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Species composition, Ecology, Seasonal abundance and Vectorial importance of the sub-family Phlebotominae (Diptera: Psychodidae) in Diteta Village, Konso Woreda of Southern Ethiopia.

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ABSTRACT

A survey aimed to study species composition, seasonal abundance and infection rate of phlebotomine sandfly species were carried out in a village called Diteta, where new cases of visceral leishmaniasis (VL) have been recently observed by researchers and health centers/hospitals in the region. Sandfly densities monitored for six months (January-June, 2012) using CDC light traps and sticky paper traps yielded a total of 8091 sandflies belong to two genera: genus *Phlebotomus* (n=118) and genus *Sergentomyia* (n=7873). Three *Phlebotomus* species representing two subgenera; *Anaphlebotomus* (*P. rodhaini*) and *Synphlebotomus* (*P. martini* and *P. celiae*) together with ten *Sergentomyia* species in three subgenera were identified.

Among *Phlebotomus* species, *P.* (*An.*) *rodhaini* and *P.* (*Sy*.) *martini* were shown similar abundance with a slight dominance of the former species and *P. celiae* were the least. Five *Sergentomyia* species: *S. (Sergentomyia.) schewtzi*, *S. (Se.) bedfordi,* and *S. (Sintonius) suberecta, S. (Parratomyia.) africana* and *S. (Se.) antennata* were the most common species. A relative increase in population of the three *Phlebotomus* species were observed during the wet season (March-May) of the year. Fluctuations in sandfly densities were seen in four most common *Sergentomyia* species during the six month period with highest densities during the dry months (January-February and June) whereas *S. suberecta* had its peak in wet month (May).

A marked habitat preference was observed in all *Phlebotomus* species collected by both traps. The termite mounds were found to be most preferable habitat for all *Phlebotomus* as well as for most *Sergentomyia* species. However, the observation of low densities of all three *Phlebotomus* during the study period does not seem to explain adequately the occurrence of VL in the area. More detailed epidemiological and entomological studies are thus required to reveal the real VL situation in the area.

1. INTRODUCTION

Phlebotomine sandflies are major biting pests of man and are vectors of several viruses such as a bacterial disease called *Bartonellosis* (caused by *Bartonella bacilliformis*) and several arboviruses (Ivovic *et al*., 2007) and most importantly, the protozoan parasites of the genus *Leishmania* that cause leishmaniasis. Worldwide, there are 2 million estimated new cases of leishmaniasis annually, and 12 million people are currently believed to be infected (WHO, 2010).

Leishmaniasis is one of the neglected tropical diseases (NTDs) that affect the lives of millions worldwide and threaten the health of millions more. In recent years, there has been a progressive increase in new cases of leishmaniasis from areas which were regarded as *Leishmania*-free areas due to several factors such as population migration, environmental and climate change, and socioeconomic factors (Ngure *et al*., 2009; WHO, 2010).

It is evident that efforts in combating further spreading of the disease and know the true incidence and prevalence mainly requires exhaustive understanding of the vectorial capacity and vector-parasite interaction (Carvalho *et al*., 2006). Control methods targeting on the disease vectors are reported as one among the five strategies recommended by WHO (2003) towards prevention and control of NTDs for the well-being of humans (WHO, 2010). WHO designated leishmaniasis as emerging and yet uncontrolled disease. Vector control requires a prior knowledge on the taxonomy and geographical distribution of the responsible vectors of the disease (WHO, 2003).

Leishmaniasis is transmitted by the bite of previously infected female phlebotomine sandflies which are prevalent throughout the tropics and sub-tropical regions of the Old World (southern Europe, the Mediterranean, Asia and Africa) and the New World (South and Central America (Banuls *et al*., 2007, Gavgani *et al*., 2008).

The phlebotomine sandflies belong to family Psychodidae, subfamily Phlebotominae, of the order Diptera. The subfamily comprises two medically important genera: *Lutzomyia* in the New World and *Phlebotomus* in the Old World which include several species serving as vectors of the different forms of leishmaniasis throughout the World (Killick-Kendrick, 1999).

1.1. Taxonomy of phlebotomine sandflies

Phlebotomine sandflies are characterized by their wing venation (the presence of numerous parallel veins running to wing margin) and the presence of dense hairs on the wings and thorax and are called phlebotomine. They are also characterized by the presence of biting mouth parts, five-segmented palps, 15-segemted cylindrical hairy antennae, hairy body and a characteristic V-shaped wing posture at rest (Lane, 1993).

An estimated, 800 species of sandflies classified into six genera are known to exist throughout the World. Of these, *Phlebotomus*, *Sergentomyia*, and *Chinus* are found only in the Old World whereas *Lutzomyia*, *Warileya*, and *Brumptomyia* are known as New World genera (WHO, 2010). Species of the three genera: *Phlebotomus*, *Lutzomyia* and *Sergentomyia* suck blood from vertebrates. Members of the genus *Phlebotomus* can often be distinguished from those within genus *Sergentomyia* by the pattern of abdominal dorsal hairs which are erect in *Phlebotomus* and recumbent in *Sergentomyia* (Lane, 1993).

Adult phlebotomine sandflies are recognized by three main features; when at rest, they characteristically hold their wings at an angle above the abdomen; they are hairy and have a characteristic hopping type of flights (Killick-Kendrick, 1990). In addition, they are minute in their size (1.3-3.5mm in length), with relatively large black compound eyes and their body color ranging from almost white to almost black. Both sexes require sugar meal for their nutrition, but only the females engorge blood meal to facilitate oogenesis (Maroli *et al.,* 2012; Volf and Volfova, 2011).

Out of the total described number of sandfly species, about half of them are contained only in two genera: genus *Lutzomyia* (with 26 subgenera) and genus *Phlebotomus* (with 12 subgenera), in which medically important vectors of human disease where belong (WHO, 2011; Maroli *et al.,* 2012).

Phlebotomine sandflies are distributed in five zoogeographical regions of the World extending mainly between latitude of 50° N to 40° S with exception in the New Zealand and the Pacific islands and an altitudinal range extending from below sea level (by the Dead Sea) to over 2800 meter above sea level in the Andes and Ethiopian highlands (Lane, 1993; Umakant and Sarman, 2008).

Old World genera are found mainly in three subzones: Mediterranean-Middle Asian, Indian, and East African subzones associated with ecology of savanna areas of low rainfall, *Phlebotomus* being the dominant genus comprising most man-biting vector species of medical importance (Killick-Kendrick, 1999**;** Umakant and Sarman, 2008; WHO, 2010). New World genera are found distributed in Neotropical regions of the North, South and Central America inhabiting mainly of forest areas with higher rainfall (Lane, 1993; WHO, 1997; Umakant and Sarman, 2008).

1.2. Biology and ecology of Sandflies

Sandflies are holometabolous insects proceeding through four developmental stages (egg, larva, pupa and adult). With a few exceptions of autogenus species, all female sandflies need blood-meal during the gonotrophic cycle to facilitate oogenesis and complete oviposition (WHO, 2010; Volf and Volfova, 2011).

Immature stages (egg, larvae and pupa) live in moist microhabitats rich in organic matter. Eggs are deposited around a potential breeding site such as in small cracks and holes in the ground, tree holes, termite mounds, rodent burrows and leaf litter. At each oviposition, some 30-70 minute eggs are laid which are more or less ovoid in shape and usually brown or black in color (Jacobson, 2003). In moist microhabitat with high humidity, eggs will hatch within 4-20 days to first instar larvae or diapauses in wetter habitats (Tesh *et al*., 1998; WHO, 2010).

Freshly emerged larva is creamy white having hairs on its body. It is voracious, feeding on organic matter present in the soil. Development of the larva depends on the availability of food, ambient temperature and humidity (Killick-Kendrick, 1999; WHO, 2010). Up on feeding, the 1st stage larva moults 3 times to form the 4th stage larva and pupae in 25–59 days (Srivastava, 2006; Volf and Volfova, 2011) or overwinter in the forth instar in regions with cool winters (Azar and Nel, 2003).

Sandfly pupa is elongated comma shaped, non-feeding and non-motile stage. The larval skin is not completely cast off rather remains attached to the end of the pupa and the presence of this skin with its characteristic two pairs of caudal bristles aids in the recognition of phlebotomine pupae and after about a week, the adult sandfly will emerge out from the pupae within 35–69 days (Azar and Nel, 2003; WHO, 2010).

The sandfly larval habitats have been identified for only a very few species. In the Old World, immature stages of *P. argentipes*, *P. papatasi*, *P. ariasi*, *P. perfiliewi* and *P. langeroni* have been recovered from soil taken from inside human dwellings or domestic animal shelters (Killick-Kendrick, 1987; Doha *et al*., 1990). Larvae of *P. papatasi* and *P. duboscqi* have consistently been recovered from soil taken from inside of rodent burrows (Doha *et al*., 1990; Perfil'ev, 1968; Artemiev *et al*., 1972).

In Kenya, termite hills and rodent burrows have been shown to be breeding sites of some *P. martini* and several *Sergentomyia* species by incubating soils from such habitats (Mutinga and Kadu, 1986). Caves have also been reported as the main breeding and resting sites for *P. sergenti*, the vector of *L. tropica* in the Old World (Moncaz *et al*., 2012). Caves were also implicated as probable breeding sites of *P. pedifer*, vectors of CL caused by *Le. aethiopica* in Ethiopia and Kenya (Mutinga and Odhiambo, 1986) in Kenya.

In the New World, livestock shelters have also been shown to be larval habitats for *Lu. longipalpis* and *Lu. intermedia* (Rutledge and Ellenwood, 1975). Larvae of other species (*Lu. trapidoi*, *Lu. umbratalis*, *Lu. anduzei* and *Lu. whitmani*), have been found among soil and leaf litter on the forest floor (Rutledge and Ellenwood, 1975; Arias and Freitas, 1982; Casanova, 2001).

However, for many of the species listed above, very few immature specimens have been recovered, and thus the importance of these as larval habitats is unclear. On the other hand, for some species, enough evidence has been compiled to make more definitive conclusions about their larval habitat. For example, the primary habitat of *P. papatasi* immature outside urbanized areas is considered to be rodent burrows as it also for *P. duboscqi* (Feliciangeli, 2004).

During daylight hours, adult sandflies prefer to stay in resting sites which are comparatively cool, humid niches, including bedrooms; latrines; cellars; stables; caves; fissures in walls, rocks or soil; dense vegetation; tree holes and buttresses; burrows of rodents and other mammals; birds' nests; and termite mounds (WHO, 2011).

Many species of female sandflies are predominantly exophagic and exophilic and their activities are usually restricted to the vicinity of larval breeding sites which is mainly associated with flight and dispersal range, often affected by wind speed (Maroli *et al.,* 2012; Azar and Nel, 2003). Flight speed of phlebotomine sandflies is considerably slower than mosquitoes and is < 1 meter per second and their speed of flight is limited at the wind speed exceeding this rate (Maroli *et al.,* 2012).

1.3. Diseases transmitted by Phlebotomine sandflies

Phlebotomine sandflies are of considerable public health importance because of their ability to transmit several viral, bacterial, and protozoal disease-causing pathogens of humans and other animals (Azar and Nel, 2003). From the six genera of family Phlebotominae, genus *Phlebotomus*, *Lutzomyia* and *Sergentomyia* are hematophagus on vertebrates. However, only the former two contain species which are vectors of human diseases including viral (sandfly or papatasi fever), bacterial (bartonellosis) and protozoal (leishmaniasis) (WHO, 2011; Maroli *et al*., 2012).

Sandfly fever (also known as papatasi fever or three-day fever), caused by a virus belonging to the genus *Phlebovirus* (Lane, 1993). It is a mild febrile disease transmitted mainly by female *P. papatasi* and possibly by other species such as *P. perniciosus* and *P. chinensis* (Azar and Nel 2003). Bartonellosis (also known as Carrion's disease) is a disease caused by an aerobic and gram- negative bacterium called *Bartonella bacilliformis*. In humans, the disease shows two different clinical features including muscle and joint pain, fever, headache (in the acute phase) followed by eruptive phase in which patients develop a cutaneous rash (Maroli *et al*., 2012). Phlebotomine sandflies of the genus *Lutzomyia*: *Lu. verrucarium* is considered as the vector and its closely related species, *Lu. colombiana* is thought to be the vector in Colombia (Lane, 1993).

1.4. Leishmaniasis

Leishmaniasis is one of an old, yet remains neglected vector-borne diseases caused by blood and tissue dwelling protozoan flagellates of genus *Leishmania* (order Kinetoplastida, family: *Trypanosomatidae*) (Almeida *et al*., 2003). From 30 so far described species of the genus *Leishmania*, at least 23 of them are known to be pathogenic to humans (Ngure *et al*., 2009; WHO, 2011).

The clinical manifestations of the disease vary in humans, and are grouped into two major forms: those which have the tendency to cause cutaneous lesions that lead to the disease form known as cutaneous leishmaniasis (CL), and those showing systemic infections with predominantly visceral lesions causing the form known as visceral leishmaniasis (VL, also known as kala-azar) (Ximenes *et al*., 2000; Emami and Yazdi, 2008; WHO, 2008).

Other manifestations such as: post-kala-azar dermal leishmaniasis (PKDL) and leishmaniasis recidivans (LR) are also described to exist in some parts of the World (Lawyer *et al*., 2004; Gravelink and Lerner1996). Leishmania-HIV co-infection has now surged as a major emerging form which demands urgent attention. In Africa, particularly in Ethiopia and Sudan from the total number of VL patients, 70% are reported to be *Leishmania*-HIV coinfection (Ngure *et al*., 2009).

Globally, leishmaniasis is estimated to cause the ninth largest disease burden among individual infectious disease and second protozoal disease, only surpassed by malaria afflicting mainly the world's poorest population in tropical and subtropical regions of the Old World and New World (WHO, 2010). Recent survey revealed the distribution of the disease in 98 countries or territories among which 72 are from Old World of which, 13 are among the poorest and least developed countries (Mac Morris, 2008; WHO, 2010).

Though intricate to generate precise figure on the global burden of the disease, it is believed that worldwide more than 350 million people are reported to be at risk and a prevalence of 2 million new cases per year accounts for the two major forms of the disease (~1.5 million cases of cutaneous leishmaniasis (CL) and 0.5 million case of visceral leishmaniasis (VL) is estimated (Umakant and Sarman, 2008; WHO, 2008, 2010).

With the exception of Antarctica, *Leishmania* species have been reported on every continent and major disease forms are primarily endemic in tropical and sub-tropical regions. In the Old World, it is most notably found distributed in the Mediterranean basin countries whereas in the New World, it occurs in the Central, North and South America (WHO, 2006).

Many of the *Leishmania* species are known to be associated with the first form where the parasite only affect the skin to cause skin lesions being mainly confined to the cutaneous tissues (Emami and Yazdi, 2008). In the Old World, members of the *L. tropica* species complex (*L. tropica, L. major* and *L. aethiopica*) along with other members of *L. donovani* complex (*L. donovani* and *L. infantum*) are known as the major causative agents for CL and VL, respectively (WHO, 2011; Alvar *et al*., 2012).

While in the New World, members of the two *Leishmania* complex groups: *L. brazilensis* complex (*L. brazilensis, L. panamensis*/*L. guyanensis*, *L. shawi*, *L. peruviana*) and members *L. mexicana* complex (*L. mexicana, L. amazonensis, L. venezuelensis*) together with other strains such as *L. lainsoni*, *L. naiffi* and *L. lindenbergi* (for CL) and only one species of the *L. donovani* complex; *L infantum* (for VL) are major etioletic agents (Emami and Yazdi, 2008; WHO, 2010).

The epidemiology of leishmaniasis is governed mainly by the presence of specific vector species and probable animal reservoir host that are capable of supporting the development of a pathogen (Umakant and Sarman, 2008). The *Phlebotomus* and *Lutzomyia* species facilitate the development of *Leishmania* parasite through the interaction with the midgut molecules of the vector that provides attachment of the parasite to the surface which is vital for the *Leishmania* life cycle (Svobodova *et al*., 2006; Myskotva *et al*., 2007).

In East Africa, the two major forms of the disease are known to be endemic mainly in countries: Ethiopia, Kenya, former Sudan, Somalia and Uganda (Desjeux, 2004; Chappuis *et al*., 2007; WHO, 2011). In the region, several epidemics of the disease have occurred, particularly since the 1990s (Seaman *et al*., 1996; Marlet *et al*., 2003; Alvar *et al*., 2007).

1.4.1. Leishmaniasis in Ethiopia

In Ethiopia both forms of the disease are known to occur. Four *Leishmania* species; *L. donovani* (causing VL), *L. aethiopica, L. major* and *L. tropica* (causing CL) are identified as etioletic agents (Hailu *et al*., 2006) found widely distributed in different ecological settings and altitudinal ranges of the country.

1.4.2. Visceral leishmaniasis

A proper documentation of visceral leishmaniasis in Ethiopia goes back to the time of World War II as reported by Cole *et al*., (1942) and Anderson (1943) as reviewed by Hailu *et al*., (2006). In Ethiopia, VL is generally known to be distributed throughout the lowlands with varying degrees of endemicity (Ayele and Ali, 1984; Hailu, *et al*., 2006), but are recently appearing in some highland areas in the north (2000m above sea level) (Alvar *et al*., 2007).

In the country, data collected during the years 2004-2008 showed a case report of 1860 VL cases per year and an estimated annual VL incidence of about 3700-7400 (Alvar *et al*., 2012). Important endemic foci include the northwest (Metema, Humera, Wolkayit, Libo-Kemekem/Fogera (Ashford *et al*., 1973; Mengesha and Abuhoy, 1978; Hailu and Frommel, 1993; Alvar *et al*., 2007) and south and southwest (Dawa, Genale, Gelana, Segen, Woito, Konso and Omo Valley) and areas adjoining Kenya and Gambella–Sudan border (Fuller *et al*., 1979, Ali and Ashford, 1994; Hailu *et al*., 2006). The Awash Valley (Rift Valley), in northeast Ethiopia up to the Djibouti border is a highly suspected VL focus (Ali *et al*., 2004; Hailu and Berhe, 2002).

To date, no definite animal reservoir of VL has been identified in Ethiopia, although there have been several investigations (Gebre-Michael and Balkew, Pers. Comm.).

1.4.3. Cutaneous leishmaniasis

In Ethiopia, CL is caused by three different species of *Leishmania* parasites which includes *L. aethiopic*a, *L. major* and *L. tropica*. However, the disease is mainly caused by the former species which is typically found in the highlands of Ethiopia between 1700m -2700m above sea level and in almost all regions of the country (Negera *et al*., 2008; Hailu *et al*., 2006). The latter two species are very rare being detected in very few patients in the lowlands: *L major* in the lower Omo valley and *L. tropica* in the Awash valley (Hailu *et al*., 2006).

The distribution and prevalence of CL (*L. aethiopica*) has only been studied in few localities or endemic foci of the country, but it is believed to have a wider distribution affecting thousands of people than is currently known. The major foci investigated so far includes the highland areas of Meta-Abo (Sebeta), Aleku (Wollega), Kutaber (Wollo), Ochollo (Gamo-Gofa), Silte Woreda and even in some localities of peripheral Addis Ababa (Ashford *et al*., 1973; Gemetchu *et al*., 1990; Hailu *et al*., 2006; Negera *et al*., 2008). The disease has three different clinical forms: localized CL (LCL), mucocutaneous CL (MCL) or diffuse CL (DCL) (Hailu *et al*., 2006). The parasite is naturally harbored by rock hyraxes (*Procavia* and *Heterohyrax* species) (Hailu *et al*., 2006) from which it is transmitted to humans living close to hyrax habitats by at least two phlebotomine sandflies (see below). The hyrax habitats include rocky habitats, cliffs, caves and trees living in close association with the sandfly vectors (Ashford *et al*, 1973).

Presently, no effective drugs are available to cure the disease; hence, the disease remains a major challenge for the affected communities as well as for the health authorities (Hailu *et al*., 2006).

1.4.4. Vectors of Leishmaniasis in Ethiopia

In Ethiopia at least about 40 species of phlebotomine sandflies are so far recorded with few more new species or records being reported from time to time (Ashford, 1974; Gebre-Michael *et al*., 1986, 1996; Gebre-Michael and Balkew, 2002, 2003). Of these, 19 belong to the genus *Phlebotomus* and 21 to the genus *Sergentomyia*. The criteria set out by WHO (2011) for incriminating a sandfly species as vector comprises the following:-, the species must be anthropophilic, must bite reservoir host(s), and must be naturally infected with the same *Leishmania* species that is causing disease in the same locality. Based on these criteria, at least eight species of *Phlebotomus* have been incriminated as vectors of the *Leishmania* species in Ethiopia.

The incriminated vectors of VL so far include *P. orientalis* in the lower Omo valley (Hailu *et al*., 2006), *P. martini* and *P. celiae* in southern Ethiopia of Segen Valley (Gebre-Michael and Lane, 1996), where the former two being the principal vectors (Hailu *et al*., 2006). The three species have a much wider distribution than the disease in lowland regions of Ethiopia. *P. orientalis* is mainly found in the north, northwest, southwest and eastern Ethiopia, whereas *P. martini* and *P. celiae* are mainly found in the south and southwest (Gebre-Michael and Balkew, Pers. Comm.).

Ecologically, these vectors are found in distinct ecological settings. *P. orientalis* occurs in woodland savanna areas associated with cracking vertisol soil type (black clay soil) covered predominately by *Acacia* and *Balanties* vegetation (Gemetchu, *et al*., 1976; Hailu, *et al*., 1995; Hailu *et al.*, 2006). The cracks in the soil are thought to be ideal breeding and resting habitats for the vector, although this has not been demonstrated conclusively.

The known areas infested with *P. orientalis* include endemic areas of northwest (Metema and Humera lowlands), southwest Ethiopia (Lower Omo valley), northern Ethiopia, and the Awash valley (Gemetchu, *et al*., 1976; Gebre-Michael *et al.*. 2003; Gebre-Michael *et al*., 2007). This species is also the major vector of VL in Sudan (WHO, 2011) where much of the endemic areas and the vector distribution are contiguous with endemic areas of northwest Ethiopia. However, the vector status of *P. orientalis* in north and northwest Ethiopia remains to be confirmed, although it is highly suspected.

P. martini and *P. celiae,* on the other hand are widely distributed in the lowlands of south and southeast Ethiopia wherever there are termite mounds. They are said to be very rare or absent in areas without termite mounds. The ventilation holes of termite mounds are believed to be the preferred breeding/resting sites of the two species (Gebre-Michael and Lane, 1996). The two species were first incriminated as vectors of VL in the Aba Roba focus (Segen valley) in Konso district of southern Ethiopia (Gebre-Michael and Lane, 1996). Both *Phlebotomus* species are now highly suspected to be vectors in other endemic areas of south and southeast Ethiopia where termite mounds and the species are abundant and VL is endemic (Negelle and Hammer areas) (Jemal, 2010; Prof. Asrat Hailu, Pers. Comm.), although no parasites have yet been detected in them in these areas. *P. martini* is also the major vector of VL in Kenya, and suspected to be so in Somalia (WHO, 2010).

CL caused by *L. aethiopica* is known naturally to be transmitted by two closely related *Phlebotomus* species in Ethiopia: *P. longipes* and *P. pedifer* (WHO, 2010). The two species have never been found together. However, *P. longipes* has a much wider distribution in Ethiopia than *P. pedifer* and hence, it is the major vector of CL *(L. aethiopica*) in the highlands Ethiopia. *P. pedifer* has a localized distribution, but an important vector of the disease in a highly endemic area of the disease in Ochollo, near Arba Minch (Hailu *et al*., 2006). The species has also been recorded in highland areas near Jimma and Wolyita Sodo (Gebre-Michael and Balkew, Pers. Comm.). *P. pedifer* is also the vector of CL (*L. aethiopica*) in Kenya.

In Ethiopia, recently, the parasite has also been isolated from *P. sergenti* near Metahara where the disease has never been detected in the human population (Gebre-Michael *et a*l., 2004). It is believed that the parasite is probably circulating among some wild animals in the area (Hailu *et al*., 2006).

L. major, which is so far a rare parasite in Ethiopia (but a major cause of CL in the Old World) has been isolated from *P. duboscqi* in the Segen Valley where the disease is yet absent in the human population in the area (Hailu *et al*., 2006). The species is also the vector of CL (*L. major*) in Kenya, Sudan and West Africa (WHO, 2010).

Two closely related species *P. sergenti* and *P. saevus* have recently been incriminated as vectors of *L. tropica* in eastern Ethiopia (Upper Awash and Middle Awash) where the disease (CL) is apparently rare or absent (Hailu *et al*., 2006). The infection rate in both species was relatively high, suggesting some wild animal serving as a reservoir of the parasite (Gebre-Michael *et al*., 2004). *P. sergenti* is a well known vector of CL caused by *L. tropica* in the Mediterranean region, southern Europe and Asia where the disease is predominantly anthroponotic (WHO, 2011). However, in Kenya, southern Africa and Israel, *L. tropica* has also been found in hyraxes (Jacobson *et al*., 2003; WHO, 2011) suggesting that the same zoonotic cycle involving the hyraxes might exist in the Awash Valley. Hyraxes were commonly observed in their study areas of the Awash Valley (Gebre-Michael *et al*., 2004).

1.5. Control measures against sandflies

In the absence of any effective vaccine and ideal drug, control strategies targeting to the disease vector and reservoir host are considered as vital in combating vector-borne disease such as human leishmaniasis (Sharma and Singh, 2008; Davies *et al*., 2003; WHO, 2011). This rely on enough knowledge on the biology of vector species, its habitats (peridomestic or sylvatic), flight range, host feeding preferences, resting sites, circadian rhythms and seasonality (WHO, 2010). Since little is known about the breeding and daytime resting sites of the immature stages, most control measures depend on combating adult phlebotomine sandflies (Casanova, 2001; Sirak-Wizeman *et al*., 2008).

Yet no single and effective strategy developed to control sandfly vectors and current control efforts focus on the use of holistic approaches under the principle of integrated vector management (IVM) (WHO, 2011). IVM advocate the use of different control methods including: chemicals, environmental management and personal protection jointly under the parasol of basic precautions in designing IVM (WHO, 2010).

The main methods for controlling sandflies with insecticides includes: indoor residual spraying (IRS); Spraying of resting sites of sylvatic species; use of insecticide-impregnated materials such as bed nets (ITN) and curtains; and pyrethroids impregnated dog collars are commonly recommended (WHO, 2011). IRS synthesized from organophosphates such as Malathion in a paint formulation (polyvinyl acetate-based suspension of Malathion), carbamates like propoxur and synthetic pyrethroids such as deltamethrin and ƛ-cyhalothrin, and parametrine (as diffusible insecticide). For sylvatic sandflies, chemical control is impractical (Alexander *et al*., 1995) although it was reported that spraying termite hills and rodent burrows with Cyflulrin reduced *P. martini* in Kenya (Alvar *et al*., 2007) but problems associated with difficulties in achieving adequate coverage with insecticides, persistence of the chemical and threat with non-target organisms are still remains unsolved (Alexander and Maroli, 2003).

ITNs with recommended mesh size and treated with synthetic pyrethroids (Permethrin, Deltametrin and Lambda-Cyhalothrin) are reported as effective due to the repellent effects of the insecticides (El-Naiem *et al*., 1999; WHO, 2008) and understanding of the sleeping traditions of the population and the biting habits of the local vector (Malaria consortium, 2010; WHO, 2008). However the most challenging aspects of vector control involving IRS or ITNs is that both methods work best only when the vectors are endophilic and endophagic. Unfortunately, most sandfly vectors are exophilic and exophagic.

Repellents, whether natural or synthetic can be used as personal protection methods, either indoors or outdoors. Natural repellents extracted from locally available plants such as Citronella, E*ucalyptus* and Neem have been found effective for most biting Dipterans including sandflies (Alexander and Maroli, 2003; Kebede *et al*., 2010). Synthetic repellent such as diethyl-phenyl-acetamide (DEET) and N, N-diethyl phenyl acetamide (DEPA) are also effective against a variety of biting insects including sandflies (Alexander and Maroli, 2003, Naucke *et al*., 2006).

Concurrent with the above mentioned control strategies, manipulating the environment to make it unsuitable for breeding or resting of sandfly vectors through mechanical destruction of microhabitats such as by ploughing the possible breeding/resting sites of sandflies (Alexander and Maroli, 2003; WHO, 2008), plastering and filling of cracks and crevices on walls by mud and lime are reported as feasible in reducing some endophilic sandflies in some regions (Yaghoobi-Ershadi *et al*., 2000; Kishore *et al*., 2006).

In Ethiopia, there has not been any systematic vector control against leishmaniasis yet, except for an experiment study in a VL focus in southern Ethiopia by spraying termite hills with DDT and a pyrethroids insecticide which resulted in a limited success for a very short period of time (Gebre-Michael, unpub. data). The only means to deal with leishmaniasis to date is to treat patients with drugs. This capacity is however limited to few specialized hospitals, research organizations, and NGOs which are usually located far from endemic areas. The current IRS and ITNs campaigns against malaria in the country are unlikely to affect the transmission of leishmaniasis since the vectors are predominantly exophillic. Therefore, attention should be given to target the sandfly vectors for which more detailed studies on the biology, behavior and ecology of the vectors are necessary.

2. STATEMENT OF THE PROBLEM

VL is endemic in many tropical and subtropical regions of the world, occurring mostly in isolated foci. Endemic areas are currently expanding into previously *Leishmania*-free areas and there has been a sharp increase in the number of cases in both forms of the disease (WHO, 2010).

In Ethiopia, the disease is recently being expanding to the areas found surrounding to the known endemic foci in Southwest part of the country particularly within Konso woreda and its surroundings, where it has never been reported before. Among the areas, in the remote village called Diteta, found near a special woreda called Kolme, new case of VL is being reported for the last seven consecutive years (2005-2012) with a record of more pediatric cases than adults (Prof. Asrat Hailu, Pers. Com; Konso woreda health office data). Despite the fact, yet there is no or little entomological efforts has been conducted in view of determining the sandfly fauna and identify the *Phlebotomus* species responsible as disease vector(s) in the area which is crucial for the efforts to be done in association with designing and implementing of control strategies targeting to the disease vector(s) in the village.

The significance of the study is therefore; to explore the species composition and determine the ecology and host preference of phlebotomine fauna found in the village with the aim of incriminating the possible disease vectors in Diteta village and bridge the gap of knowledge through providing entomological informations for further efforts to be done in reducing the spreading of leishmanial disease and trim down man-vector contact in the area.

3. OBJECTIVE OF THE STUDY

3.1 General Objective

To determine the prevailed species composition of Phlebotomine fauna and investigate the transmission dynamics of *Leishmania* disease revealed in the study area with respect to incrimination of the possible *Phlebotomus* vectors, their ecology, abundance and determine the degree of man-vector contact in the new VL foci, Diteta village (Konso Woreda) SNNPR.

3.2. Specific Objective

- To determine species composition of phlebotomine sandflies in the study area.
- To investigate the vectorial importance of sandfly species in the transmission of VL in the area.
- To determine the monthly density of *Phlebotomus* species in the study period.
- To study habitat and host preference of *Phlebotomous* species in the area
- To determine the correlation between meteorological variables (temperature, relative humidity, rainfall, and wind speed) and phlebotomine sandfly densities in the study period.

4. MATERIALS AND METHODS

4.1. Description of the Study Area

This study was conducted in Diteta village, Konso woreda (05^0 20'N, 037^0 26'E) with an elevation of 1200-1470 meters above sea level is located 596 KM south from Addis Ababa. The administration town is located in the Segen people's zone, Ali Woreda. According to a statistical data of the year 2011 (a report taken from the Konso woreda Population Affair Coordination and Implementation Office) an estimated 236,897 people are living in the Konso woreda, of which about 11,782 (5%) account for Gelgele-kolmele Kebele where the actual study locality is found.

There is a health center in Kolme special kebele, about 12 kilometers from the study area. In the health center one health extension officer and 10 nurses are put in charge to provide health care for the people (Konso Woreda Health office). The natural vegetation cover of the study area was dominated by *Acacia* trees and thorny bushes. Nearby compounds, a common food tree, *Moringa stenopthalia* (local name Haleko) and cereal crops such as maize, sorghum, soybean, and cotton plants are found.

During the preliminary survey, about 30 termite hills were recorded distributed around hut's, farm fields, hillsides, and forests in the area. However, there could have been more termite hills but the hilly topography of the area made it difficult to make comprehensive surveys.

Termite mounds are favorable resting/breeding sites for some East African vectors of VL in the region (Wijers and Minter, 1962; Minter, 1964; Gebre-Michael and Lane, 1996). At dusk, children in the village are often seen playing on termite mounds found near their huts. These conditions together with the presence of livestock kept in their backyards might increase human-sandfly that could lead to the risk of contacting VL.

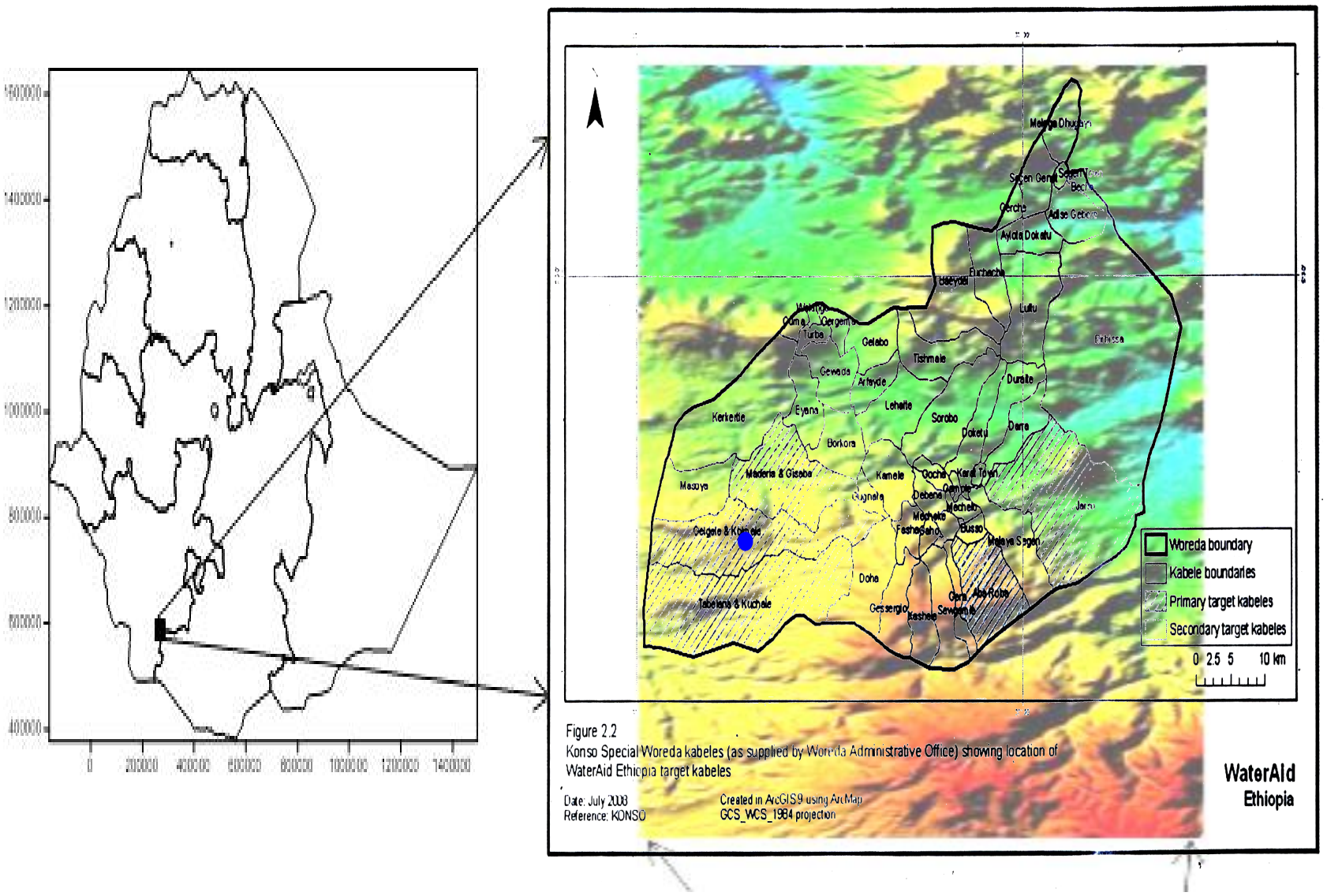

Figure 1. Map of the study area

4.2. Collection of Sandflies

Sandflies were collected regularly in every two weeks sampling interval for 6 months (from January, 2012 to June, 2012) using two methods; Center for Disease Control and Prevention (CDC) miniature light traps and sticky paper traps in selected sample sites both outdoors and indoors. The outdoor collection sites included termite hills, farm fields and compounds combined with indoor collection.

4.2.1. CDC light trap collection

In every two weeks sampling intervals, four CDC light traps, powered by 6 volt dry cell batteries were used to collect sandflies from termite mounds, farm fields and compounds. The light traps where suspended about 50cm off the ground or ventilation shaft of termite mounds and ran from 1800hrs to 0600hrs. In the early mornings, the sandflies attracted and fell to the mesh-collection bag of each trap were collected in the following morning with aspirator (Alexander, 2000).

They were then transferred to test tubes plugged with cotton pads, anaesthetized by chloroform vapor, and placed on filter papers in Petri dishes to separate *Phlebotomus* females from the rest (*Phlebotomus* males and *Sergentomyia* species) under a dissecting microscope based on Abonnenc and Minter (1965). The head of each *Phlebotomus* females were carefully severed and slide-mounted in polyvinyl acetate (PVA) mountant for later species identification and the remaining body (thorax and abdomen) were individually preserved covered by soft paper and placed in empty antibiotic capsules with silica gel grains inside, bearing a corresponding label with the mounted specimen on the slide either for PCR detection of *Leishmania* parasites or for blood meal analysis (see 4.4 below). Whereas, *Phlebotomus* males and all *Sergentomyia* species were preserved in 70% ethyl alcohol until mounted in the laboratory (see 4.3 below).

4.2.2. Sticky trap collection

Yellow polythene sheets (20x30cm) coated with motor oil (No 30) on both sides were used regularly in every two weeks interval for collection of sandflies from indoor and termite mounds. Sticky traps were preferred over CDC light traps to collect sandflies from indoors since they are non-attractive and are only interceptive traps (Lane, 1993).

Light traps if placed inside houses might attract sandflies from outdoors and may not collect the true host seeking endophilic sandflies.

Thus, based on their history of having at least one VL case, five huts were selected for regular bimonthly sampling using five sticky traps per hut: an overall of 25 such traps per sampling time. For this purpose, each of the five sheets of sticky traps was pinned on a nylon string serially with paper clips at intervals of about 30-40 cm between them and was suspended about a meter above and parallel to the side of the head position of the bed or mat during sunset.

In outdoor collections, about 25-30 sticky traps were used in each sampling period for collection of sandflies from termite mounds. Accordingly, 3-5 sticky traps were placed on ventilation shafts of about 8-10 termite hills around sunset during each sampling period. The traps (from indoors and outdoors) were collected early morning and the sandflies were picked off with fine forceps or *Acacia* thorns and preserved in absolute alcohol (99.9%) with proper labeling until processing and identification in the laboratory later (see 4. 3 and 4.4 below).

Plate 1. A CDC light trap positioned at the ventilation shaft of termite hill for collecting exophilic Phlebotomine sandflies in the study area.

Plate 2. A CDC light trap placed in a farm field for sampling Phlebotomine sandflies.

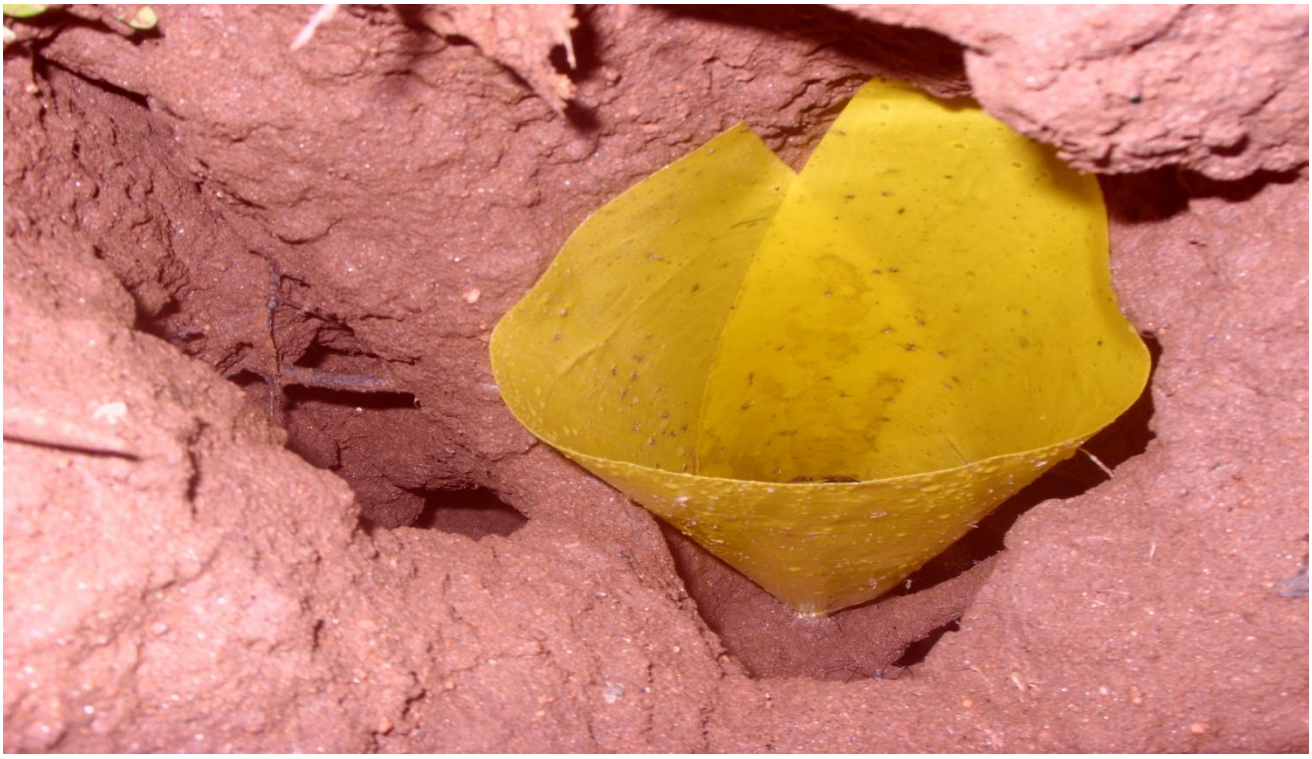

Plate 3. Oil smeared sticky paper trap placed inside an opening of a termite hill for collecting Phlebotomine sandflies in the microhabitat.

Plate 4. Oil smeared sticky paper traps positioned inside a resident hut above headboard for collecting endophilic/endophagic Phlebotomine sandflies in the study area.

4.3. Sandfly processing

In the laboratory sample specimens Plate 5collected by sticky traps were dipped in a detergent solution and left for an overnight to remove the oil from the samples and wetted in distilled water before soaked to 70% alcohol to remove the detergent. Both samples (treated sticky trap samples and CDC samples) were then transferred to Nesbit's solution separately where they are left for an overnight to remove the alcohol and dirt and clean the reticular substances of the sample to make internal structures easily seen (Gebre-Michal and Lane 1996) and were slide-mounted in polyvinyl acetate (PVA) mountant under individual cover glasses and allowed to dry for identification to species based on keys used above and other morphological criteria.

4.4. Identification of sandflies

All mounted sandfly specimens (*Phlebotomus* and *Sergentomyia* species) were identified to species. Identification was based on keys and illustration by Abonnenc and Minter (1965), Abonnenc (1972), and Lewis (1982). Males were identified mainly from the characters of the external genitalia, while females were mainly identified by the morphology of the spermathecae, cibarium, the relative lengths of the antennal, labral and palpal segments and other morphological characteristics. The identifications of doubtful specimens were confirmed by my advisors.

Plate 5. Sandflies collected from the study area where being identified to species level in a laboratory (Aklilu Lemma Institute of Pathobiology, Vector Biology Research Unit).

4.5. Meteorological data

In order to assess the correlation between sandflies density and meteorological variables, monthly weather datas (rain fall, temperature, relative humidity and night wind speed) of the study period were obtained from nearest Meteorological station located at Konso (Karat) town. Monthly variation in the first three bioclimatic data (Fig.1) depicted a rainy season between February and May which is typical of southern Ethiopia. Though data on the monthly average night wind speed were insignificant and not showed on the figure, during the study period, minimum and maximum wind speed recorded were 0.63 m/s in May and 1.32 m/s in February respectively.

4.6. Data analysis

Data entry and analysis was done using Microsoft excel 2007 and SPSS Inc. Version 17 respectively. The association of sandfly density and monthly meteorological variables and to assess the effect of habitat, species and their interaction on sandfly density were analysed using Pearson correlation, two-way analysis of variance (ANOVA) and Tukey's HSD. Whereas, Chi-square (X^2) analysis was also used to test for sex bias from an assumed 1:1 ratio of each *Phlebotomus* species.All analysis values are considered to be statistically significant if P values were < 0.05.

5. RESULTS

5.1. Species composition of sandfly fauna

A total of 8091 phlebotomine sandflies were collected by both CDC and sticky traps and were identified (Table 1) of which *Phlebotomus* species represented 1.45% (n=118) of the total catches. They comprised three *Phlebotomus* species in two subgenera (*Synphlebotomus* and *Anaphlebotomus*) and 10 *Sergentomyia* species (n=7973) in three subgenera (*Sergentomyia*, *Sintonius* and *Parratomyia*). The *Phlebotomus* species included *P. (An.) rodhaini* (0.58%) which was slightly more abundant than the two other species; *P. (Sy.) martini* (0.53%) and *P. (Sy.) celiae* (0.35%). The great majority of all the sandflies collected were those of *Sergentomyia* species, of which *S. (Se.) schewtzi* (60.7%) was the dominant followed by *S. (Se.) bedfordi* (13%) and *S. (Si.) suberecta* (10.4%), and least abundant was a single specimen of *S. (Se.) schoutedeni* (0.01%).

Of the total 8091 phlebotomine sandflies, the majority (n= 4970; 41.3%) were collected by sticky traps, and the rest (n=3212; 39.7%) by CDC light traps (Tables 2 and 3). All the three *Phlebotomus* species were collected by both methods of collection, while eight species of *Sergentomyia* were represented in both, although two *Sergentomyia* species (different species) were either present or absent in one or the other collection method. However, more specimens of each species of *Phlebotomus* and *Sergentomyia* were collected by sticky traps than by the light traps and *Sergentomyia schwetzi* was the predominant species in both collection methods.

5.2. Sex ratio

The overall sex ratio was slightly in favour of males (51.6%) than females (48.4%). However, this ratio varied between individual species where some species have different sex ratio in each method of collection (Tables 2 and 3) though the difference was insignificant in all cases ($P > 0.05$).

In sticky trap collections, overall male to female ratio was 52.7% to 47.3% (Table 2) and by habitat, there was no significant difference among sex ($P> 0.05$) and more males were collected from termite hills than females whereas the reverse was true in indoors. In contrast, in CDC trap collections, this ratio was almost 1 to1 (Table 3) with no significant difference in the overall total as well as within each habitats ($P >0.05$).

Table 1. Species and sex composition of phlebotomine sandflies collected by two methods from different habitats in Diteta (January- June 2012).

Species	Male No. (%)	Female No. (%)	Overall Total (%)
P. (*Anaphlebotomus*) *rodhaini*	21 (44.7)	26 (55.3)	47 (0.58)
P. (*Synphlebotomus*) *martini*	31 (72.1)	12 (27.9)	43 (0.53)
P. (*Synphlebotomus*) *celiae*	21 (75.0)	7 (25.0)	28 (0.35)
S. (*Sergentomyia*) *schwetzi*	2930 (59.6)	1983 (40.4)	4913 (60.72)
S. (*Sergentomyia*) *bedfordi*	333 (31.7)	716 (68.3)	1049 (12.97)
S. (*Sergentomyia*) *schoutedeni*	0	1 (100.0)	1 (0.01)
S. (*Sergentomyia*) *multidens*	2 (50.0)	2 (50.0)	4 (0.05)
S. (*Sergentomyia*) *antennata*	176 (39.5)	270 (60.5)	446 (5.51)
S. (*Sintonius*) *suberecta*	336 (39.9)	507 (60.1)	843 (10.42)
S. (*Sintonus*) *clydei*	73 (64.0)	41 (36.0)	114 (1.41)
S. (*Sintonius*) *affinis*	47 (56.6)	36 (43.4)	83 (1.03)
S. (*Sintonus*) *adleri*	3 (75.0)	1 (25.0)	4 (0.05)
S. (*Parrotomyia*) *africana*	206 (39.9)	310 (60.1)	516 (6.38)
Total	**4179**	**3912**	**8091**

Table 2. Species and sex composition of phlebotomine sandflies collected by Sticky paper traps (January- June 2012).

	Termite hill		Indoor		Total		Overall total
Species	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**(M+ F) [%]**
P. (*Anaphlebotomus*) *rodhaini*	14 (48.3)	15 (51.7)	-	1 (100)	14 (46.7)	16 (53.3)	30 (0.6)
P. (*Synphlebotomus*) *martini*	20 (83.3)	4 (16.7)	1 (50)	1 (50)	21 (80.8)	5 (19.2)	26 (0.5)
P. (*Synphlebotomus*) *celiae*	11 (84.6)	2 (15.4)	-	1 (100)	11 (78.6)	3 (21.4)	14 (0.3)
S. (*Sergentomyia*) *schwetzi*	1641 (58.3)	1172 (41.7)	139 (53.1)	123 (46.9)	1780 (57.9)	1295 (42.1)	3075 (61.9)
S. (*Sergentomyia*) *bedfordi*	197 (54.3)	166 (45.7)	44 (20.1)	175 (79.9)	241 (41.4)	341 (58.6)	582 (11.7)
S. (*Sergentomyia*) *antennata*	97 (38.6)	154 (61.4)	28 (30.1)	65 (69.89)	125 (36.3)	219 (63.7)	344 (6.9)
S. (*Sergentomyia*) *multidens*	-	-	-	-	-	-	-
S. (*Sergentomyia*) *schoutedeni*	-	-	-	-	-	-	-
S. (*Sintonus*) *adleri*	3 (75.0)	1 (25.0)	-	-	3 (75)	1 (25)	4 (0.1)
S. (*Sintonus*) *clydei*	43 (76.8)	13 (23.2)	2 (40)	3 (60.0)	45 (73.8)	16 (26.2)	61 (1.2)
S. (*Sintonius*) *affinis*	19 (90.5)	2 (9.5)	5 (83.3)	1 (16.67)	24 (88.9)	3 (11.1)	27 (0.5)
S. (*Sintonius*) *suberecta*	203 (35.7)	365 (64.3)	15 (50)	15 (50)	218 (36.5)	380 (63.5)	598 (12.0)
S. (*Parrotomyia*) *africana*	88 (65.2)	47 (34.8)	50 (67.6)	24 (32.43)	138 (66.0)	71 (34)	209 (4.2)
Total	**2336**	**1941**	**284**	**409**	**2620**	**2350**	
Overall total	**4277**		**693**		**4970**		**4970**

Table 3. Species and sex composition of phlebotomine sandflies collected by CDC light traps (January- June 2012).

	Termite hill		Compounds		Farm flied		Total		Overall total
Species	**M**	**F**	**M**	**F**	**M**	**F**	**M (%)**	**F (%)**	**(M+ F) [%]**
P. (*Anaphlebotomus*) *rodhaini*	7 (50)	7 (50)	-	-	-	3 (100)	7 (41.2)	10 (58.8)	17 (0.54)
P. (*Synphlebotomus*) *martini*	8 (57.1)	6 (42.9)	2 (66.7)	1 (33.3)	-	-	10 (58.8)	7 (41.2)	17 (0.54)
P. (*Synphlebotomus*) *celiae*	10 (71.4)	4 (28.6)	-	-	-	-	10 (71.4)	4 (28.6)	14 (0.45)
S. (*Sergentomyia*) *schwetzi*	581 (66.7)	290 (33.3)	351 (62.6)	210 (37.4)	218 (53.7)	188 (46.3)	1150 (62.6)	688 (37.4)	1838 (58.89)
S. (*Sergentomyia*) *bedfordi*	27 (18.4)	120 (81.6)	36 (20)	144 (80)	29 (20.7)	111 (79.3)	92 (19.7)	375 (80.3)	467 (14.96)
S. (*Sergentomyia*) *antennata*	18 (41.9)	25 (58.1)	20 (60.6)	13 (39.4)	13 (50)	13 (50)	51 (50.0)	51 (50.0)	102 (3.27)
S. (*Sergentomyia*) *multidens*	1 (50)	1 (50)	-	-	1 (50)	1 (50)	2 (50.0)	2 (50.0)	4 (0.13)
S. (*Sergentomyia*) *schoutedeni*	-	-	-	1 (100)	-	-	-	1 (100.0)	1 (0.03)
S. (*Sintonius*) *suberecta*	49 (42.6)	66 (57.4)	39 (54.9)	32 (45.1)	30 (50.8)	29 (49.2)	118 (48.2)	127 (51.8)	245 (7.85)
S. (*Sintonius*) *affinis*	14 (48.3)	15 (51.7)	9 (37.5)	15 (62.5)	-	3 (100)	23 (41.1)	33 (58.9)	56 (1.79)
S. (*Sintonus*) *clydei*	11 (61.1)	7 (38.9)	10 (40)	15 (60)	7 (70)	3 (30)	28 (52.8)	25 (47.2)	53 (1.70)
S. (*Sintonus*) *adleri*	-	-	-	-	-	-	-	-	-
S. (*Parrotomyia*) *africana*	31 (20.7)	119 (79.3)	20 (19)	85 (81)	17 (32.7)	35 (67.3)	68 (22.1)	239 (77.9)	307 (9.84)
Total	**757**	**660**	**487**	**516**	**315**	**386**	**1559**	**1562**	**3121**
Overall total	**1417**		**1003**		**701**		**3121**		

5.3. Sandflies distribution among different habitats

Using sticky traps, collection was only made from termite hills (outdoors) and indoors (houses). Of the total 4970 sandflies, the great majority of all sandflies (n=4277) were collected from termite hills than indoors (n=693) (Table 2). Of the total 70 specimens of three *Phlebotomus* species, 94% were collected from termite hills (outdoors) and very few (6%) were collected from indoors of which 4 of 6 were females. The great majority of *Sergentomyia* species collected by sticky traps were also from termite hills with the majority being males than from indoors, where females were the dominant.

Of the total 3,121 sandflies collected from three habitats using CDC light traps, the majority were again from termite hills (n=1417) followed by compounds (n=1003) and farm fields (n=701) (Table 3). Most *Phlebotomus* and all three species were also collected from termite hills (42 of 48) as were the *Sergentomyia* species, while only one species was collected from compounds (*P. martini*) or farm fields (*P. rodhaini*). As in sticky traps collection, *Sergentomyia* species were the predominant from termite mounds.

5.4. *Phlebotomus* sandfly density comparison between habitats and their preference

In both collection methods (CDC light trap and sticky paper traps), significant differences were recorded in *Phlebotomus* sandfly densities between termite hill and other habitats showing a significant habitat effect ($F=30.343$, $P= 0$; and $F=14.577$, $P= 0.001$ by analysis of variance [ANOVA]; Table 4 and 5). Of the habitats survey by CDC light trap method, termite hill were recorded as the most productive site with highest mean density value of all *Phlebotomus* species (Tukey's HSD test, $P= 0$) while no significant difference was observed in the remaining habitats (Tukey's HSD test, $P> 0.05$; Appendix 3). Similarly, in sticky trap collection, termite hill were showed highest productivity with the uppermost mean density value than its complement indoor habitat (Tukey's HSD test, $P< 0.05$; Appendix 4).

Though the result of ANOVA operated for habitat preference of *Phlebotomus* species collected by both methods (CDC light trap and sticky paper traps), showed insignificant result on 'Habitat by Species' interaction ($F= 0.247$, $P= 0.910$; and $F= 0.762$, $P= 0.475$ respectively; Table 4 and 5) strong correlation with highest mean density were recorded in each *Phlebotomus* species with termite hill than other two habitats ($P= 0$, Fig. 6 and 7).

Table 4. Two-way analysis of variance [ANOVA] testing the effect of habitat and species type on sandfly density collected by CDC light trap (number/trap/month).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	0.570	1	0.570	43.700	0.000
Habitat	0.792	2	0.396	30.343	0.000
Species	0.001	2	0.000	0.034	0.967
Habitat * Species	0.013	4	0.003	0.247	0.910

Table 5. Two-way analysis of variance [ANOVA] testing the effect of habitat and species type on sandfly density collected by Sticky paper traps (number/trap/month).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	0.037	1	0.037	19.615	0.000
Habitat	0.028	1	0.028	14.577	0.001
Species	0.003	2	0.002	0.832	0.445
Habitat * Species	0.003	2	0.001	0.762	0.475

5.5. Monthly variations in population of *Phlebotomus* species

Variation on the monthly population densities in the three *Phlebotomus* species were observed in both traps during the six month study period. Generally, there were a slight increase in densities of the three *Phlebotomus* species (*P. martini*, *P. celiae* and *P. rodhaini*) in both CDC and sticky traps during the wet months (February –May) of southern Ethiopia (Fig. 2 and Fig. 3). However, the numbers collected were too low to see any distinct seasonal pattern.

5.6. Monthly variations in population of five relatively abundant *Sergentomyia* species

Similar monthly pattern was observed among the population of three most abundant *Sergentomyia* species: *S. schwetzi*, *S. bedfordi*, and *S. africana*, where all reached their peak in February (early in the wet season) followed by a steady decline during the wet months (March to May) (Appendix 8). *S. suberecta* tended to increase during the wet months (March to May), whereas *S. antennata* showed no obvious fluctuations throughout the study period except for slight increase during March (a wet month) (Fig. 4).

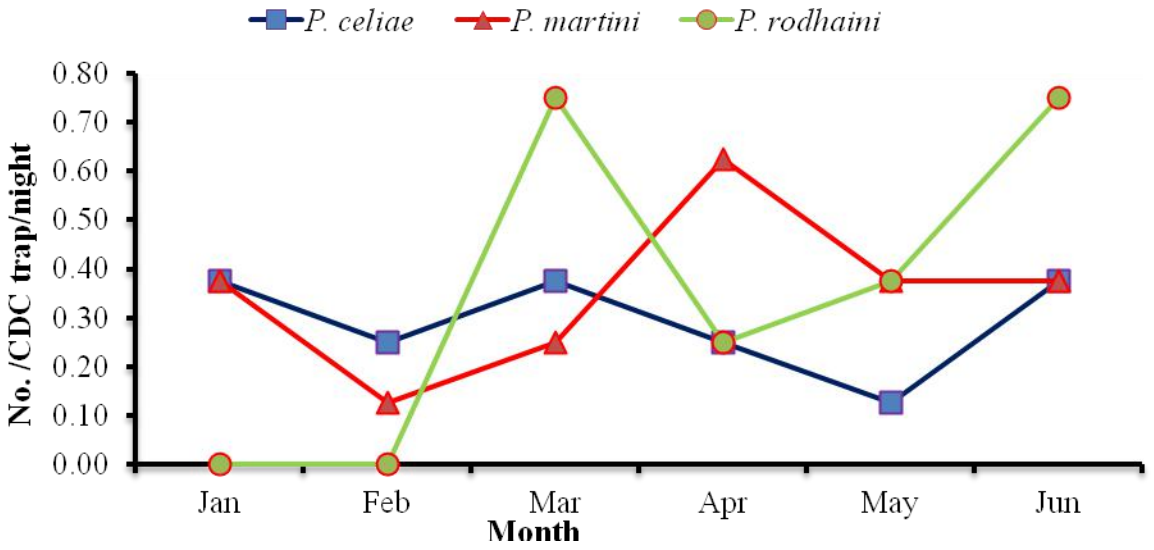

Figure 2. Monthly distribution in densities of three *Phlebotomus* sandflies collected from Diteta using CDC light traps (January-June 2012).

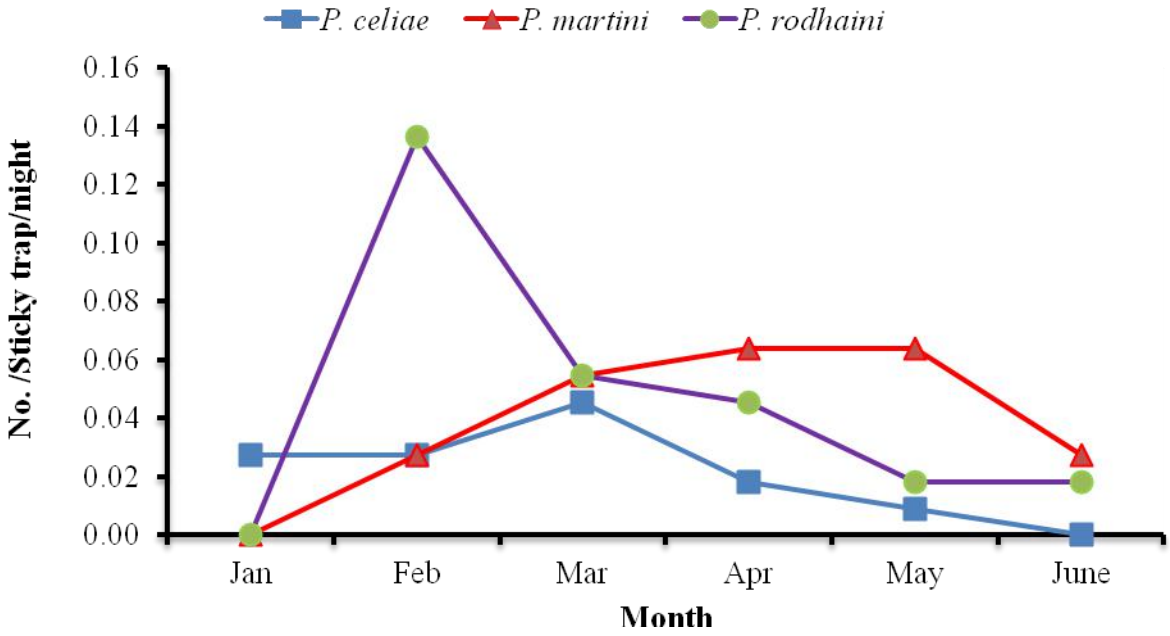

Figure 3. Monthly distribution in population density of three *Phlebotomus* sandflies collected from Diteta using sticky paper traps (January-June 2012).

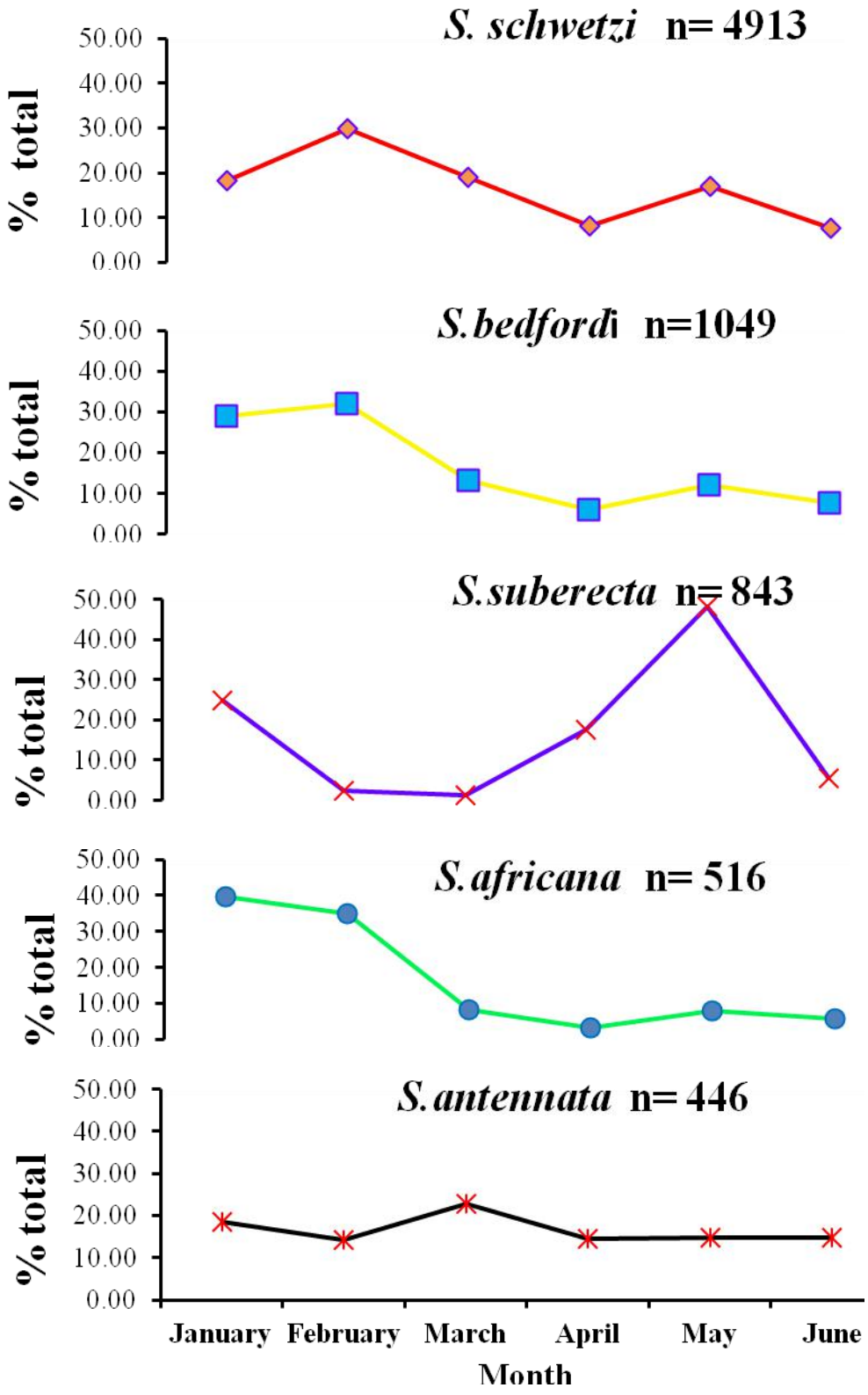

Figure 4. Monthly distribution of five common *Sergentomyia* species collected in both methods from Diteta (January-June 2012).

5.7. Influence of weather variables on density of *Phlebotomus* species

The result of Pearson correlation analysis done for CDC light trap collections (as summarised in Table 6) on densities of three *Phlebotomus* sandflies and bioclimatic factors (monthly total rainfall, relative humidity and average monthly temperature) depicted negative correlation with average monthly temperature, although it was insignificant in all species ($p > 0.05$). *P. celiae* was the only species showing negative correlation with all bioclimatic variables (monthly total rainfall, relative humidity and average monthly temperature). Strong positive correlation was observed between density of *P. martini* and monthly total rain fall ($r = 0.801$, $p = 0.05$) while *P. rodhaini,* showed negative correlation ($r = -0.014$, $p = 0.979$).

In sticky trap collection, unlike the case for CDC light trap, positive correlations were observed in density of all *Phlebotomus* species with average monthly temperature. *P. celiae* showed significant positive correlation with average monthly temperature ($r = 0.917$, $p = 0.010$) but negatively correlated with total monthly rain fall and mean relative humidity ($r = -0.077$ and -0.56 respectively) *P. martini* showed positive correlation with average monthly temperature, monthly total rainfall and relative humidity ($r = 0.246$, 0.598, 0.60 and $p = 0.639$, 0.210, 0.600) respectively. On the other hand, *P. rodhaini* showed negative correlation with the later two meteorological factors, though it was insignificant (Table 7).

5.8. Collection of *Phlebotomus* female specimens for detection of *leishmania* by PCR

As the collection of total *Phlebotomus* females were very few, only a total of 21 female *Phlebotomus* species: *P. rodhaini* ($n = 10$), *P. martini* ($n = 7$) and *P. celiae* $n = (4)$ were collected for determination of *leishmania* infection using PCR. However, PCR analysis could not be conducted for the present report due to delays in procurement of the necessary reagent (*Leishmania* primers) hence, the specimens remain preserved.

Table 6. Pearson correlation analysis result depicting effect of meteorological factors on the density of *Phlebotomus* sandflies collected by CDC light traps in Diteta (January-June 2012).

	Temperature		**Rainfall**		**Relative humidity**	
Species	r	p	r	p	r	p
P. celiae	-0.069	0.897	-0.240	0.647	-0.470	0.347
P. martini	-0.328	0.526	0.801	0.055	0.649	0.163
P. rodhaini	-0.245	0.640	-0.014	0.979	0.523	0.287

r= Pearson correlation coefficient, p= level of significance

Table 7. Pearson correlation analysis result depicting effect of meteorological factors on the density of *Phlebotomus* sandflies collected by Sticky paper traps in Diteta (January-June 2012).

	Temperature		**Rainfall**		**Relative humidity**	
Species	r	p	r	p	r	p
P. celiae	0.917**	0.010	-0.077	0.885	-0.560	0.248
P. martini	0.246	0.639	0.598	0.210	0.793	0.600
P. rodhaini	0.479	0.336	-0.039	0.942	-0.369	0.471

r= Pearson correlation coefficient, p= level of significance, ** = correlation is significant at the 0.01 level (2-tailed).

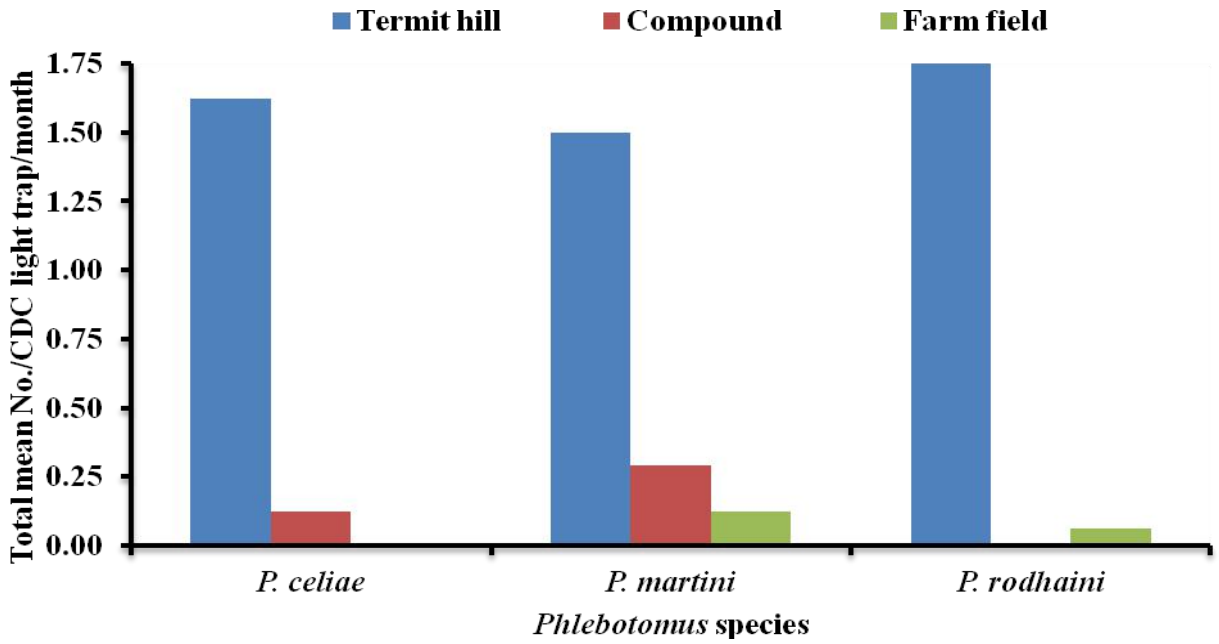

Figure 5. Habitat preference of different *Phlebotomus* sandflies collected by CDC light trap from the study area (Diteta).

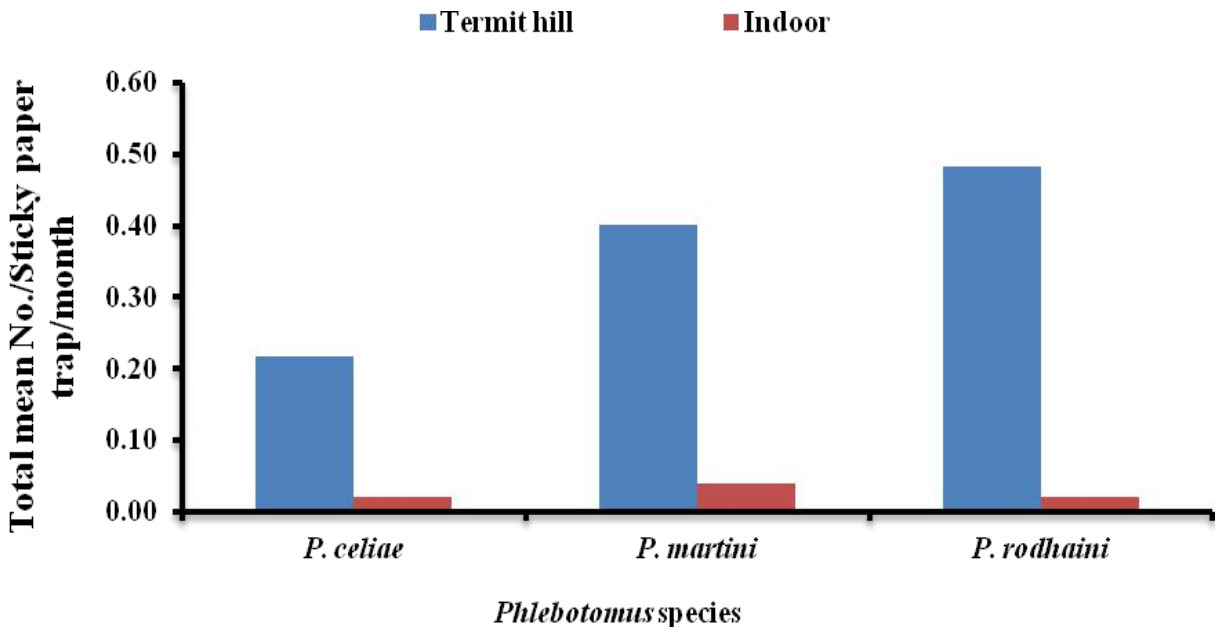

Figure 6. Habitat preference of different *Phlebotomus* sandflies collected by sticky paper trap in the study area (Diteta).

6. DISCUSSION

It is evident that understanding the species of sandflies present, their ecology and biology in a given endemic area is very crucial for combating Leishmaniasis. The present study carried out between January and June, 2102, documented the presence of some sandfly species and their ecology in an endemic locality (Diteta, Konso in SNNPR), not previously surveyed. Thirteen species, which included three *Phlebotomus* species and 10 *Sergentomyia* species were documented. All of these species are known species occurring in several other endemic regions of southern Ethiopia such as in Aba Roba and the lower Omo Plains (Gebre-Michael and Lane, 1996; Balkew *et al*., 1999).

The sandfly fauna of Diteta is typically Afrotropical with essentially the same species found elsewhere in East Africa (Minter, 1964; Quate, 1964). However, the relative scarcity of the three *Phlebotomus s*species, particularly *P. martini* and *P. celiae* in Diteta may be difficult to explain in view of the importance of visceral leishmaniasis in the area (Prof. Asrat Hailu, Prof. Com.), but could be associated with the low number of termite hills found in the village (~ 25 termite hills). In two endemic sub-villages of Aba Roba (Galga and Goinada) census of termite hills revealed the presence of at least 200 termite hills in each village (Gebre-Michael and Lane, 1996; Gebre-Michael, Unpublished Data), thus*, P. martini* and *P. celiae* were far more abundant than in Diteta during the present study. The three East African *Synphlebotomus* species (*P. martini*, *P. celiae* and *P. vansomeranae)* are closely associated with the mounds of the termite *Macrotermes* species for resting and breeding (Minter, 1964, Gebre-Michael and Lane, 1996).

Although the three *Phlebotomus* species were generally very scarce in Diteta, *P. rodhaini* and *P. martini* were of similar abundance, with a slight dominance of the former species. *P. celiae* was the least abundant. Based on abundance therefore, both *P. rodhaini* and *P. martin*i might be equally be important in the transmission of visceral leishmaniasis in the area. *P. martini* has long been confirmed as the most important vector of VL in East Africa: Kenya (WHO, 2011) and Southern Ethiopia (Gebre-Michael and Lane, 1996). It is also a highly suspected vector of VL in Somalia and Uganda (WHO, 2011), whereas, *P. celiae* and P. *vansomerenae* are secondary vectors in East Africa (Minter and Wijers, 1963; WHO, 2011). *P. celiae* has naturally been found infected in Aba Roba, where it is believed to serve as secondary vector (Gebre-Michael

and Lane, 1996) as well as in Woyto valley of southern Ethiopia (Balkew *et al*., 1999). Thus, *P. celiae* could as well be a secondary vector of visceral leishmaniasis in Diteta. As was observed in the present study, *P. rodhaini* is generally considered a rare species in Ethiopia although it occurs in several arid lowlands of Ethiopia (Ashford *et al*., 1974; Gebre-Michael and Lane, 1996; Balkew *et al*., 1999, Balkew *et al*, 2002; Gebre-Michael *et al.,* 2010). It also occurs in several tropical African countries. The species is known to feed on rodents, but is reported to bite man rarely in Sudan and Ethiopia (Hoogstraal and Heyneman, 1969; Ashford, 1974). However, it has recently been implicated in the transmission of visceral leishmaniasis in Sudan, based on the detection of *L. donovani* in wild caught *P. rodhaini* by molecular methods (Elnaiem *et a*l., 2011). Thus, this species might also contribute to the transmission of the disease in Diteta area in addition to the role played by *P. martini* and *P. celiae*.

Apart from *Phlebotomus* species, ten species of *Sergentomyia* species were recorded in the present study. The most abundant of these was *S. schwetzi,* while four others (*S. bedfordi, S. suberecta, S. africana and S. antennata*) were also common species; the remaining five species were scarcely found. All the *Sergentomyia* species recoded in the present study have also been recorded in several localities in Ethiopia before (Ashford, 1974; Gemetchu *et al,* 1976; Gebre-Michael and Lane 1996; Balkew *et al*., 1999, 2002) and elsewhere in East Africa (Minter, 1964; Quate, 1964).

Although there are several species of *Sergentomyi*a which bite man, none of these have been incriminated as vectors of mammalian *Leishmania* species to date (WHO, 2011). However, some *Sergentomyia* species have been implicated in the transmission of visceral leishmaniasis. For example, *S. garnhami* in Kenya was suggested as a vector of visceral leishmaniasis because of its high infection rate with promastigotes in the anterior mid gut (16.4%), the high degree of its anthropophilic behavior as well as its ability of being infected when fed on VL patients (Mutinga and Odihambo, 1982). However, the identity of the *Leishmania* parasites from wild caught sandflies was unconfirmed. Recent evidence has also emerged on the possible role of some species of *Sergentomyia* in the transmission of *Leishmania* parasites in humans. Berdjane-Brouk *et al.,* (2012) have recently detected natural infections of *L. major* (cause of CL) in *S. darlingi* in Mali (West Africa) by molecular methods and suggested

the potential involvement of this species in the transmission of *L. major* in Mali. *Sergentomyia darlingi* is also found in eastern Ethiopia (Dr. Teshome, Pers. Comm*).* Thus, the above few observations on *Sergentomyia* species elsewhere, suggest that attention should be given to these groups of sandflies, particularly, on those exhibiting anthrophilic tendencies for their potential roles in the transmission of mammalian leishmaniasis. *Sergentomyia schwetzi, S. multidens, S. adleri*, and *S. clydei* recorded in the present study are known occasional man biters in Ethiopia as well as elsewhere in Africa (Wijers, 1963; Mutinga, 1986; Gebre-Michael and Lane, 1996).

Climatic factors (such as rainfall, temperature and wind conditions), appears to have important effects on the distribution and abundance of sandflies. Some species are widely distributed and found throughout the year, while others are limited to certain seasons of the year. Unfortunately, the present study was limited to only six months observation, thus, the true seasonal occurrence or incidence of the sandflies could not be clearly understood. Nevertheless, some indications of population fluctuations could be observed by both methods of collection (CDC and sticky trap collections) in the present study.

Thus, all the three *Phlebotomus* species slightly increased in abundance during the wet months in the area (March - May), although variations were observed between the trapping methods (CDC light and sticky traps) which might be attributed to differences in the average night wind speed during different nights of sampling. Dispersal and flight range of sandflies can be altered by wind speed (Maroli *et al.,* 2102). Furthermore, rainy nights during the trapping nights have affected both the CDC and sticky traps on several occasions in the rainy months. Thus, no clear and consistent correlations of weather variables with the density of the three *Phlebotomus* species were detected. However, the slight increase in population of the three *Phlebotomus* species during the wet months is similar to the observation made in Aba Roba focus (Gebre-Michael and Lane 1996) and foci in Kenya (Minter, 1964). With the exception of a few species (e.g. *P. orientalis)* most sandfly species in tropical areas exhibit increased population density during the wet season (Hoogstraal and Heyneman, 1969; Tesh, 1998). However, sampling of sandflies with concurrent recording of the meteorological data of the area for at least one year is essential to appreciate distinct seasonal patterns of the sandflies.

With regards to the five most common *Sergentomyia* species, dissimilar pattern of rainfall and fluctuations of sandfly density was observed except for *S. suberecta*. Thus, *S. schwetzi*, *S. bedfordi*, *S. africana*, and *S. antennata* were present throughout the six months period of investigation with higher densities during the dry months (January–February, and June). *S. suberecta* had its peak during the wet month (May). Wijers and Minter, (1962) discussed the seasonal occurrence of sandflies dividing them as perennial and rainy season groups. Most of the *Sergentomyia* species recorded here can be regarded as perennial; although a year-long longitudinal study has not been made in the preset study.

As far as microhabitats are concerned, termite hills were the most productive microhabitat for the *Phlebotomus* species compared to other habitats as shown by both CDC light traps and sticky paper traps. Termite hills have long been known as the most suitable resting and possibly breeding sites for sandflies of the *Synphlebotomus* (*P. martini, P. celiae* and *P. vansomerenae)* and many other sandflies in Ethiopia and elsewhere in Africa (Minter, 1964; Gebre-Michael and Lane 1996; Balkew *et al.,* 1999). It is also believed that the termite hill habitat is most likely the breeding microhabitat although numerous investigations by examining excavated soils from termite mounds failed to show any or very few larvae (Minter, 1964; Mutinga and Kamau, 1986). This failure was attributed to the nature of the termite mounds which are predominantly subterranean making inspection of soil samples for larvae very tedious and difficult.

Wijers and Minter (1962) and Minter (1964) described four types of termite mounds found in Kenya: the castellated type, the eroded type, the closed type and the pinnacled type. The castellated types have multiple ventilation shafts, which are favorable for many sandflies, especially *Sergentomyia* species but with very little or no *Synphlebotomus* species. The eroded type is believed to have derived from the castellated type by normal weathering when building activities of the termite colony has ceased or declined. Such termite mounds are also favorable for the species of *Synphlebotomu*s as well as for many *Sergentomyia* species. The pinnacled type is a chimney type which has a low mound at the base from which a single (sometimes two) ventilation shaft rises, like a pinnacle or chimney to heights up to 8 meters. Such mounds are also favorable habitats for numerous *Sergentomyia* species but for only *P. martini* of the *Synphlebotomus* subgenu*s.* Finally, the closed type (with no ventilation shafts) is usually less common and is unsuitable for sandflies.

In Diteta, although termite mounds were generally very few in number, only the castellated and the eroded type of termite mounds were present, the former being the most abundant. Elsewhere in Ethiopia, all the four types of termite mounds are present in various endemic areas of southern and southeastern Ethiopia with differences in their distribution. For example, the long chimney type is predominant in southern and southeastern Oromia and Somali Regions, whereas the castellated and eroded types are predominant in much of the Konso and Hammer districts (Dr. Teshome, Pers. Com). Apart from sandflies, many other animals, particularly rodents, mongooses, reptiles (snakes and lizards) and various arthropods live in the ventilation shafts of termite mounds, some of which (rodents and mongooses) are suspected as reservoir hosts of leishmaniasis. Thus, the termite hill ecology plays a significant role in the epidemiology of leishmaniasis in East Africa, although much remains to be studied.

7. CONCLUSION

The study area was rich in sandfly fauna comprising 13 species (three species of *Phlebotomus* and 10 species of *Sergentomyia*) the latter were dominant. All the three *Phlebotomus* species (*P. rodhaini*, *P. martini* and *P. celiae*), the most likely species involved in transmission of VL, were very low in abundance during the six-month study period for reasons not very obvious. For this reason and limited time of sampling (6 months), no distinct seasonal pattern was observed, although slightly more *Phlebotomus* occurred during the wet season in the area (March-May). Whether some species of man-biting of *Sergentomyia* (e.g. *S. schwetzi*) which are much more abundant than *Phlebotomus* are also involved in the transmission remains to be investigated. Some species of *Sergentomyi*a have recently been implicated as vectors of leishmaniasis (CL and VL) elsewhere in Africa.

Similar to some foci in southern Ethiopia and Kenya, termite hills were the most favourable resting sites for both *Phlebotomus* and *Sergentomyia* species than other sites observed. These sites are also probably the breeding sites for the sandflies, which could be targeted for vector control in the area.

8. RECOMMENDATION

Though the present entomological report provides baseline information about the species composition and *Phlebotomus* fauna found in the study area, there are many issues that are not answered yet. Detailed epidemiological studies on the magnitude of the visceral leishmaniasis are required. From the entomological point of view, since the current study was conducted only for a very short period of time (six months) in depth longitudinal vector study need to be carried out for an extended study period to come up with enough knowledge with regards to vector incrimination, seasonality, host preference and identification of the breeding and resting habitats of the Phlebotomine fauna identified in the study area as well as the possible vector(s). Furthermore, attention should be given for the possible role of some anthropophilic *Sergentomyia* species captured from the area in considering of their abundance and unusual man-biting behaviour exhibited in relationship with any vectorial role they might play in the transmission of the disease is also worth investigating.

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APPENDICES

Appendix 1. Two-way analysis of variance (ANOVA) testing the effect of habitat and species type on sandfly density collected by CDC light trap (number/trap/month) in Diteta village.

Source	Type III Sum of Squares	df	Mean Square	F	P
Intercept	.570	1	.570	43.700	.000
Species type	.001	2	.000	.034	.967
Habitat	.792	2	.396	30.343	.000
Habitat *Species type	.013	4	.003	.247	.910
Total	1.964	54			

Appendix 2. Multiple comparisons between *Phlebotomus* species collected by CDC light trap using Tukey's HSD test.

(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
celiae	*martini*	-.0094	.03808	.967	-.1017	.0829
	rodhaini	-.0022	.03808	.998	-.0945	.0901
martini	*celiae*	.0094	.03808	.967	-.0829	.1017
	rodhaini	.0072	.03808	.980	-.0851	.0995
rodhaini	*celiae*	.0022	.03808	.998	-.0901	.0945
	martini	-.0072	.03808	.980	-.0995	.0851

Homogeneity of variance is marinated (P= 0.013).

Appendix 3. Multiple comparisons of *Phlebotomus* sandfly densities (CDC light trap) between three sites using Tukey's HSD test.

A. Descriptive Statistics

Species	Habitat	Mean No. of sandflies/trap/month	Std. Deviation	N
	Termite hill	.2750	.12309	6
celiae	Compound	.0217	.05307	6
	Farm field	.0000	.00000	6
	Termite hill	.2533	.11183	6
martini	Compound	.0500	.06387	6
	Farm field	.0217	.05307	6
	Termite hill	.2933	.28197	6
rodhaini	Compound	.0000	.00000	6
	Farm field	.0100	.02449	6
	Termite hill	.2739	.17833	18
Total	Compound	.0239	.04972	18
	Farm field	.0106	.03298	18

N= species type x number of sample test

B. Multiple Comparisons

(I) Habitat	(J) Habitat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Termite hill	Compound	.2500*	.03808	.000	.1577	.3423
	Farm field	.2633*	.03808	.000	.1710	.3556
Compound	Termite hill	-.2500*	.03808	.000	-.3423	-.1577
	Farm field	.0133	.03808	.935	-.0790	.1056
Farm field	Termite hill	-.2633*	.03808	.000	-.3556	-.1710
	Compound	-.0133	.03808	.935	-.1056	.0790

*. The mean difference is significant at the .05 level, Homogeneity of variance is marinated (P= 0.013).

Appendix 4. Two-way analysis of variance (ANOVA) testing the effect of habitat and species type on sandfly density collected by sticky paper trap (number/trap/month) in Diteta village.

Source	Type III Sum of Squares	df	Mean Square	F	P
Intercept	.037	1	.037	19.615	.000
Habitat	.028	1	.028	14.577	.001
Species	.003	2	.002	.832	.445
Habitat * Species	.003	2	.001	.762	.475
Total	.128	36			

Appendix 5. Multiple comparisons between *Phlebotomus* species collected by sticky paper traps using Tukey's HSD test.

Multiple Comparisons

(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
celiae	*martini*	-.0175	.01782	.594	-.0614	.0264
	rodhaini	-.0217	.01782	.453	-.0656	.0223
martini	*celiae*	.0175	.01782	.594	-.0264	.0614
	rodhaini	-.0042	.01782	.970	-.0481	.0398
rodhaini	*celiae*	.0217	.01782	.453	-.0223	.0656
	martini	.0042	.01782	.970	-.0398	.0481

Homogeneity of variance is marinated (P= 0.002).

Appendix 5. Multiple comparisons of *Phlebotomus* sandfly densities (collected by sticky paper trap) between three sites using Tukey's HSD test.

A. Descriptive Statistics

Habitat	Species	Mean No. of sandflies/trap/month	Std. Deviation	N
Termite hill	*celiae*	.0350	.02739	6
	martini	.0667	.04885	6
	rodhaini	.0783	.08976	6
Indoor	*celiae*	.0033	.00816	6
	martini	.0067	.01033	6
	rodhaini	.0033	.00816	6
Total	*celiae*	.0192	.02539	12
	martini	.0367	.04599	12
	rodhaini	.0408	.07229	12
	Total	.0322	.05100	36

N= species type x number of sample test

B. Multiple Comparisons

(I) Habitat	(J) Habitat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Termite hill	Indoor	.056*	.015	.001	.026	.085
Indoor	Termite hill	-.056*	.015	.001	-.085	-.026

*. The mean difference is significant at the .05 level, Homogeneity of variance is marinated (P= 0.002).

Appendix 6. Meteorological data of Diteta area (January-June, 2012).

Meteorological factors	Month					
	January	**February**	**March**	**April**	**May**	**June**
Mean Temp.	22.98	24.3	25.85	23.28	22.5	19
Rainfall	0	3.4	35.2	217.3	32.1	17.6
RH	43.1	44.3	56.3	70.36	71.5	64.7
AWSN	1.17	1.32	1.18	1.17	0.63	1.07

Note: Mean Temp = Mean monthly temperature, RH= Mean monthly relative humidity, AWSN = Monthly average wind speed of the night.

Appendix 7. Monthly mean number of *Phlebotomus* sandflies collected by sticky paper trap (number/trap/ night).

Month	***P. celiae***		***P. martini***		***P. rodhaini***	
	TH	Indoor	TH	Indoor	TH	Indoor
January	0.05	0	0.00	0	0.00	0
February	0.03	0.02	0.03	0.02	0.25	0
March	0.08	0	0.08	0.02	0.08	0.02
April	0.03	0	0.12	0	0.08	0
May	0.02	0	0.12	0	0.03	0
June	0.00	0	0.05	0	0.03	0
Total	0.22	0.02	0.40	0.04	0.48	0.02

Note: TH = Termite hill

Appendix 8. Monthly mean number of *Phlebotomus* sandflies collected by CDC light trap (number/trap/ night).

Month	***P. celiae***			***P. martini***			***P. rodhaini***		
	TH	COM	FF	TH	COM	FF	TH	COM	FF
January	0.38	0.00	0.00	0.13	0.04	0.00	0.00	0.00	0.00
February	0.25	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00
March	0.38	0.00	0.00	0.25	0.00	0.00	0.38	0.00	0.06
April	0.13	0.13	0.00	0.38	0.13	0.13	0.25	0.00	0.00
May	0.13	0.00	0.00	0.25	0.13	0.00	0.38	0.00	0.00
June	0.38	0.00	0.00	0.38	0.00	0.00	0.75	0.00	0.00
Total	1.63	0.13	0.00	1.50	0.29	0.13	1.75	0.00	0.06

Note: TH = Termite hill, COM = Compound, FF= Farm field.

Appendix 8. Monthly variation in temperature, rainfall and relative humidity during the study period (January- June 2012) taken from the nearest meteorological station (Konso) of the study area.