinton)

Though minor variations in morphometric measurements of taxonomic characters were found when compared present specimens with that of other territories; however, present work is in conformity with the findings of Lewis (1967) and Artemiev (1978). The effect of different ecological factors such as temperature, relative humidity ecological niche on the growth and size of structures of flies cannot be ruled out. Belazzoug *et al.* (1982), while working in different ecological zones of Algeria, have shown that number of cibarial teeth varies according to certain climatic factors, mainly humidity.

It is hoped that present findings would provide the base for further research on sand flies taxonomy and also on other aspects essential for the control of sand flies and the disease leishmaniases. Keeping in view of wide distribution in plains and foothills and specially its presence in human residences in the areas of cutaneous leishmaniasis, vectoral role of *S. clydei* also needs to be investigated.

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