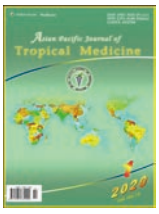

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Sandfly fauna and ecological analysis of *Phlebotomus orientalis* and *Phlebotomus martini* in the lowland foci of visceral leishmaniasis in Somali Regional State, southeast Ethiopia

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ABSTRACT

Objective: To identify the sandfly fauna and analyze ecology of sandfly vector(s) of visceral leishmaniasis in three districts of Somali Regional State, southeast Ethiopia.

Methods: Sandflies were collected from four sampling habitats, including indoor, peri-domestic, farm field and mixed forest using light and sticky traps in July 2016, and February and April 2017 in Liben and Dawa zones in the Somali Regional State, southeastern Ethiopia.

Results: In total, 4 367 sandfly specimens, belonging to 12 species (three *Phlebotomus* spp. and nine *Sergentomyia* spp.) were identified. *Phlebotomus (P.) heischi*, *P. orientalis*, and *P. martini* constituted 45.7%, 31.1%, and 23.1% of the sandfly collection, respectively. There were significant differences in the median number of *P. orientalis*, and *P. martini* captured per CDC trap/night between the three sampling districts (P<0.05). In light trap capture, collection habitats had significant effects on the abundance of *P. orientalis*, and *P. martini* (P<0.05). More median numbers of *P. orientalis*, and *P. martini* species were collected in agricultural fields followed by mixed forest and peri-domestic habitats. *P. orientalis* and *P. martini* were caught more in outdoor than indoor habitats, suggesting exophilic behaviour.

Conclusions: The study demonstrated that the presence of *P. orientalis* and *P. martini* are probable vectors of visceral leishmaniasis in this new focus. The findings of our study will improve the understanding of the dynamics of visceral leishmaniasis transmission and will facilitate the implementation of integrated disease control measures based on ecological knowledge of visceral leishmaniasis vector in Liben and Dawa zones and its surrounding regions.

KEYWORDS: Ecology; *Phlebotomus orientalis*; *Phlebotomus martini*; Sandfly fauna; Visceral leishmaniasis

1. Introduction

In Ethiopia, visceral leishmaniasis (VL) caused by *Leishmania (L.) donovani* complex is a public health threat, with approximately 3.2 million people at risk and 3 400-5 000 cases occurring annually[1,2]. This disease is known to be endemic in the semi-arid Humera and Metema plains in the northwest[2,3], and the south-western lowlands, which include Omo and Aba-Roba plains, Woyto, Segen, Dawa and Genale river valleys[3,4]. Moreover, VL cases were reported in the south-eastern semi-arid lowlands of Afder, Liben and Gode zones from the Somali Regional State[5,6].

Like any other vector-borne disease, VL is transmitted by the bite of infected female sandflies of genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World[7,8]. In the Old World, proven or suspected sandfly vectors constitute a total of 42 species, of which 26 are implicated in VL transmission caused by *L. donovani* and *L. infantum*[9].

Determining the diversity and distribution of sandfly species in different ecologies of *Leishmania* is an important step in the identification of the proven or suspected vectors. With this in mind, investigations to identify the sandfly fauna and vectors

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responsible for the transmission of two forms of leishmaniases were undertaken in Ethiopia. This research on sandfly fauna has resulted in identification of 22 *Phlebotomus* species, representing seven subgenera (*Larroussius*, *Synphlebotomus*, *Phlebotomus*, *Paraphlebotomus*, *Anaphlebotomus*, *Adlerius*, and *Parvidens*)[10-13]. Specifically, *Phlebotomus (P.) orientalis*, *P. martini*, and *P. celiae* are regarded as the proven vectors of VL in different endemic regions of the country[10,14-16]. In southern Ethiopia, the principal vector of VL is *P. martini*, which breeds and rests in termite mounds[17]; whereas in north and northwest Ethiopia, *P. orientalis* is implicated as a vector that is mostly confining to *Acacia-Balanites-Zyzphus* spp. forest and deeply cracking 'black cotton clay' soils[11,18].

In recent years, VL has spread to Liben and Dawa zones of Somali Regional State, in south-eastern Ethiopia, areas where it was previously non-endemic (Somali Region Health Bureau, unpublished data)[5]. In this new VL focus, seven cases caused by *L. donovani* were diagnosed and treated by Medecins Sans Frontieres (MSF), of which most cases occurred within the children of the pastoralist community, suggesting autochthonous transmission. In addition, in the neighbouring Bakool region of Somalia, around 1 671 VL patients were admitted to the Huddur Center of MSF Holland from January 2002 until December 2006[19]. In spite of the public health significance and the recent occurrences of VL in these particular foci, no entomological studies to determine the importance of *Phlebotomus* species were conducted. Therefore, the current study was carried out to identify the sandfly fauna and bionomics of the suspected vector(s) of VL in Liben and Dawa zones of Somali Regional State, southeast Ethiopia.

2. Materials and methods

2.1. Study areas

Entomological investigations were undertaken in two districts (*i.e.* Filtu and Deka-Softu) of the Liben zone, and one district (Hudet) of Dawa zone in the Somali Regional State, southeastern Ethiopia (Figure 1) in the months of July 2016, February and April 2017. Filtu district is one of the six districts in Liben zone. In Filtu district, the altitude ranges from 400 m along the rivers to 1 540 m at mount Fiil, east of Filtu town. The town is also located about 715 km southeast of Addis Ababa. Another district in the zone is Deka Softu that is bordered on the west with Fitu district. The centre of Deka Softu is 655 km from Addis Ababa. On the other hand, the third sampling district, Hudet is found in Dawa zone, with a latitude and longitude of 4°45'0" N and 39°13'60" E, respectively. The district is located at an elevation of 912 m above sea level.

Figure 1. Map of the study villages in Liben and Dawa zones of Somali Regional State, southeastern Ethiopia.

The climatic condition of the area is generally ranged from arid to semi-arid, with an annual rainfall of about 200-600 mm per year. The rain season starts at the middle of April and ends at beginning of June. The maximum and minimum rainfall intensities are recorded in April and October, respectively. The maximum temperature varies from 25 ℃ to 40 ℃ in March to the coolest month of July with 19 ℃-26 ℃. The vegetation comprises drought resistant bushes and *Acacia* spp., *Commiphora* spp., and *Boswellia* spp. family trees in pastoral areas. The inhabitants of the study areas are mainly pastoralists, who make their livelihood by raising camel, cattle, goats, and sheep as an essential supplement to the agro-pastoral economy. During the wet seasons, the communities grow maize and sorghum depending on the rain intensity. Squirrels, rodents, white-tailed mongoose (*Ichneumia* spp.) and foxes are some of the wildlife fauna that are commonly seen in the study villages.

2.2. Sandfly sampling

For different aspects of entomological study, three representative districts, namely Filtu, Deka-Softu, and Hudet were selected. From the three districts, six villages (Mesajid and Bekeka in Filtu; Sora and Takathager in Deka-Softu; Mersha and Luchole in Hudet) were chosen based on VL occurrence. Within the sampling villages, four representatives trapping habitats indoor, peri-domestic, farm field and mixed forest were identified and used for the entire sandfly species collection. Sandflies were trapped for two consecutive nights at each sampling village, constituting a total of 18 collection nights.

CDC light traps (CDC-LTs): Sandflies were collected in CDC light traps (LT). LTs were deployed at peri-domestic, agricultural fields, and mixed forest land. Three LTs were set in representative sites of peri-domestic habitats such as cracked walls, stone piles, and animal enclosures. Another three LTs in each village were positioned to

sample sandflies in agricultural fields, where they were positioned in open fields, dry riverbeds, and the edge of farmlands. LTs were suspended in mixed forest with the fan 0.4-05 m above the ground level. The traps were deployed 1 h before sunset and collected at dawn the next morning. Afterwards, the sandflies were sorted by sex and genus (*Phlebotomus* or *Sergentomyia* spp.), and preserved in 70% ethanol for later species identification.

Sticky traps (ST): White STs of polypropylene sheets, measuring 21 cm width and 29 cm length, coated with sesame oil were used for capturing sandflies. Four STs were installed inside five different houses in each village to intercept and capture any indoor resting (endophilic) sandflies. Similarly, another four STs were randomly installed horizontally on cracked walls, stone piles, and animal enclosures in the peri-domestic biotopes. At the same time, the remaining four STs were deployed in representative sites of agricultural fields, and mixed forest land. Two sets of STs were placed horizontally on the cracks of agriculture fields while the other two sets of STs were hung vertically in a row 30 cm above the ground supported by metal pegs. Each morning, sandflies from STs were removed using forceps and stored in 96% ethyl alcohol in labelled vials for identification.

2.3. Mounting and identification of sandflies

Sandflies collected during the study were mounted on microscope slides in Hoyer's medium with their heads separated from thoraces and abdomens. Sandflies were identified to the species level based on the morphology of external genitalia of males, pharynx and antennal features, and spermathecae of females, using different keys[20] and other publications[21].

2.4. Statistical analysis

Statistical analyses were conducted using IBM SPSS statistics, version 20 for Windows (SPSS Inc., Chicago, IL, USA), and Microsoft® Office Excel 2007. Sandfly numbers were checked for normality by Kolmogorov-Smirnov test before data analysis. When trapping data did not conform to the normal distribution, the non-parametric equivalent tests of Kruskal-Wallis and Mann-Whitney-*U* were applied. Kruskal-Wallis test (K-W) was followed to compare the median numbers of *P. orientalis* and *P. martini* collected in the three sampling villages using CDC-LTs. Likewise, Kruskal-Wallis test was used for habitat preference comparisons for CDC-LT captures. For multiple comparisons of medians, multiple-Mann-Whitney *U*-test was used and, *P*-values were adjusted with the Bonferroni correction to adjust for the inflation of type I errors when several Mann-Whitney tests are performed[22]. Mann-Whitney test (*U*) was also used for comparing sex ratio of *P. orientalis* and *P. martini* in trapping methods. Statistical analyses were considered significant when $P<0.05$ unless stated.

3. Results

3.1. Species composition and relative abundance of sandflies

A total of 4 367 sandfly specimens comprising 12 species in two genera (*Phlebotomus* and *Sergentomyia*) were collected using LTs and STs during three surveys (Table 1). Overall, 3 and 9 sandfly species in the subgenera *Phlebotomus* and *Sergentomyia* were identified, respectively. In the three sampled districts, 1 399, 1 702 and 1 266 specimens of sandfly were identified in Filtu, Deka-Softu and Hudet districts, respectively. In Filtu district, 11 species of sandfly were caught, comprising *Phlebotomus heischi*, *Phlebotomus orientalis*, *Phlebotomus martini*, *Sergentomyia squamipleuris*, *Sergentomyia schwetzi*, *Sergentomyia bedfordi* group, *Sergentomyia clydei*, *Sergentomyia antennata*, *Sergentomyia adleri*, *Sergentomyia adami*, and *Sergentomyia dubia*. In Deka-Softu district, however, 8 species of sandflies, belonging to the genus *Phlebotomus* and *Sergentomyia* were trapped, which constituted 39.0% of the total collection. *Sergentomyia africana* was only collected in Hudet district while *P. heischi* was absent from the faunal list of the district.

Table 1. Relative abundance and fauna of sandflies collected from three districts in Liben and Dawa zones, July 2016, February and April, 2017.

Sandfly species	Type of collections methods								Overall total				Relative frequency (%)
	Light traps				Sticky traps								
	Filtu	Deka-Softu	Hudet	Total	Filtu	Deka-Softu	Hudet	Total	Filtu	Deka-Softu	Hudet	Total	
Phlebotomus heischi	234	625	0	859	33	159	0	192	267	784	0	1 051	24.1
Phlebotomus orientalis	84	293	81	458	18	239	1	258	102	532	225	716	16.4
Phlebotomus martini	176	89	224	489	13	12	20	45	189	101	101	534	12.2
Sergentomyia squamiplueris	230	27	219	476	33	10	9	52	263	37	228	528	12.1
Sergentomyia bedfordi group	140	0	175	315	25	19	25	69	165	19	200	384	8.8
Sergentomyia clydei	111	38	160	309	20	21	11	52	131	59	171	361	8.3
Sergentomyia schwetzi	107	53	144	304	13	16	25	54	120	69	169	358	8.2
Sergentomyia antennata	108	45	96	249	32	27	14	73	140	72	110	322	7.4
Sergentomyia adleri	20	29	28	77	0	0	0	0	20	29	28	77	1.8
Sergentomyia adami	0	0	14	14	1	0	0	1	1	0	14	15	0.3
Sergentomyia africana	0	0	13	13	0	0	3	3	0	0	16	16	0.4
Sergentomyia dubia	0	0	4	4	1	0	0	1	1	0	4	5	0.1
Total	1 210	1 199	1 158	3 567	189	503	108	800	1 399	1 702	1 266	4 367	100.0

Males of *Sergentomyia antennata* and *Sergentomyia dubia* are morphologically difficult to distinguish with certainty[12].

Table 1 depicts the relative abundance of different sandfly species collected from the three sampling districts. Of the 2 301 *Phlebotomus* specimens, *P. heischi* constituted 45.7% of sandflies captured, followed by *P. orientalis* (31.1%) and *P. martini* (23.2%). *Sergentomyia squamipleuris* was the most predominant species, accounting for 25.6% and 12.1% of *Sergentomyia* species and the entire sandfly collection, respectively. The remaining species in descending order were *Sergentomyia bedfordi* group (8.8%), *Sergentomyia clydei* (8.3%), *Sergentomyia schwetzi* (8.2%), *Sergentomyia antennata* (7.4%), *Sergentomyia adleri* (1.8%), *Sergentomyia africana* (0.4%), *Sergentomyia adami* (0.3%), and *Sergentomyia dubia* (0.1%).

3.2. Abundance of Phlebotomus species in the three sampling districts

Table 2 shows the median numbers of *P. orientalis* and *P. martini* trapped from the three sampling districts (Filtu, Deka-Softu, and Hudet) using CDC Lts during the three collections periods. There was a significant difference in the median number of *P. orientalis* captured per CDC trap/night in the three sampling districts [Kruskal-Wallis test ($\chi_{2K\text{-}W}$=5.42); *df*=2; *P*<0.05]. Similarly, the sampling districts significantly differed in their median number of *P. martini* caught in light trap collections [Kruskal-Wallis test ($\chi_{2K\text{-}W}$=5.95); *df*=2; *P*<0.05]. Deka-Softu was the most productive sampling village for *P. orientalis* compared to Filtu and Hudet districts. In contrast, *P. martini* was trapped in large numbers in the district of Hudet followed by Filtu. The number of *P. orientalis* and *P. martini* collected in STs in all sampling habitats were fewer so that no statistically analyses were run.

Table 2. Median numbers (IQR) of *Phlebotomus orientalis* and *Phlebotomus martini* collected by CDC light traps from three different sampling districts.

Sampling districts	Median No. (IQR) of sandflies/trap/night	
	Phlebotomus orientalis	*Phlebotomus martini*
Filtu	1.51 (2.4)[a]	2.92 (3.1)[ab]
Hudet	2.33 (2.4)[a]	4.83 (2.2)[b]
Deka-Softu	5.00 (0.96)[b]	1.10 (0.7)[a]

Median values followed by different letters in the same column are statistically significant (Multiple-Mann Whitney *U*-test; *P*<0.01).

3.3. Habitat preferences of P. orientalis and P. martini

A significant difference was recorded in the median density of *P. orientalis* among the three sampling habitats using CDC Lts [Table 3; Kruskal-Wallis test ($\chi_{2K\text{-}W}$=11.94); *df*=2; *P*<0.05]. Higher median number of *P. orientalis* per CDC light/night was caught in agricultural fields [3.17 (0.43) per trap/night] than mixed forest habitats [1.46 (0.55) per trap/night], and peri-domestic [0.18 (0.12) per trap/night]. Similarly, habitat type had significant effects on the density of *P. martini* in light trap collections [Kruskal-Wallis test ($\chi_{2K\text{-}W}$=6.91); *df*=2; *P*<0.05]. The median density of *P. martini* collected per CDC light traps per night in peri-domestic, mixed forest, and agricultural fields were 0.40 (0.11), 1.47 (0.45), and 3.85 (0.23) per trap/night, respectively.

Table 3. Median numbers (IQR) of *Phlebotomus orientalis* and *Phlebotomus martini* collected in CDC light traps/trap/night from different sampling habitats.

Sampling habitats	Median No. (IQR) of sandflies/trap/night	
	Phlebotomus orientalis	*Phlebotomus martini*
Peri-domestic	0.18 (0.12)[a]	0.40 (0.11)[a]
Mixed forest	1.46 (0.55)[a]	1.47 (0.45)[a]
Agricultural field	3.17 (0.43)[ab]	3.85 (0.23)[ab]

Median values followed by different letters in the same column are statistically significant (Multiple-Mann Whitney *U*-test; *P*<0.01).

3.4. Indoor and outdoor abundance of P. orientalis and P. martini

In total, 30 and 33 specimens of *P. orientalis* and *P. martini* were captured on sticky traps deployed outdoor, respectively (Table 4). However, sticky traps placed indoors were not productive in collecting a single specimen of *P. orientalis* and *P. martini*.

Table 4. Number of *Phlebotomus orientalis* and *Phlebotomus martini* captured by sticky traps indoor and outdoor

Habitat types	Number of sandfly specimens trapped					
	Phlebotomus orientalis			*Phlebotomus martini*		
	Male	Female	Total	Male	Female	Total
Indoor	0	0	0	0	0	0
Outdoor	25	5	30	22	11	33

*Outdoor: combined collections of peri-domestic, agricultural field and mixed forest.

3.5. Sex ratio

Sex ratios (males: females) for different sandfly species showed that males caught by all methods combined was slightly higher than that of females (2 220 males: 2 147 females), with an overall sex ratio of 1.03:1 (Table 5). For *P. orientalis*, the sex ratio in LTs was 0.72:1, which was not statistically different [*U*(1)=156.50, *Z*=-0.18, *P*>0.05]. However, a relatively high ratio of male versus female (2.53:1) on the sticky traps was observed. *P. martini* had the sex ratios slightly in favour of males with 1.17:1 and 2.00:1 in light and sticky traps, respectively; while the former was statistically not significant (Mann Whitney *U*-test, *P*>0.05).

Table 5. Sex ratio of sandfly species collected from different habitats using CDC light traps and sticky traps in Filtu, Deka-Suftu and Hudet districts.

Sandfly species	Type of collection methods					
	Light traps			Sticky traps		
	Male	Female	Sex ratio	Male	Female	Sex ratio
Phlebotomus heischi	455	404	1.13	101	91	1.11
Phlebotomus orientalis	192	266	0.72	185	73	2.53
Phlebotomus martini	264	225	1.17	30	15	2.00
Sergentomyia squamiplueris	160	316	0.51	36	16	2.25
Sergentomyia bedfordi group	130	185	0.70	48	21	2.29
Sergentomyia clydei	161	148	1.09	33	19	1.74
Sergentomyia schwetzi	152	152	1.00	33	21	1.57
Sergentomyia antennata	142	107	1.33	41	32	1.28
Sergentomyia adleri	34	43	0.79	0	0	NA
Sergentomyia adami	10	4	2.50	0	1	NA
Sergentomyia africana	10	3	3.33	3	0	NA
Sergentomyia dubia	0	4	NA	0	1	NA
Total	1 710	1 857	0.92	510	290	1.76

4. Discussion

A cross-sectional entomological investigation was carried out in three districts of Somali Regional State, southeast Ethiopia. This study showed that 12 species of sandflies were distributed in this region and that 3 were *Phlebotomus* and 9 were *Sergentomyia*. To the best of our knowledge, this is the first report of sandfly fauna in this region of Ethiopia. Overall, the sandfly fauna in the present study area has distinctive Afrotropical elements, with essentially the same species found elsewhere in East Africa[8,23]. Three species of *Phlebotomus* were identified in the study areas, where *P. heischi* was the dominant, constituting 24.1% (1051/4367) of total sandfly captures. Nevertheless, the role played by this species in the epidemiology of the VL is yet unclear.

The two sandfly species, *P. orientalis* and *P. martini*, are the proven vectors of VL in East Africa[23]. *P. orientalis* is a known vector of *L. donovani* in Sudan, South Sudan[24,25], southwestern Ethiopia[14], and the northern Ethiopia[15,16]. *P. martini* has been implicated as the principal vector of VL in southern Ethiopia[10] and Kenya[26,27]. The dominance of *P. orientalis* and *P. martini* in our study was previously reported in various parts of Ethiopia[11,28,29]. These sandfly species apparently play a significant role in the epidemiology of VL in this focus area.

In addition to *Phlebotomus* spp., 9 species of *Sergentomyia* were collected and identified. The most abundant species was *Sergentomyia squamiplueris*, while the other four (*Sergentomyia bedfordi* group, *Sergentomyia clydei*, *Sergentomyia schwetzi*, and *Sergentomyia antennata*) were also common; the remaining four species were rarely trapped. In general, the sandfly fauna encountered in the present study is consistent with previous reports in other parts of Ethiopia[11,12,28–30].

The abundance of *P. orientalis* and *P. martini* differed among the three study districts. For *P. orientalis*, Deka-Softu was the most abundant sampling district while Filtu and Hudet were the least. The vast area of Deka-Softu and its environs is consisted of a vertosol soil type. This soil type is characterized by extensive cracking during dry season that contributes to a favourable habitat for resting/breeding of *P. orientalis*[18]. This factor has contributed to the moderate abundance of *P. orientalis* in the district. In contrast, *P. martini* was trapped in large numbers in the districts of Hudet, followed by Filtu. The ecological conditions in Hudet are mainly characterized by the presence of red soils, consisting of large number of pinnacle-type termite mounds. Many species of sandflies rest in the airshafts of termite hills, and *P. martini*, which occupies old shaded termite mounds, is the vector of "termite hill kala-azar" in Kenya[27] and southern Ethiopia[17]. These may explain the predominant abundance of *P. martini* in this district.

With respect to microhabitats, agricultural fields were the most productive microhabitat for the *Phlebotomus* spp. compared to other habitats as shown by light trap captures. Higher numbers of *P. orientalis* and *P. martini* were trapped in agricultural fields than other habitats, which could be related to the dominance of black cotton soil (vertosolic). Higher humidity in combination with stable temperatures maintained throughout dry season, and the availability of organic matter that provides food for immature development in the deeper layers of cracked vertisol, creating a suitable microclimate for the *P. orientalis*[18,31]. In addition, the presence of some vegetation in the farm field could provide shade and source of sugar for adult populations[31]. Earlier studies in eastern Sudan and northern Ethiopia also showed that *P. orientalis* is mainly associated to forest area with large expanses of vertisol[11,31] and rarely associated with human dwellings.

In Hudet, agricultural fields consist of large numbers of pinnacle-type termite mounds that provide ideal breeding and resting sites for the three East African species sandflies of the subgenus *Synphlebotomus* (*P. martini*, *P. celiae* and *P. vansomerenae*) and many other sandflies in southern Ethiopia and different parts of Kenya[17,26,33]. Moreover, various species of animals, particularly rodents, mongooses, and reptiles (snakes and lizards) live in the ventilation shafts of termite mounds, some of which (rodents and mongooses) are suspected as reservoir hosts of leishmaniasis[34]. As a result, the termite hill ecology requires more researches to fully understand the significance in the epidemiology of VL.

Attempts to sample indoor resting sandfly species in general and *Phlebotomus* spp. (*P. orientalis* and *P. martini*) in particular yielded fewer captures. In the same way, trapping endophilic *Phlebotomus* species using pyrethrum spray catches did not yield any specimens (result not indicated). This observation suggests that both sandfly species have a propensity towards exophilic (outdoor resting) behaviour, a finding which is in agreement with earlier studies in some districts in northern Ethiopia[11,30]. However, this finding is unlike that of Lambert *et al*.[35] where 79% of the total collected *P. orientalis* by light traps were from inside human dwellings in eastern Sudan. From epidemiological point of view, this observation has important significance in planning VL control measures based on conventional vector control methods, such as indoor residual spraying.

In our study, *P. orientalis* and *P. martini* had slightly higher proportion of male to female both in CDC light traps and sticky traps. Similar trends were also noted for other sandfly species [11,29]. Given a normal sex ratio of 1, then skewed ratios in favour of males might be related to the fact that the traps were placed near emergence sites, where males are generally abundant.

In conclusion, our data showed the occurrence of 12 species of sandflies, belonging to two genera: *Phlebotomus* and *Sergentomyia* in the southeast VL focus of Ethiopia. *P. orientalis* and *P. martini*

reported herein are the two principal vectors of VL, which could probably contribute to the VL transmission in this new VL focus. The study also revealed that *P. orientalis* and *P. martini* exhibit exophilic behaviour. This knowledge is important in the selection and application of effective vector control interventions. Programs of the exophilic behaviour of *P. orientalis* and *P. martini* should be designed and taken into account. Moreover, further studies to determine the infection rates and host preference analysis of *P. orientalis* and *P. martini* should be conducted in the current study area and other VL endemic foci in the Somali Regional State.

Conflict of interest statement

The authors declare they have no conflict of interest.

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Authors' contributions

AG and SY conceived the investigation. AG, SY, and EA performed the field work and identified the sandflies. AG, SY, and EA analyzed the data and prepared the draft manuscript. All authors read and approved the final version of the manuscript.

References

[1] Tsegaw T, Gadisa E, Seid A, Abera A, Teshome A, Mulugeta A, et al. Identification of environmental parameters and risk mapping of visceral leishmaniasis in Ethiopia by using geographical information systems and a statistical approach. *Geospat Health* 2013; **7**: 299-308.

[2] Leta S, Dao THT, Mesele F, Alemayehu G. Visceral leishmaniasis in Ethiopia: An evolving disease. *PLoS Negl Trop Dis* 2014; **8**: e3131.

[3] Hailu A, Gebre-Michael T, Berhe N, Balkew M. Leishmaniasis. In: Berhane Y, Hailemariam D, Kloos H, (eds.) *Epidemiology and ecology of health and disease in Ethiopia*. Addis Ababa: Shama Books; 2006, p. 615-634.

[4] Assefa A. Leishmaniasis in Ethiopia: A systematic review and meta-analysis of prevalence in animals and humans. *Heliyon* 2018; **4**: e00723.

[5] Marlet MV, Sang DK, Ritmeijer K, Muga RO, Onsongo J, Davidson RN. Emergence or re-emergence of visceral leishmaniasis in areas of Somalia, northeastern Kenya, and southeastern Ethiopia in 2000-2001. *Trans R Soc Trop Med Hyg* 2003; **97**: 515-518.

[6] Malaria Consortium. *Leishmaniasis control in Eastern Africa: Past and present efforts and future needs. Situation and gap analysis.* 2010. [Online] Available from: http://www.malariaconsortium.org. [Accessed on 22 May 2014].

[7] Maroli M, Feliciangeli MD, Bichaud L, Charrel RN, Gradoni L. Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. *Med Vet Entomol* 2013; **27**: 123-147.

[8] Akhoundi M, Kuhls K, Cannet A, Vot pka J, Marty P, Delaunay P, et al. A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLoS Negl Trop Dis* 2016; **10**: e0004349.

[9] Ready PD. Biology of phlebotomine sandflies as vectors of disease agents. *Annu Rev Entomol* 2013; **58**: 227-250.

[10] Gebre-Michael T, Lane R. The roles of *Phlebotomus martini* and *P. celiae* (Diptera: Phiebotominae) as vectors of visceral leishmaniasis in the Aba Roba focus, southern Ethiopia. *Med Vet Entomol* 1996; **10**: 53-62.

[11] Gebresilassie A, Kirstein OD, Yared S, Aklilu E, Moncaz A, Tekie H, et al. Species composition of phlebotomine sand flies and bionomics of *Phlebotomus orientalis* (Diptera: Psychodidae) in an endemic focus of visceral leishmaniasis in Tahtay Adiyabo district, Northern Ethiopia. *Parasites & Vectors* 2015; **8**: 248.

[12] Yared S, Gebresilassie A, Akililu E, Deribe K, Balkew M, Warburg A, et al. Diversity and altitudinal distribution of phlebotomine sandflies (Diptera: Psychodidae) in visceral leishmaniasis endemic areas of northwest Ethiopia. *Acta Trop* 2017; **176**: 1-10.

[13] Yared S, Gebresilassie A, Akililu E, Balkew M, Warburg A, Hailu A, et al. Habitat preference and seasonal dynamics of *Phlebotomus orientalis* in urban and semi-urban areas of kala-azar endemic district of Kafta Humera, northwest Ethiopia. *Acta Trop* 2017; **166**: 25-34.

[14] Hailu A, Balkew M, Berhe N, Meredith S, Gemetchu T. Is *Phelotomus (Larroussius) orientalis* a vector of visceral leishmaniasis in southwest Ethiopia? *Acta Trop* 1995; **60**: 15-20.

[15] Gebresilassie A, Abbasi I, Kirstein OD, Aklilu E, Yared S, Moncaz A, et al. Physiological age structure and *Leishmania* spp. detection in *Phlebotomus (Larroussius) orientalis* (Parrot, 1936) (Diptera: Psychodidae) at an endemic focus of visceral leishmaniasis in northern Ethiopia. *J Trop Medicine* 2015; **2015**: 710528.

[16] Kirstein OD, Skrip L, Abassi I, Iungman T, Horwitz BZ, Araya-Gebresilassie A, et al. A fine scale eco-epidemiological study on endemic visceral leishmaniasis in north Ethiopian villages. *Acta Trop* 2018; **183**: 64-77.

[17] Gebre-Michael T, Malone JB, Balkew M, Ali A, Berhe N, Hailu A, et al. Mapping the potential distribution of *Phlebotomus martini* and *P. orientalis* (Diptera: Psychodidae), vectors of kala-azar in East Africa by

use of geographic information systems. *Acta Trop* 2004; **90**: 73-86.

[18]Moncaz A, Kirstein O, Gebresilassie A, Lemma W, Yared S, Gebre-Michael T, et al. Characterization of breeding sites of *Phlebotomus orientalis*-the vector of visceral leishmaniasis in northwestern Ethiopia. *Acta Trop* 2014; **139**: 5-14.

[19]Raguenaud ME, Jansson A, Vanlerberghe V, Van der Auwera G, Deborggraeve S, et al. Epidemiology and clinical features of patients with visceral leishmaniasis treated by an MSF clinic in Bakool Region, Somalia, 2004-2006. *PLoS Negl Trop Dis* 2007; **1**: e85.

[20]Abonnenc E, Minter DM. Bilingual keys for the identification of the sandflies of the Ethiopian Region (in Fr. and Eng.). *Cah Off Rech Sci Tech Outre–Mer Entomol Med (Ser Entomol Med Parasitol)* 1965; **5**: 1-63.

[21]Gebre-Michael T, Lane R. Distinguishing the females of *Phlebotomus* (*Synphlebotomus*) *martini* and *Phlebotomus* (*Synphlebotomus*) *celiae* (Diptera: Phlebotominae), vectors of visceral leishmaniasis in southern Ethiopia. *Bull Entomol Res* 1993; **83**: 353-360.

[22]Dytham C. *Choosing and using statistics: A biologist's guide.* 3rd ed. UK: Wiley-Blackwell; 2011.

[23]Lane RP. Sandflies (Phlebotomidae). In: Lane RP, Crosskey RW. (eds.) *Medical Insect and Arachnids*. London: Chapman- Hall; 1993, p. 78-119.

[24]Elnaiem DE. Ecology and control of the sand fly vectors of *Leishmania donovani* in East Africa, with special emphasis on *Phlebotomus orientalis*. *J Vector Ecol* 2011; **36**(Suppl 1): S23-S31.

[25]Elnaiem DA, Ward RD, Hassan HK, Miles MA, Frame LA. Infection rates of *Leishmania donovani* in *Phlebotomus orientalis* from a focus of visceral leishmaniasis in eastern Sudan. *Ann Trop Med Parasitol* 1998; **92**: 229-232.

[26]Perkins PV, Githure JI, Mebrahtu Y, Kiilu G, Anjili C, Ngumbi PS, et al. Isolation of *Leishmania donovani* from *Phlebotomus martini* in Baringo District, Kenya. *Trans R Soc Trop Med Hyg* 1988; **82**: 695-700.

[27]Tonui WK. Situational analysis of leishmaniases research in Kenya. *Afr J Health Sci* 2006; **13**: 7-21.

[28]Balkew M, Gebre-Michael T, Berhe N, Ali A, Hailu A. Leishmaniasis in the middle course of the Ethiopian Rift Valley: II. Entomological observations. *Ethiop Med J* 2002; **40**: 271-282.

[29]Lemma W, Tekie H, Balkew M, Gebre-Michael T, Warburg A, Hailu A. Population dynamics and habitat preferences of *Phlebotomus orientalis* in extra-domestic habitats of Kafta Humera lowlands-kala azar endemic areas in Northwest Ethiopia. *Parasites & Vectors* 2014; **7**: 359.

[30]Aklilu E, Gebresilassie A, Yared S, Kindu M, Tekie H, Balkew, M, et al. Studies on sandfly fauna and ecological analysis of Phlebotomus orientalis in the highland and lowland foci of kala-azar in northwestern Ethiopia. *PLoS One* 2017; **12**: e0175308.

[31]Elnaiem DA, Conners S, Thmoson M, Hassan MM, Hassan KH, Aboud MA, et al. Environmental determinants of the distribution of *Phlebotomus orientalis* in Sudan. *Ann Trop Med Parasitol* 1998; **92**: 877-887.

[32]Elnaiem D, Hassan H, Ward R. Associations of *Phlebotomus orientalis* and other sandflies with vegetation types in the eastern Sudan focus of kala-azar. *Med Vet Entomol* 1999; **13**: 198-203.

[33]Gebre-Michael T, Balkew M, Yimer M, Hailu A. First record of *Phlebotomus (Synphlebotomus) vansomerenae* (Diptera: Psychodidae) in Ethiopia. *J Med Entomol* 2013; **50**: 659-663.

[34]Elnaiem DA, Hassan MM, Maingon R, Nureldin GH, Mekawi AM, Miles M, et al. The Egyptian mongoose *Herpestes ichneumon* is a possible reservoir host of visceral leishmaniasis in eastern Sudan. *Parasitology* 2001; **122**: 531-536.

[35]Lambert M, Dereure J, El-Sail SH, Bucheton B, Dessein A, Boni M, et al. The sandfly fauna in the visceral leishmaniasis focus of Gedaref in the Atbara-river area of eastern Sudan. *Ann Trop Med Parasitol* 2002; **96**: 631-636.