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Short Communication

First Report of Sand Fly (Diptera: Psycodidae: Phlebotomine) Immature Males and Various Reproductive Stages of Females in Upper and Lower Dir Districts, Khyber Pakhtunkhwa Pakistan

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ABSTRACT

Study on phlebotomine sand flies was carried out in four tehsils of Upper and Lower Dir. The collected sand flies were examined to study different physiological status regarding immature stages of male and blood fed/half fed and gravid/semi gravid females. Immature male individuals belonging to three different species including *P. salengensis*, *P. sergenti*, and *P. andrejevi* were found in the collection from sheds of cow, goat and sheep in three tehsils; Timergara, Khall and Balambat. A total of 1703 female sand flies were dissected revealing 30 fed, 59 half fed and 19 gravid/semi gravid flies. The sites where immature stages or blood fed or gravid female flies were found can be considered as possible breeding sites of sand flies, consequently could be used for their source reduction in control measures.

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Authors' Contribution
NA designed and supervised the project. KK executed the experimental work and wrote the article. SW and AS helped in preparation of manuscript.

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Sand flies (Diptera: Psycodidae: Phlebotomine) are small hematophagous insects comprised of 1000 known species and subspecies (Killick-Kendrick, 1999; Kakar and Suleman, 2004). Three genera of sand flies, *Phlebotomus*, *Sergentomyia* and *Lutzomyia* are very important from medical point of view. The former two genera are present in Old World (Afro-Eurasia) while the latter is the principal genus in New World (North and South America).

Phlebotomine sand flies are responsible for transmission of several human diseases, including bartonellosis, leishmaniasis and sand fly fever viruses in many parts of the world (Service, 1996). Leishmaniasis is one of the top emerging and neglected tropical disease in the world including Pakistan. Key factors in the spread of leishmaniasis are distribution of the vectors and reservoir animals (Kakar and Suleman, 2009). No detailed and authentic information on biology of sand flies of Pakistan is available, although several attempts have been made to describe sand fly fauna from its different areas (Qutubuddin, 1951; Nasir, 1958; Burney and Lari, 1986; Barnett and Suyemoto, 1961; Ahmad and Burney, 1962; Munir, 1994; Nasir, 1964; Lewis, 1967, Burney *et al.*, 1979). An extensive study on sand flies of Baluchistan (South West Pakistan) was conducted by Kakar (2004), while Aslamkhan (1996), Aslamkhan *et al.* (1997) worked on the biodiversity of sand flies of Pakistan.

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An increase in cutaneous leishmaniasis has been reported from Pakistan (Rowland *et al.*, 1999; Reithinger *et al.*, 2003; Brooker *et al.*, 2004; Anwar *et al.*, 2007) and neighboring countries, Afghanistan (Ashford, 1992) and Iran (Yaghoobi-Ershadi *et al.*, 2004; Razmjou *et al.*, 2009). The disease is observed to be spreading continuously from endemic areas to non-endemic areas of Pakistan (Kakar and Suleman, 2011).

Grassi (1907) was the first to report an immature stage of phlebotomine sandfly in natural condition in Rome and was described as a new species, *Phlebotomus mascittii*. The first breeding site was reported at the base of a tree in Brazil (Ferreira *et al.*, 1938). Sand flies immatures stages have been found in a wide range of ecotopes and many species of sand flies employ rodent burrows, on buttress roots of trees in Panama, in termite hills in Kenya, in caves and rocks in East Africa and in the earthen floor of human habitations (Feliciangeli, 2004). Ecotopes occupied by immature phlebotomines are usually organically rich moist soils or contaminated soil of animal shelters. Despite the medical importance of leishmaniasis, scarce information is available on the ecology of the developmental stages and natural breeding sites of sand flies (Casanova, 2001) as searching for sand flies developmental stages is an extremely difficult and tedious job (Killick-Kendrick, 1987; 1999; Feliciangeli, 2004). The control efforts are therefore mainly confined to adult sand flies and personal protection (Alexander and Maroli, 2003).

Isolation of immature stages from a place will help to identify and describe types of breeding sites used by sand flies. Information on sand fly breeding sites can be used to facilitate their control by source reduction leading

to reduced density of the vectors and eventually control of the disease (Warburg and Faiman, 2011; Young and Duncan, 1994). Further information on their larval habitats could help in successful establishment of sustainable laboratory colonies that could make contribution to experimental parasitology and medical entomology.

The present study reports the first finding of immature male sand flies in natural habitats at district Dir. Reproductive stages in sand flies after insemination and before oviposition in natural conditions are also described.

Material and methods

The present entomological survey was carried out in four Tehsils, three (Timergara, Balambat, Khall) in Lower Dir and one (Warae) in Upper Dir. Panjkora is the main river which passes through both districts. There are distinct summer and winter seasons with heavy snowfall in many areas of Dir. Annual rainfall in the region is 241.23mm, with minimum and maximum temperatures 34.45°C and -2.39°C. Mostly the area is hilly surrounded by high mountains of Hindukush Range.

Collection was carried out in different indoor and outdoor habitats for which A-4 white paper (20 × 30 cm) smeared with castor oil on both sides (Sticky trap) was fixed at a height of 30 cm above the ground. Sticky traps were placed before 7:00 PM and were recovered before 7:00 AM. Captured sand flies were washed in 90% ethanol for 2-5 min. to remove oils and other debris and were preserved in 70% alcohol in a separate labeled Eppendorf tubes. These sand flies were then transferred to micro Eppendorf tubes containing Berlese's media (Hoyer's fluid) kept for 10 to 20 days, for complete removal of bristle, hairs and dust particles.

Permanent slides of sand flies were made using the same media. Fly head was separated and placed ventrally to expose the mouth parts (cibarium and pharynx) while the abdominal part was laterally placed on the slide as suggested by Aslamkhan and Aslamkhan (2000), required in taxonomic identification. Primary help in taxonomic identification of sand flies was sought from the valid and authentic keys of Lewis (1967) and Artemiev (1978). Female sand flies were dissected for the determination of whether they are blood fed/half fed/unfed and gravid/semi gravid. Juvenile stages in male sand flies were identified by the incomplete rotation of the genitalia.

Results

Like other Dipterans, male sand flies rotate their external genitalia on the longitudinal body axis through 180° in the 16-26 h of adult hood to become fully mature (external genitalia rotated). Juvenile males, about to mature with unrotated external genitalia were collected from cow sheds, belonging to three species, *P. sergenti*, *P. salengensis* and *P. andrejevi* (Table I). Male immature stages were rare as compared to mature stages (48/4375). *P. sergenti* juvenile were observed from July to October, while those of *P. salengensis* were captured in August and September. Male immature individuals of *P. andrejevi* were sampled in August only. These three species were found in the captures from three studied tehsils except Warae (Table II).

Female flies require blood (post insemination) from the vertebrate host including humans for the development of eggs. Female sand flies were separated into different categories; blood-fed, half-fed, un-fed and gravid/semi gravid on the basis of their anatomical examination. Unfed and half-fed sand flies were high as compared to blood-fed and gravid/semi graved. Gravid flies, blood–fed and half-fed flies collected from indoor sites were high as compared to collection from outdoor sites (Table III).

Discussion

Collection from different sites in and around human dwellings was made in four tehsils of district Dir, where frequent cases and outbreaks (Timergara) of cutaneous leishmaniasis (CL) were previously reported (Rowland *et al.*, 1999). In adult sand flies collection some individuals were recognized as immature male, identified by the incomplete rotation of genatailia (most probably in the first night of adulthood). As the rotation process is the last stage of male maturation, these males were therefore still very close to the oviposition sites (Moncaz *et al.*, 2012). Immature male sand flies herein are the first report not only from Pakistan but also its bordering countries, Afghanistan, Iran and India.

Majority of the surveyed houses had domestic animals (mostly bovine species) the probable hosts for female sand flies. Both sexes of *P. salengensis* and *P. sergenti* (with blood fed, gravid/ semi graved females) were collected from cattle droppings and junks accumulated in open areas around human dwellings. Such sites probably serve as favourable breeding habitats for the early developmental stages of sand flies (Killick-Kendrick, 1999). *P. sergenti* collected from cow dung is the suspected vector of CL in the area (Rowland *et al.*, 1999), also reported to be breeding in sheep pens by other worker (Branco *et al.*, 2013; Rijal *et al.*, 2010).

Alencer *et al.* (2011) reported greater number of immature forms in soil with litter, between roots and under fallen trunks. Resting or breeding sites are expected in the nearby places, where adult flies are captured as their flight capacity is limited and they tend

Table I.- Immature male of sand fly belonging to three *Phlebotomus* species collected from cattle sheds.

Species	Balambat			Timergara			Khall			Total
	Cowshed	Sheep shed	Goat shed	Cowshed	Sheep shed	Goat shed	Cowshed	Sheep Shed	Goat shed	
P. sergenti	2	-	-	20	-	-	4	-	-	**26**
P. salengensis	13	-	-	-	-	-	2	-		**18**
P. andrejevi	-	-	-	-	-	-	-	-	4	**4**
Total	**15**	**-**	**-**	**20**	**-**	**-**	**6**	**-**	**7**	**48**

Table II.- Seasonal distribution of mature and immature (in parenthesis) males of three sand fly species.

Species	May	June	July	August	September	October	Grand Total
P. sergenti	118	245	795 (3)	888(16)	134(6)	54(1)	**2234(26)**
P. salengensis	104	265	693	812(17)	171(1)	59	**2104(18)**
P. andrejevi	3	5	12	17(4)	-	-	**37(4)**
Total	**225**	**515**	**1500(3)**	**1717(37)**	**305(7)**	**113(1)**	**4375(48)**

Table III.- Reproductive stages of wild caught female sand flies regarding blood feeding and maturation of eggs.

Location	Collection site	Number examined	Number dissected	Blood-fed females (%)	Half-feed (%)	Unfed (%)	Graved/semi graved (%)
Timergara	Indoor	902	892	18(1.056)	45(2.64)	832(48.76)	7(0.40)
	Outdoor	60	58	3(0.176)	5(0.29)	50(2.94)	2(0.11)
Balambat	Indoor	379	371	06(0.35)	04(0.23)	361(21.23)	8(0.46)
	Outdoor	19	19	-	-	19(1.11)	-
Khaal	Indoor	214	213	02(0.117)	05(0.29)	206(12.11)	1(0.058)
	Outdoor	8	7	-	-	7(0.41)	1(0.058)
Warae	Indoor	140	140	01(0.058)	-	139(8.176)	-
	Outdoor	-	-	-	-	-	-

to remain localized (Chaniotis *et al.*, 1974). Scanty literature about sand fly larvae is available from diverse habitats including caves, crevices, animal burrows, termite mounds, and cracks in the soil, domestic animal shelters, cracked walls, tree-holes, birds' nests and leaf litter (Sharma and Singh, 2008). This study was an attempt to provide preliminary information regarding premature sand flies and reproductive stages to identify the most probable breeding sites which could be used as a base line for further investigation.

Information regarding physionchemical nature and physical structure of natural breeding sites can be used to control immature of sand flies in a particular study area. For example, in Sudan and Ethiopia *P. orientalis* has been known to breed in cracked black-cotton soil, which can be modified by adding gypsum for disease control (Warburg and Faiman, 2011; Gomes *et al*., 1982; Pal *et al*., 2006). Knowledge about natural breeding sites of phlebotomine sand flies is scanty an thus future studies should focus on collecting numerous substrate samples that may contain various larval or pupal stages of these insects. In addition, sampling and observation efforts should be extended to identify phlebotomine breeding sites in urban habitats to implement control strategies on larger scale.

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Statement of conflict of interest

Authors have declared no conflict of interest.

References

Ahmad, N. and Burney, M.I., 1962. *Pak. Armed Forces med. J.*, **12:** 1-11.

Alencar, R.B., DeQueiroz, R.G. and Barrett, T.V., 2011. *Acta Trop.*, **118**: 204–208.

Alexander, B. and Maroli, M., 2003. *Med. Vet. Ent.*, **17**: 1–18.

Anwar, M., Hussain, M.A., Ur-Rehman, H., Khan, I. and Sheikh, R.A., 2007. *East. Medit. Hlth. J.*, **13:** 1212–1215.

Artemiev, M.M., 1978. *Sand flies (Diptera, Psychodidae, Phlebotominae) of Afghanistan*, Kabul.

Aslamkhan, K., 1996. *Biodiversity of Sand flies (Phlebotominae) of Pakistan.* M.Sc. thesis Govt. College, Lahore, Punjab University, Pakistan, pp. 1-143.

Aslamkhan, K. and Aslamkhan, M., 2000. *Pakistan J. Zool.*, **32**: 181–182

Aslamkhan, K. Aslamkhan, M. and Azizullah, 1997. *Pakistan J. Zool.*, **29:** 351-360.

Ashfford, R., 1992. *Ann. Trop. Med. Parasitol*., **86**: 361–71.

Barnett, H.C. and Suyemoto, W., 1961. *Trans. N.Y. Acad. Sci.*, **23:** 609-617.

Branco, S., Alves-Pires, C., Maia, C., Cortes, S., Cristovão, J.M., Gonçalves, L., Campino, L. and Afonso, M.O., 2013. *Acta Trop.*, **125** 339–348

Brooker, S., Mohammed, N., Aadil, K,. Agha, S. and Reithinger, R., 2004. *Emerg. Infect. Dis.*, **10**: 1581-1584.

Burney, M. I. and Lari, F.A., 1986. *Pak. J. med. Res.*, **25**: 101-108.

Burney, M.I., Wazir, Y. and Lari, F.A., 1979. *Trop. Doct.* **9:** 110-116.

Casanova, C., 2001. *Mem. Inst. Oswaldo Cruz*, **96**: 273–275.

Chaniotis, B.N., Correa, M.A., Tesh, R.D. and Johnson, K.M., 1974. *J. med. Ent.*, **11**: 363-375.

Gomes, A., Rabello, E., Santos, J. and Galati, E., 1982. *Rev. Saud. Publ.*, **16**:149-59.

Grassi, B. and Richerce Sui Flebótomi, **1907**. *Mem. Soc. Ital. Sci. Nat.*, **14:** 353-394.

Feliciangeli, M.D., 2004. *Med. Vet. Ent.*, **18:** 71–80.

Ferreira, L.C., Deane, L. and Mangabeira, F.O., 1938. *O'Hospital*, **14:** 3–6.

Kakarsulemankhel, J.K., 2004. *Pak. J. biol. Sci.*, **7:** 593-596.

Kakarsulemankhel, J.K., 2009. *Int. J. Agric. Biol*., **11**: 125–130.

Kakarsulemankhel, J.K., 2011. *Int. J. Agric. Biol.*, **13:** 611-620.

Killick-Kendrick, R., 1987. In: *The Leishmaniases*. Academic Press, London, volume 1, pp. 473-497.

Killick-kendrick, R., 1999. *Clin. Dermatol.*, **17**: 279–289.

Lewis, J.D., 1967. *Bull. Br. Mus. nat. Hist. Ent. London*, **19**: 1-57.

Moncaz, A., Faiman, R., Kirstein, O. and Warburg, A., 2012. *PLoS Negl Trop. Dis.*, **6**: e1725.

Munstermann, L.E., 2004. In: *Biology of disease vectors* (eds. W.C. Marquardt, W. C. Black, J. E. Frier, H. H. Hagedorn, J. Hemingway, H. Higgs, A. A. James, B. Kondratieff and C.G. Moore. 2nd Ed, Elsevier, San Diego CA, pp. 141-151.

Munir, M.A., 1994. *Systematics and Biology of Phlebotomine Sand flies of the Visceral Leishmaniasis foci of Northern Pakistan*. Ph.D. thesis, Department of Medical Parasitology, Faculty of Science University of London, School of Hygiene and Tropical Medicine, London, pp. 301.

Nasir, A.S., 1958. *Pak. J. Hlth*., **8:** 21-22.

Nasir, A.H., 1964. *Pak. J. Hlth*., **14**: 26-30.

Pal, D., Bhattacharyya, S., Ray, P., Chandran, P., Srivastava, S. and Bhuse, S., 2006. *Geoderma*, **136**:210-28.

Qutubuddin, M., 1951. *Pak. J. Hlth.*, 1: 34-36.

Razmjou, S., Hejazya, H., Motazedianb, MH., Baghaeia, M., Emamyc, M. and Kalantaryb, M., 2009. *Trop. Med. Hyg.*, **103:** 727-730.

Reithinger, R., Mohsen, M., Aadil, K., Sidiqi, M., Erasmus, P. and Coleman, P.G., 2003. *Emerg. Infect. Dis.*, **9:** 727-729.

Rijal, S., Uranw, S., Chappuis, F., Picado, A., Khanal, B., Paude, I., Andersen, E.W., Meheus, F., Ostyn, B., Das, M.L., Davies, C. and Boelaert, M., 2010. *Trop. Med. Int. Hlth.*, **15**: 21–28.

Rowland, M., Munir, A., Durrani, N., Noyes, H. and Reyburn, H., 1999. *Trans. R. Soc. trop. Med. Hyg.*, **93**: 1-4.

Service, M.W., 1996. *Medical entomology for students*. Champman and Hall London, Ist ed., pp. 2-13

Sharma, U. and Singh, S., 2008. *J. Vector Borne Dis.*, **45:** 255–272.

Warburg, A. and Faiman, R., 2011. *J. Vect. Ecol*., **36**: S10–S16.

Yaghoobi-Ershadi, M.R., Jafari, R. and Hanafi-Bojd, A.A., 2004. *Ann. Saud. Med*., **24:** 98–101.

Young, D.G. and Duncan, M.A., 1994. *Mem. Am. Ent. Inst*., **54**: 88.