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1. Phlebotominae sand flies (Diptera: Psychodidae): Main vectors in Europe and their distribution with special emphasis for Turkey

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Insects of the family Psychodidae are relatively small (2 to 5 mm) and can be distinguished by the dense covering of long narrow scales on head, thorax, legs, and wing veins. Members of the subfamily Phlebotominae are delicate midges with relatively long legs and antennae, in contrast to the squatter and more robust appearance of the other psychodid flies. Moreover they differ by bearing piercing mouthparts allowing taking a bloodmeal. Currently, New World species (some 400 spp.) are classified into four genera (*Brumptomyia*, *Hertigia*, *Lutzomyia*, and *Warleya*) while Old World species (some 430 spp.) are into five (*Austraophlebotomus*, *Chinius*, *Idiophlebotomus*, *Phlebotomus*, and *Sergentomyia*) (Léger & Depaquit, 2002).

The members of the genus *Phlebotomus* (94 spp.) show an important vector potential in the Old World, especially in the West Palearctic region. They are natural vectors of parasitic protozoa of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). Beside, they are also vectors of other human pathogens such as *Bartonella* and viruses including, for Europe, viruses belonging to the *Phlebovirus* genus (fam. Bunyaviridae) including Toscana, Sicilian and Naples sand fly fever viruses (Depaquit *et al.*, 2010). In addition, less important viruses have also been reported from Europe: Chios virus was isolated from a human case of severe encephalitis in Greece, Corfou virus from *Phlebotomus (Larrousius) neglectus* in Greece, Massilia virus from *P. (L.) perniciosus* in France and Arbia virus from *P. (L.) perniciosus* and from *P. (L.) perfiliewi* in Italy. However, so far there are no reports of human disease related to these viruses, in contrast to the numerous reported outbreaks due to Phleboviruses (Depaquit *et al.*, 2010).

As identified during the ECDC-funded 'V-borne' project ('Assessment of magnitude and importance of vector-borne diseases in Europe'), the following Mediterranean Phlebotomine vector species are identified as important for the transmission of leishmaniasis and sand fly

fevers and are considered in VBORNET: *P. (L.) ariasi*, *P. (L.) perniciosus*, *P. (L.) perfiliewi*, *P. (L.) neglectus/syriacus*, *P. (L.) tobbi*, *P. (Phlebotomus) papatasi*, and *P. (Paraphlebotomus) sergenti*. A first series of baseline distribution maps (Fig.1-8) at country level (NUTS 0) have been compiled using published historical data. These will be further finetuned with recent information provided by VBORNET members. Focus will be on identifying sub-national distribution limits at various NUTS levels (i.e. from NUTS 1 to 3). For Turkey, more detailed maps at NUTS 3 level are already available and also shown here (Fig.9-14).

Unlike for mosquitoes, the presence/absence of exotic vector species is not mapped, because sand flies have not been recorded as being transported by humans or as using winds to disperse across seas.

Distribution of the main vectors in Europe, based on published historical data

First VBORNET distribution maps for Europe at level NUTS 1 (countries) are based on published historical data from around 80 articles including the most recent from Ready (2010) and Depaquit *et al.* (2010). Figures 1-8 show the distribution of 8 medically important species in Europe with data reported in the VBORNET Vector Questionnaire: *P. ariasi*, *P. neglectus/syriacus*, *P. papatasi*, *P. perfiliewi*, *P. perniciosus*, *P. sergenti*, *P. similis*, and *P. tobbi*.

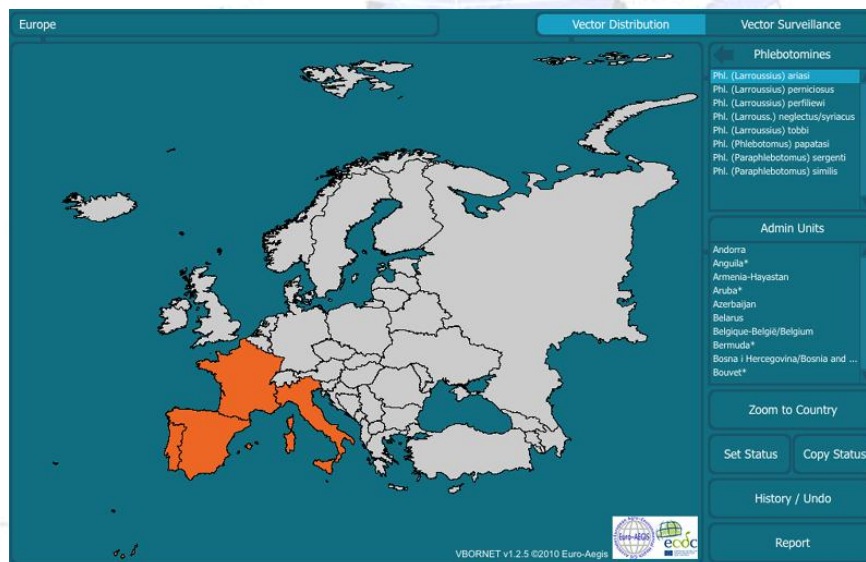


Figure 1 – Distribution of *P. ariasi* in Europe (NUTS 0)

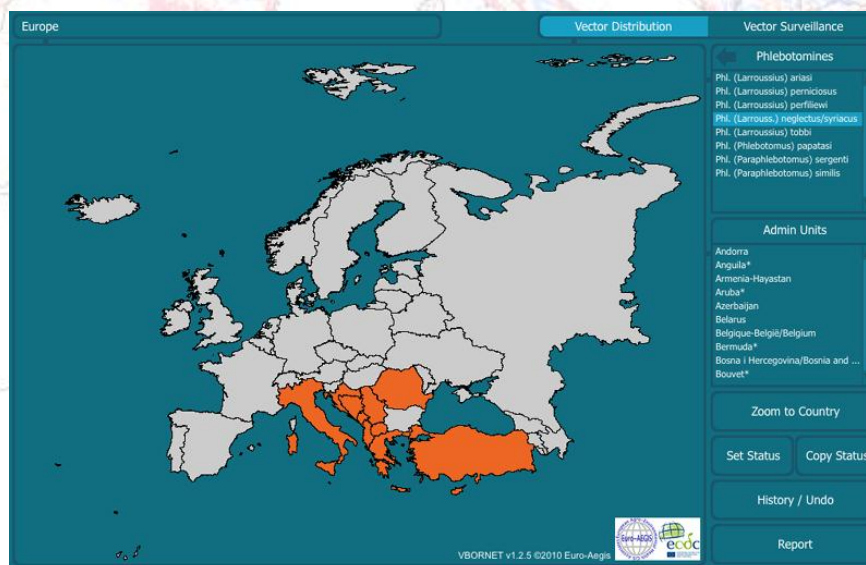


Figure 2 – Distribution of *P. neglectus/syriacus* in Europe (NUTS 0)

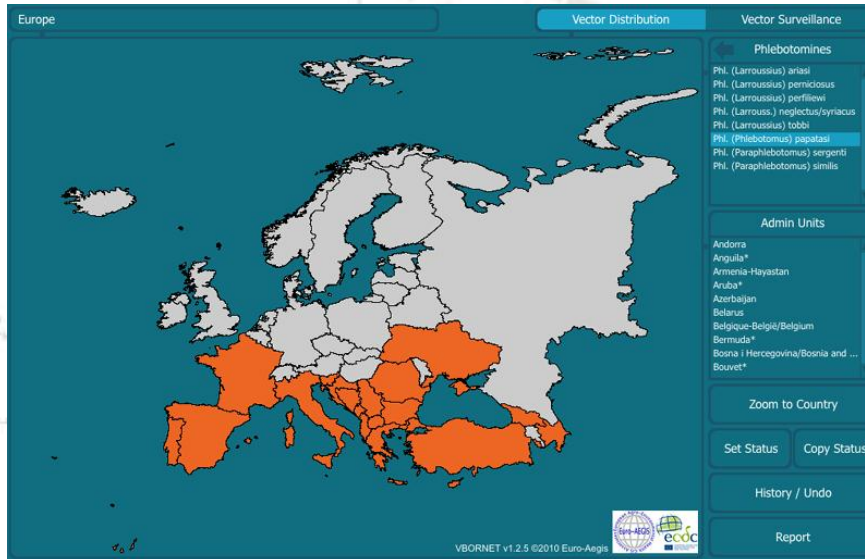


Figure 3 – Distribution of *P. papatasi* in Europe (NUTS 0)

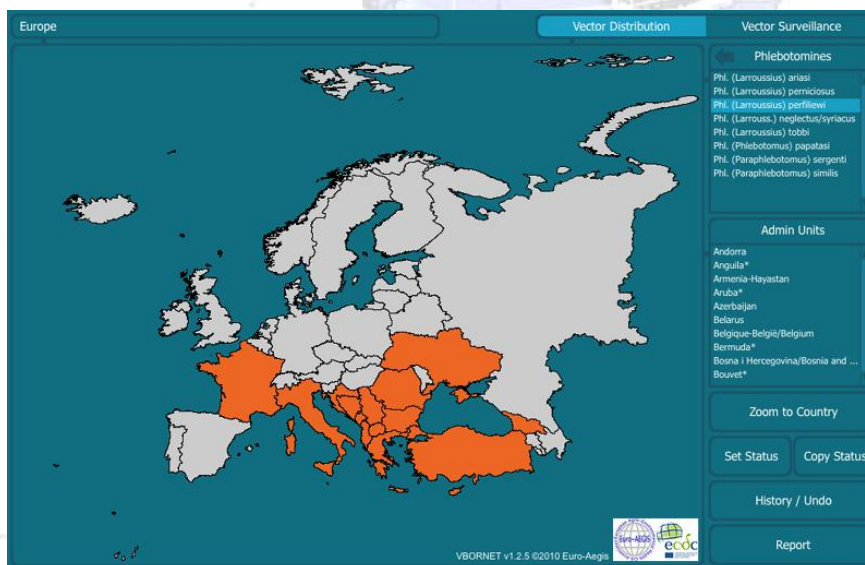


Figure 4 – Distribution of *P. perfiliewi* in Europe (NUTS 0)

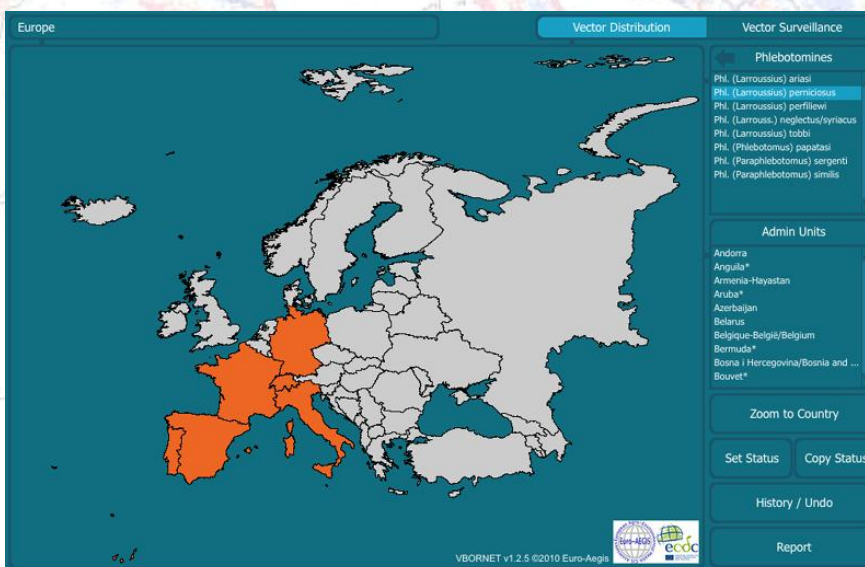


Figure 5 – Distribution of *P. perniciosus* in Europe (NUTS 0)

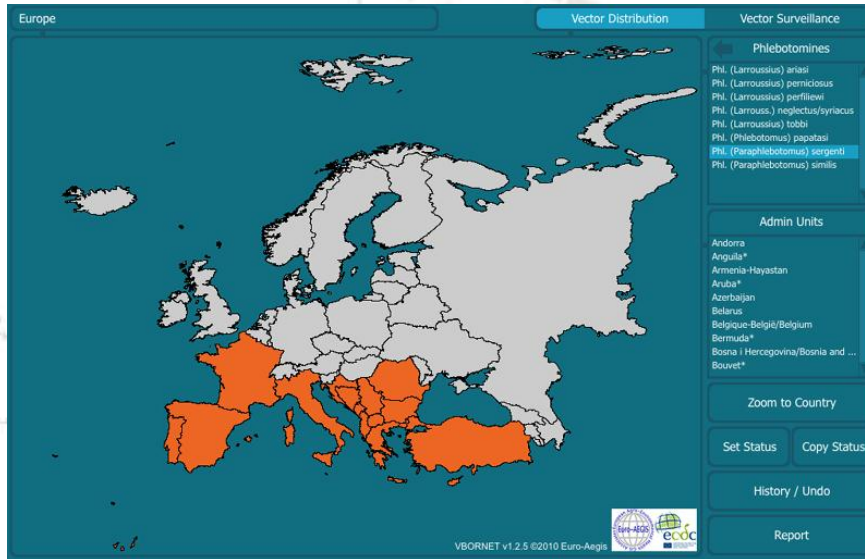


Figure 6 – Distribution of *P. sergenti* in Europe (NUTS 0)

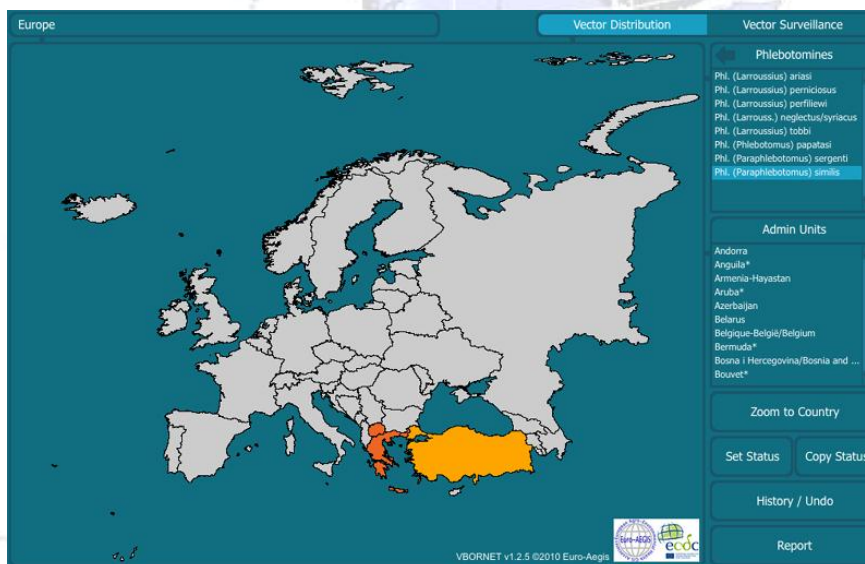


Figure 7 – Distribution of *P. similis* in Europe (NUTS 0)

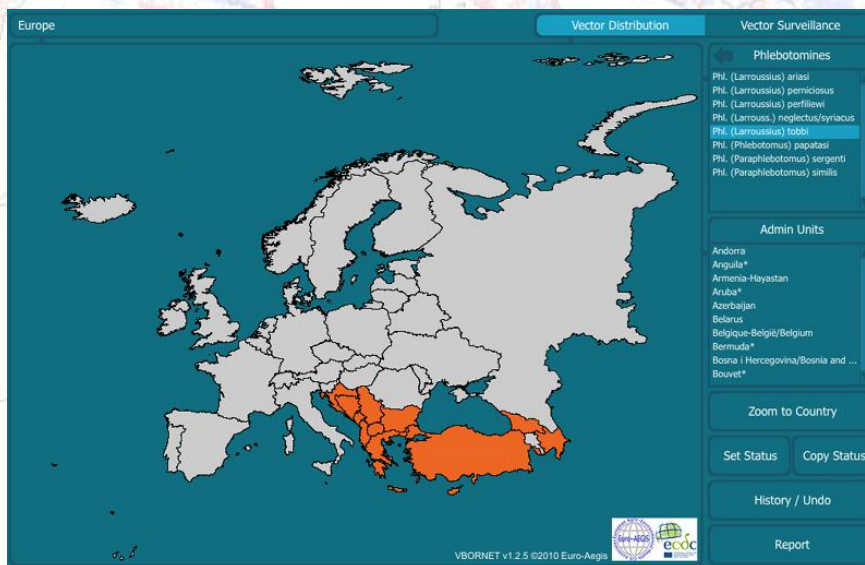


Figure 8 – Distribution of *P. tobbi* in Europe (NUTS 0)

Distribution of the main vectors in Turkey, based on current data.

To date, 24 sand fly species from five subgenera (*Phlebotomus*, *Paraphlebotomus*, *Larrousius*, *Adlerius* and *Sergentomyia*) have been reported from Turkey. Among them, six species are considered as important vectors: *P. papatasi*, *P. perfiliewi*, *P. sergenti*, *P. similis*, *P. neglectus/syriacus*, and *P. tobbi*. Reports received through the VBORNET Vector Questionnaire Tool allow the edition of updated distribution maps. Examples are given in Figures 9-14 at NUTS 3 level.

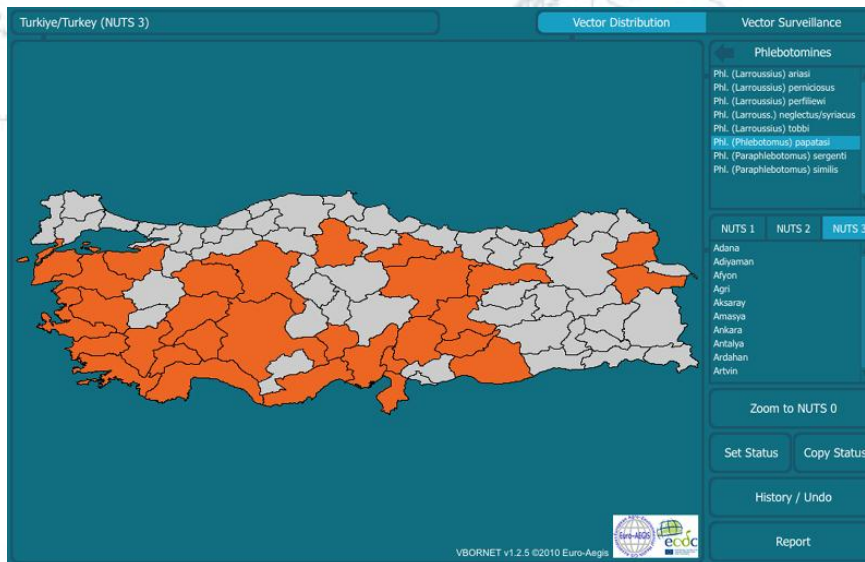


Figure 9 – Distribution of *P. papatasi* in Turkey (NUTS 3)

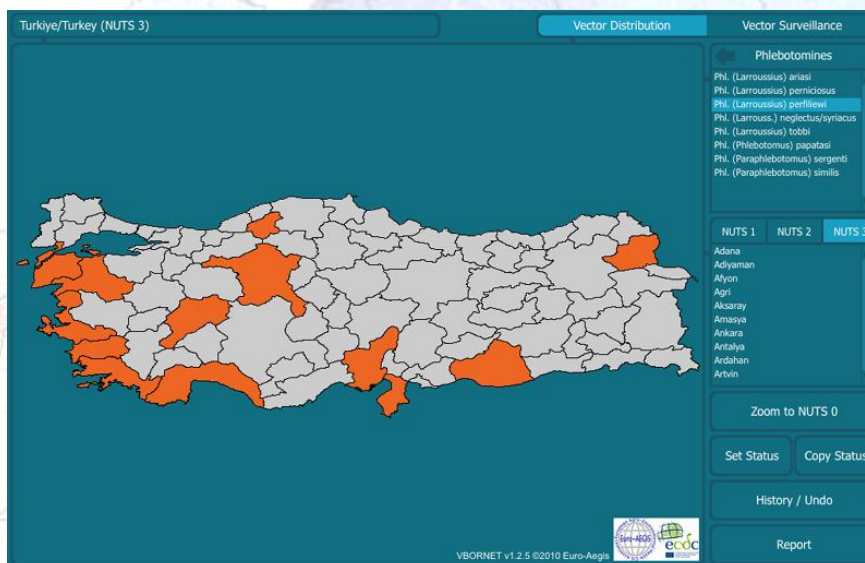


Figure 10 – Distribution of *P. perfiliewi* in Turkey (NUTS 3)

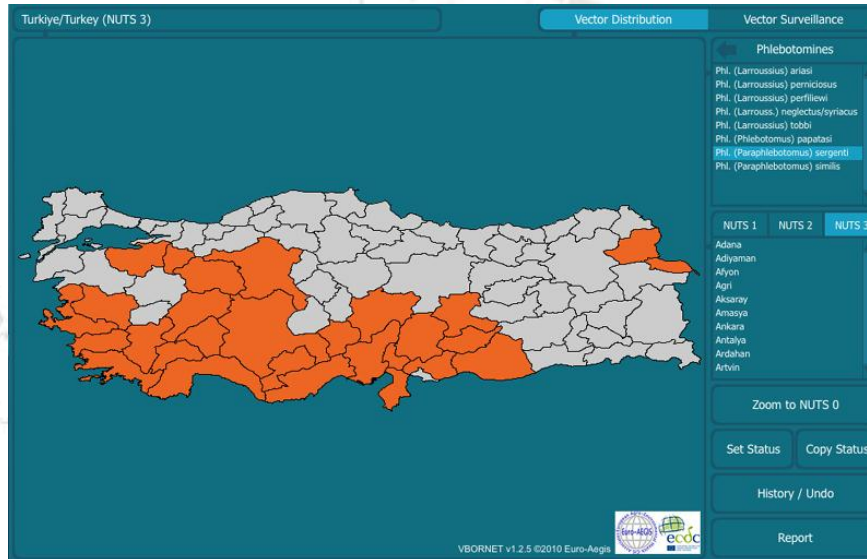


Figure 11 – Distribution of *P. sergenti* in Turkey (NUTS 3)

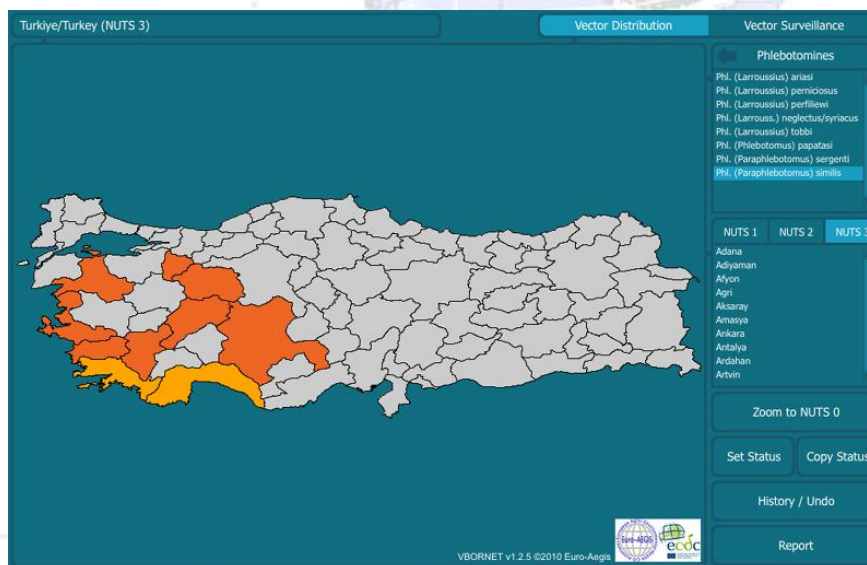


Figure 12 – Distribution of *P. similis* in Turkey (NUTS 3)

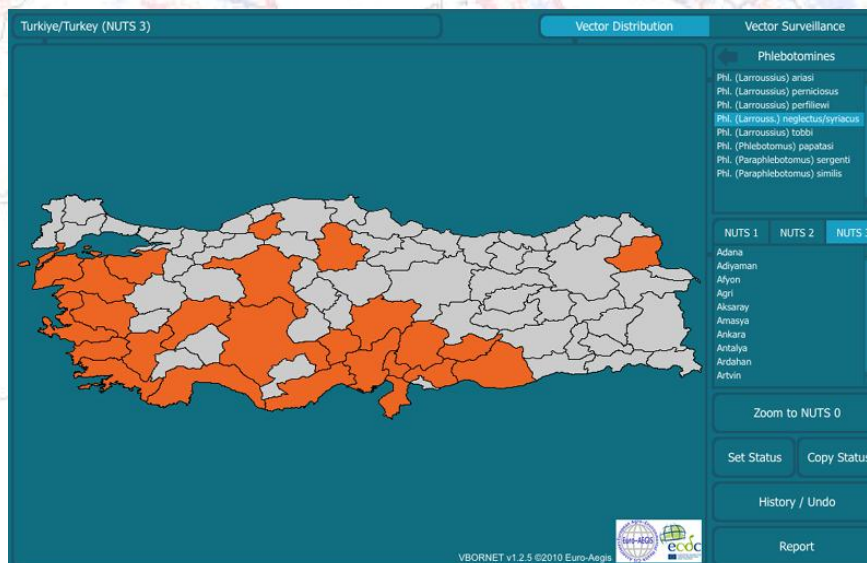


Figure 13 – Distribution of *P. syriacus/neglectus* in Turkey (NUTS 3)

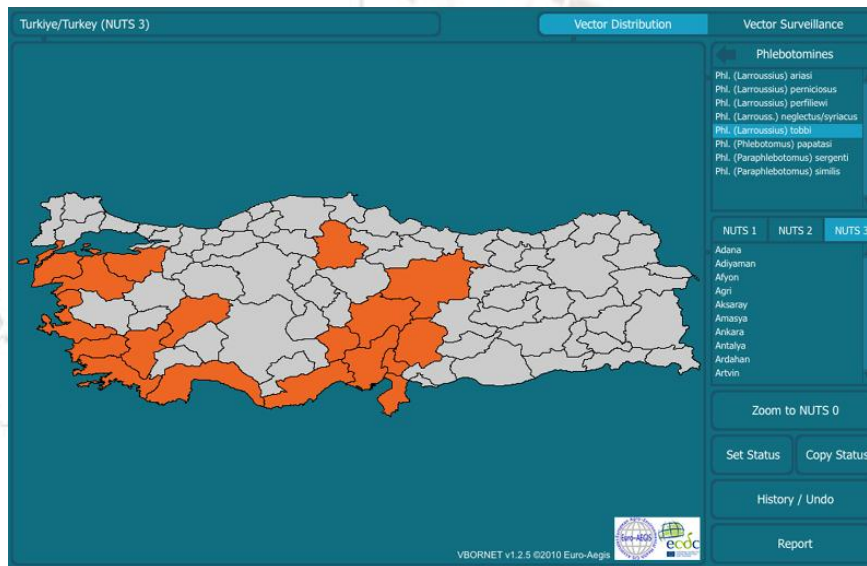


Figure 14 – Distribution of *P. tobbi* in Turkey (NUTS 3)

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2. The leishmaniasis in southern Europe

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Leishmaniasis are a large group of parasitic diseases that range over the intertropical zones of America and Africa, and extend into temperate regions of South America, Southern Europe and Asia. They are caused by protozoan members of the genus *Leishmania*, parasites infecting numerous mammal species, including humans, and transmitted by the bite of phlebotomine sand flies. Of the approximately 600 known species of sand flies, only 30 are proven *Leishmania* vectors. The clinical aspect of the diseases ranges from a simple, self-resolving cutaneous lesion (CL), to mucocutaneous (MCL), diffuse cutaneous (DCL) forms and the potentially fatal visceral leishmaniasis (VL). Among 15 well-recognized *Leishmania* species known to infect humans, 13 have a definite zoonotic nature (Gramiccia & Gradoni, 2005). For their impact on human health, underestimated for many years, leishmaniasis have now been classified by the World Health Organization among the most neglected tropical diseases. At present, an estimated 12 million people are infected in 66 Old World and 22 New World endemic countries, with an estimated yearly incidence of 1-1.5 million cases of CL forms, 500,000 cases of VL forms, at least 60,000 people that succumb yearly to VL and a loss of 2.4 million disability-adjusted life years (DALYs) (Desjeux, 2004). During the last decade, human and animal leishmaniasis have shown a wider geographical distribution than previously known and autochthonous *Leishmania* transmission has been recently recorded in traditionally non-endemic areas (Gramiccia & Gradoni, 2007). Leishmaniasis are dynamic diseases and the circumstances of transmission are changing in relation to environmental, demographic and human behavioural factors. Changes in the habitat of the natural host and vector, immunosuppressive conditions (e.g. HIV infection or organ transplantation-associated therapies) and the consequences of conflicts, all contribute to the changing leishmaniasis landscape.

Leishmaniasis are the only “tropical” vector-borne diseases that have been endemic in Southern Europe for decades. Incidence of leishmaniasis in humans is relatively low (0.02-0.49/100,000) with an estimation of about 700 reported new cases/year for Southern European countries (Dujardin *et al.*, 2008). Most of the cases consist of two entities, zoonotic VL (ZVL) and sporadic CL, both caused by different strains of *L. infantum*, transmitted from dogs to humans by the bite of phlebotomine sand flies belonging to the sub-genus *Phlebotomus* (*Larroussius*). Dogs, which may suffer from a severe canine leishmaniasis (CanL) represent the principal reservoir, but other domestic and wild mammals (cats and wild canids) may play a secondary role (Gramiccia & Gradoni, 2007). Being previously confined to coastal Mediterranean biotopes, autochthonous leishmaniasis cases appear not limited to these habitats anymore. It has spread northward, as shown by recent reports of indigenous ZVL cases in northern Italy and southern Germany (Maroli *et al.*, 2008; Harms *et al.*, 2003). Spreading of infections has been monitored by CanL investigations carried out among susceptible domestic dogs, acting as suitable sentinel hosts.

During 2002-2009, the northward spread of the disease was monitored in Italy through human, canine and entomological surveys performed in northern continental regions at the border with France, Switzerland, Austria and Slovenia. Results showed that the most competent *L. infantum* vector, *P. perniciosus*, was widespread in these territories, associated with *P. neglectus* in the sub-Alpine and with *P. perfilliewi* in sub-Apennines territories. The large Padana valley was apparently free from sand fly colonization, probably acting as natural barrier. CanL investigations confirmed the ongoing northward spread of ZVL, with a mean seroprevalence of 1.8% found in sub-Alpine sites, and an increase from 2 to 4.7% in sub-Apennines sites during the survey period. Despite the presence of a competent vector, Alto Adige-South Tyrol region at the border with Austria has been found still free from autochthonous CanL (Morosetti *et al.*, 2009). Both ZVL and CL autochthonous human cases due to *L. infantum* have been recorded in the newly endemic regions. All findings demonstrate conclusively that northern continental Italy became focally endemic for ZVL after 1990s (Gramiccia *et al.*, 2010). The description of a novel CanL focus, with *P. perniciosus* and *P. ariasi* acting as local vectors, has been recently reported in a territory of French Pyrenees outside the traditional endemic range of leishmaniasis in southern France (Dereure *et al.*, 2009). During a period of 13 years, seroprevalence rates in foothill villages increased by 10 folds as a probable consequence of the 1°C increase in the mean annual temperature. The progressive increase in CanL seroprevalence rate was also reported at elevated altitudes of 600-900m a.s.l. in the Alpujarras region of southeastern Spain, climbing from 9.2% in 1984, to 20.1% in 2006 (Martín-Sánchez *et al.*, 2009). In Germany, the detection of leishmaniasis cases in humans and animals (dog, cat, horse) that never travelled abroad, has led to the hypothesis of a recent establishment of autochthonous transmission in that country (Naucke *et al.*, 2008). A northward *L. infantum* expansion was suggested, although entomological surveys did not provide solid evidence for the presence of competent vector species.

A third leishmaniasis entity, anthroponotic CL caused by *L. tropica*, is sporadically reported in Greece (Gramiccia & Gradoni, 2007). The suspected vector is *P. sergenti*, a species incriminated to be a *L. tropica* vector in Turkey, Middle East and Morocco (Depaquit *et al.*, 2002) and whose geographical range extends to southern territories of Europe, mainly Spain, Portugal and Italy (D’Urso *et al.*, 2004). Recently, *L. donovani* was reported for the first time in Cyprus and two synantropic VL cycles, anthroponotic one by *L. donovani* and zoonotic one by *L. infantum*, have been suggested with respectively two different natural hosts, human for *L. donovani* and canine for *L. infantum* (Mazeris *et al.*, 2010).

The burden of leishmaniasis in endemic countries bordering Europe south (Afro-Mediterranean region) and southeast (Turkey, Near and Middle East) is much higher than in Southern Europe. Three to four leishmaniasis entities affect most of these territories. ZVL and sporadic CL due to *L. infantum* are found in Mediterranean biotopes of Morocco, Algeria, Tunisia, Egypt, Israel and West Bank, Lebanon and Turkey; the incidences are similar to those recorded in Southern Europe. On the contrary, thousands cases of zoonotic CL caused by *L. major* are annually recorded in semi-arid zones of all northern African and Near and Middle East countries, but not in Turkey. Furthermore, anthroponotic CL caused by *L. tropica* affects thousands individuals in urban areas of Morocco, all Middle East countries and South-

East Turkey. Sporadic *L. tropica* cases are also reported in rural areas of Tunisia, Jordan, Israel and West bank. Hence, the risk for the introduction of novel parasites into leishmaniasis-endemic zones of Southern Europe should not be underestimated, with an undisputable higher risk for *L. tropica* than *L. major* introduction, due to the absence of the rodent species acting as natural reservoir of the latter parasite. From published and unpublished information it appears that leishmaniasis cases caused by non-indigenous *Leishmania* species are indeed frequent (Gramiccia & Gradoni, 2007; Dujardin *et al.*, 2008). Patients are immigrants from endemic extra-European countries, or nationals that travelled for tourism or work, as well as military staff. Cases are mainly represented by CL forms due to *L. tropica* or *L. major*, but also to Neotropical parasites of the *L. braziliensis*, *L. amazonensis* or *L. guyanensis* complexes. Although for the latter parasites the adaptation to Old World vectors seems less probable, phlebotomine species susceptible to *L. tropica* (*P. sergenti*) or *L. major* (*P. papatasi*) are recorded in several Southern European countries (Ready, 2010). In this respect, the recent report of hybrid *Leishmania* from Portugal that shares *L. infantum* and *L. major* genetic traits suggests that adaptation of novel parasites to Southern European vectors may indeed occur (Volf, 2007).

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3. Public health importance and control of sand flies in continental Europe

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Sand flies as vectors of diseases

Sand flies (Diptera: Psychodidae) transmit several pathogens important for human health across the world. Sand fly vectors of the New World belong to the genus *Lutzomyia* and those of the Old World to the genus *Phlebotomus*. Sand fly-borne viruses are mainly from the *Phlebovirus*, *Vesiculovirus* and *Orbivirus* genera, of which some viruses are associated with outbreaks of human cases in the Mediterranean. *Phlebotomus* species are vectors for Pappataci fever, an arbovirus caused by Sand fly fever viruses such as Naples and Sicilian strains, which are members of the genus *Phlebovirus* (family Bunyaviridae), which also includes the closely related Toscana virus. The medical importance, geographical distribution, epidemiology and potential spreading of these and other sand fly-borne viruses are nicely reviewed by Depaquit *et al.* (2010).

Sand flies can also transmit *Bartonella*, a genus of Gram-negative bacteria, causing Oroya fever, a sand fly-borne disease exclusive to the New World. After the bite of an infected sand fly acute disease usually occurs after 16 to 22 days, although the incubation period may be as long as 3-4 months. Acute disease is characterized by fever, headache, musculoskeletal pain, and enlargement of lymph nodes (Scott, 1996). Invertebrate and vertebrate reservoir hosts other than humans have not been demonstrated.

Considering global incidence, the most important disease transmitted by sand flies is leishmaniasis. This parasitic disease is caused by the unicellular *Leishmania* parasite that affects worldwide millions of people. Depending on the *Leishmania* species involved, the disease can vary from ulcerative skin lesions developing at the site of the sand fly bite (cutaneous leishmaniasis, CL) to destructive mucosal inflammation (mucosal leishmaniasis, ML) and disseminated visceral infection (visceral leishmaniasis, VL), which is lethal when untreated.

Leishmaniasis is prevalent in four continents and is considered to be endemic in 88 countries, 72 of which are developing countries. In Europe it is endemic in all southern countries with approximately 700 autochthonous human cases reported each year (Dujardin *et al.*, 2008). Most of the reported cases are due to VL but CL is also present. In most regions of the world including Europe, leishmaniasis is a zoonosis with dogs playing an important role as reservoir host for different *Leishmania* species. These animals can also suffer from leishmaniasis and become very sick. Based on seroprevalence studies 25% of domestic dogs have been shown in Europe to have a *Leishmania* infection (Dujardin *et al.*, 2008). Asymptomatic infected dogs however, are usually sero-negative therefore, this percentage is an under estimation of the actual infection prevalence of dogs in Europe. Sand flies may feed on both asymptomatic and symptomatic dogs and transmit the infection.

Interestingly, autochthonous leishmaniasis appears not to be limited to southern Europe only. VL cases have been reported in northern Italy and southern Germany (Maroli *et al.*, 2008; Bogdan, 2001), indicating northern spreading of this disease.

Surveillance data indicates that the global number of leishmaniasis cases has increased during the past decades (Ashford, 2000; Desjeux, 2004; Reithinger *et al.*, 2003). The noted increases in leishmaniasis may also have been influenced by improved detection and surveillance methods, however an actual increase in numbers caused by more travel was reported (El Hajj *et al.*, 2004; Malik *et al.*, 2006; Stark *et al.*, 2008). Possibly, greater rates of HIV infection in certain regions could also have influenced the incidence (El Hajj *et al.*, 2004; Weitzel *et al.*, 2005). Increased cases of leishmaniasis particularly VL, has been also reported in non-endemic countries (Herremans, 2010).

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Interrupting contact between humans and sand flies

Besides vectors of diseases, sand flies are also a nuisance because of their uncomfortable bites. To prevent the disease transmission or nuisance, various control efforts against sand flies are developed. Since breeding sites of most sand flies are unknown, most control measures aimed at interrupting contact between humans and adult female sand flies (Faiman *et al.*, 2009). General personal protection measures against sand flies bites are advised, including wearing of long sleeves and pants, usage of (impregnated) bednets, avoiding outdoor activities in the evening or usage of insect repellents. The most common methods to control the adult sand flies fall into two broad categories of environmental modifications or usage of insecticides. Studies on destruction and modification of sand fly habitats have found to be suitable for mature sand flies (Yaman, 2008). Environmental modification can also involve the eradication of rodents, destruction of burrow systems and spraying herbicides to kill their food plants and has been demonstrated to be effective in controlling zoonotic leishmaniasis (Faiman *et al.*, 2009). Raising physical barriers such as 2m high walls (personal comm.) or vertical fine mesh nets (Faiman *et al.*, 2009) largely decrease the number of weak flying insect, such as sand flies, entering enclosures.

Chemicals killing adult sand flies are used in insecticide barrier zones in the environment, residual insecticide house-spraying or insecticide impregnated textiles (such as curtains, bednets or bed covers) (Kasali *et al.*, 2010) or vertical mesh nets (see earlier) and dog collars (Killick-Kendrick *et al.*, 1997). Sand fly control has been frequently a side effect of large malaria eradication campaigns residual house-spraying (Alexander & Maroli, 2003). Insecticide barrier zones by fogging with insecticide are effective for exophilic sand flies, but harmful to the environment and labor intensive, and therefore unsustainable and uneconomical.

Besides the common ones, some novel control approaches include rodent feed-through insecticide against sand fly larvae (Mascari & Foil, 2010), avermectin or microbial agents

spraying of the sand flies' sugar supplying vegetation (Yaman, 2008) and the use of pheromone baits (Bray *et al.*, 2010). Moreover, antibodies produced by the host against the salivary glands of sand flies shown promise in the development of vaccination in the control of both sand flies and leishmaniasis (Yaman, 2008)

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4. Sand fly species as new vector candidates for *Leishmania* transmission in Europe

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Scientists working on sand flies in the Mediterranean Basin are used to work in well-known endemic areas for investigating the fauna, seasonal activity or new aspects related to sand flies and sand fly-*Leishmania* parasite interactions.

Recently some studies started to focus on the northern limits of sand fly distribution in Europe mainly in Germany and Hungary. Climate change can affect the distribution ranges of both sand fly vectors and pathogens and they may extend northwards and into higher altitudes. In recent years, evidence of the northernmost occurrence of sand flies in Europe has gradually shifted from 47°55'05.3"N to 48°44'42.2"N and more recently to 50°19'41.2"N (Naucke & Pesson, 2000; Naucke, 2007). In the Mediterranean area, five species, *P. ariasi*, *P. neglectus*, *P. perfiliewi*, *P. tobbi*, and *P. perniciosus*, have been shown to transmit visceral leishmaniasis (VL). In addition, *P. langeroni*, present in central Spain, has been found to carry *L. infantum* in Egypt (Doha & Shehata, 1992).

For several reasons, the majority of sand fly species play no part in the transmission of leishmaniasis: i) they may never bite man; ii) their distribution may be different from that of a reservoir host; iii) their feeding preferences may not include a reservoir host; or iv) they may be unable to support the development of the parasites. In insusceptible flies, *Leishmania* parasites often grow well in the gut of the female during blood meal digestion, but are lost when the fly defecates (Killick-Kendrick, 1999).

To identify new potential vector species the distribution, population structure and vectorial competence of less known sand fly species in areas where autotaneous human or canine leishmaniasis were described should be studied. Especially since these species are now known to spread in most of European and also North African countries. These species are: *P. mascittii* (Sicily-Italy, France, Belgium, Germany, Switzerland, Turkey, Crete-Greece), *P. kandelakii* (Croatia, Slovenia, Turkey), *P. alexandri* (Greece, Spain, Turkey, Tunisia) and *P. halepensis* (Turkey).

The presence of sand fly species which may be probable vectors in some areas of Europe may contribute to an increased risk of emergence or re-emergence of leishmaniasis in northern European countries in association with (i) the introduction of exotic *Leishmania* species or strains into Europe via the increased worldwide travelling of humans and domestic dogs, (ii) the natural spread of visceral and cutaneous leishmaniasis caused by *L. infantum* and *L. tropica* from the Mediterranean region of Europe, where these species are endemic, to neighbouring temperate areas where there are vectors without disease (Desjeux, 2001; WHO, 2009; Ready, 2010). Based on published studies, we hereafter discuss the presence and the possible vectorial roles of these lesser known sand fly species in some leishmaniasis endemic areas of Europe.

***Phlebotomus (Transphlebotomus) mascittii* Grassi**

In Europe, *P. mascittii* was first found in Italy (Rome) by Grassi in 1908. Afterwards the presence of *P. mascittii* was recorded in European Mediterranean countries from Spain to Turkey (Seccombe *et al.*, 1993). *Phlebotomus mascittii* is the type species of the subgenus *Transphlebotomus* created by Artemiev & Neronov (1984).

Phlebotomus mascittii was found in western Switzerland in 1912, in southern Switzerland in 1931, in Strasbourg, near the German border, in 1950, in the southeast of Corsica island in 1954 (reviewed in Naucke *et al.*, 2008). Rioux & Golvan (1969) reported the doubtless presence of the species in Belgium, Germany and Luxembourg in 1969. It was again recorded in Germany in 1999 (Naucke & Pesson, 2000) and in Belgium in 2001 (Depaquit *et al.*, 2005). Other records include Croatia (Dalmatia) in 2006 (Bosnić *et al.*, 2006). Moreover, this species also occurs in Spain, Italy, Greece and Cyprus.

In Germany, the first sand flies were trapped in 1999 at three different locations (Naucke & Pesson, 2000). By 2007, a total of 237 specimens of *P. mascittii* had been caught in 17 locations. The location in Rhineland-Palatinate, the latter near Cochem (on the Mosel river) being the northernmost finding of a sand fly species in Europe. In addition, a breeding area in an old barn was found in the village of Neuenburg, Germany (Naucke, 2002). Since 1991, 11 cases of leishmaniasis in human and different animals were reported in Germany in which an autochthonous origin was confirmed or which were highly likely to have been of indigenous origin. Most of the cases occurred in regions where sand flies have been found (Naucke *et al.*, 2008).

The subgenus *Transphlebotomus* is closely related to the subgenera *Larroussius* and *Adlerius* which comprise the main proven vectors of *Leishmania infantum*. Two species from the latter subgenus were recently implicated in the transmission of cutaneous leishmaniasis: *P. arabicus* (in the Near East, infected by *L. tropica* in nature) (Jacobson *et al.*, 2003) and *P. halepensis* (*L. major* and *L. tropica*, experimentally infected) (Sadlova *et al.*, 2003). *Phlebotomus mascittii* has not yet been confirmed as a vector of leishmaniasis, like other *Transphlebotomus* species. Furthermore low biting rates on humans and its autogeny cast doubt on its epidemiological importance (Ready, 2010). However its vectorial competence is strongly suspected. *Phlebotomus mascittii* is the only one species found in some geographical areas where autochthonous cases of leishmaniasis were described. In Europe, Alpine mountains are thought to act as a natural barrier to the geographical distribution of Sand flies. However, there are regions in Germany with a more Mediterranean climate which are appropriate for some sand flies (Naucke *et al.*, 2008).

Naucke & Pesson (2000) clearly described the vectorial situation of *P. mascittii* after the first detection of this species in Germany. The authors stated that although *P. mascittii* is always found at a low density in *Leishmania infantum*-endemic regions compared with main local vector and little is known about its ethology, and previous field surveys evinced its anthropophilic nature. Additionally, Depaquit *et al.* (2005) also mentioned that the members of *Transphlebotomus* subgenus seem to be relatively abundant, especially in the areas where other *Phlebotomus* species are rare. In southwestern Germany and Switzerland, *P. mascittii* was caught in two sites situated close to human dwellings as well as in a wild biotope (Naucke & Pesson, 2000; Pesson *et al.*, 1985; Grimm *et al.*, 1993). Despite of the author's

suggestions to further study the vector role of this species (Naucke & Pesson, 2000), data on its natural and/or experimental infection of females are still not available.

The subgenus *Transphlebotomus* comprises three species, *P. mascittii*, *P. canaaniticus* and *P. economidesi*, which are morphologically difficult to separate (especially the first two species). To identify these three species a specific molecular marker was developed using ND4 gene by Depaquit *et al.* (2005). The authors found 9.96–10.13% variation between three species making this region suitable for determining the identity of Eastern *Transphlebotomus* species. However since this publication, no other molecular work was done on *P. mascittii* and future work could also include other markers.

A recent study show that the optimum temperature for *P. mascittii* larvae is 19°C, preferred humidity for adults 60-80% and preferred altitude is between 0 and 400 m (Naucke & Schmitt, 2004), but the biology of *P. mascittii* remains poorly documented. Furthermore, it is known to be a cavernicolous species, probably autogenous, strongly attracted by CDC light traps, which seem to be a more efficient method for catching this species than sticky papers. Based on the observed densities in nature and the fact that the completion of the *L. infantum* life cycle is clearly dependent on the number of efficient vectors, *P. mascittii* cannot maintain a *L. infantum* life cycle by itself in nature. Nevertheless, the possibility of transmission of *L. infantum* cannot be excluded, especially around imported cases of canine leishmaniasis. Therefore, the natural or experimental infection of species of *P. (Transphlebotomus)* spp. with *Leishmania* parasites should be further investigated (Depaquit *et al.*, 2005).

***Phlebotomus (Adlerius) halepensis* Theodor**

Phlebotomus halepensis was first described by Theodor in 1958 as a subspecies of *P. chinensis* and was elevated to species level by Artemiev in 1980.

Species of the subgenus *Adlerius* are suspected to transmit *Leishmania* parasites in various Eurasian countries, but the species involved in the transmission have seldomly been identified due to difficulties for morphological identifications, since most species are generally identified on morphology of males. Laboratory colonies of *Adlerius* spp. are seldom available for experimental studies on (female) vector competence and biosystematics (Sadlova *et al.*, 2003).

In Turkey, so far, *P. halepensis*, *P. simici*, *P. balcanicus*, *P. brevis* and *P. kyreniae* were reported, *P. halepensis* and *P. simici* being the more common. *Adlerius* sand flies spp. are widely considered as vectors of VL and are associated with many foci of *L. tropica* causing CL (Killick-Kendrick 1999). Jacobson *et al.* (2003) and Svobodova *et al.* (2006) have isolated *L. tropica* from *P. arabicus* in Northern Israel. Although *P. halepensis* is widespread from Turkey to Israel and Jordan, across Transcaucasia to Iran and southern Turkmenistan, and is distributed in both visceral (*L. infantum*) and cutaneous (*L. major*, *L. tropica*) leishmaniasis endemic areas, its vector status still remains unclear. In only one study, under laboratory conditions, Sadlova *et al.* (2003) showed its high susceptibility to both *L. major* and *L. tropica*. Moreover, the multiplication and development of both parasite species is as high as in the efficient vectors *P. duboscqi* and *P. sergenti* (Sadlova *et al.*, 2003). Lewis (1982) already suggested probable transmission of VL in some southern states of the former USSR and in the former Nakhichevan ASSR (transcaucasian part of southern Azerbaijan, near the Iran border) by *P. halepensis*.

In an unpublished study carried out in an endemic area for CL (Nigde province, Central Turkey) (Ozbel *et al.*, unpublished) 10 patients were diagnosed for CL by conventional PCR method, and two *Leishmania* strains were isolated from them and identified as *Leishmania tropica* MON-55 by isoenzyme analysis. In three villages belonging to Nigde province, the dominant sand fly species was *P. halepensis*, and the overall ratio of *P. halepensis* was 79%. Although promastigotes could not be checked in the sand flies by direct method, no positive fly were detected by PCR method. In preliminary studies carried out in Afyon province, another CL endemic area located in the centre of the Aegean Region, *P. halepensis* was also the most dominant species, but *Leishmania* parasites from this area are not yet identified (Ozbel, unpublished). All these preliminary studies support the possibility that *P. halepensis* is

a CL vector in the nature, but it has not yet been proven to transmit *L. major* or *L. tropica* in the field. However, CL patients are still reported in Nigde and Afyon provinces and the presence of *Leishmania* parasites in *P. halepensis* should be studied using direct microscopical method and real-time PCR. Several field works are planned in summer 2010 and 2011 in both endemic areas.

According to Killick-Kendrick (1990, 1999), four criteria should be checked to assess the role of a sand fly to transmit specific *Leishmania* species, but so far, only two of them have been fulfilled for *P. halepensis*: (1) the sand flies supports development of the parasite after the infecting bloodmeal has been digested and defecated, and (2) the sand flies feed on man and animal reservoir host(s) (Sadlova *et al.*, 2003). In addition, field data on *P. chinensis* in China (Zhang & Leng, 1997) and *P. longiductus* in Central Asia (Dergacheva & Strelkova, 1985) have also showed the anthropophilic behaviour of other members of subgenus *Adlerius*. Although the known natural habitat of *P. halepensis* are rocks, unfed and blood-fed females were also caught in houses during the preliminary study carried out in Turkey, confirming Artemiev's observations (1980), and despite of the absence of blood-meal analyses it is speculate that it bites human. Two other criteria still need to be fulfilled for *P. halepensis*: (1) the isolation of *Leishmania* parasites from wild-caught *P. halepensis*, and (2) their transmission of *Leishmania* parasites by bite. As Alexander & Maroli (2003) emphasized, the local role of a sand fly species in *Leishmania* transmission, in this case *P. halepensis*, should be clarified as much as possible, to be able to counteract by sand fly control.

***Phlebotomus (Larroussius) kandelakii* Shurenkova**

Phlebotomus kandelakii was described in 1929 and belongs to the most important subgenus, *Larroussius* which includes the majority of the vectors of leishmaniasis, mainly visceral. *Phlebotomus kandelakii* is a probable vector of *L. infantum* in Transcaucasia, Iran and Afghanistan (Killick-Kendrick, 1999) and Central Asia (Killick-Kendrick, 1990). In the studies carried out in Afghanistan, it is shown that *P. kandelakii* is very hydrophilic and moderately thermophilic, and bites human and large animals (Lewis, 1982). This Asiatic species has recently been reported from Montenegro (Ivovic *et al.*, 2004) and this finding is noteworthy for Europe. This record also supports the previously doubtful record of this species in Albania (Artemiev & Neronov, 1984).

In Turkey the species is only recorded so far in the provinces of Kars (Eastern Turkey, Armenian border) in a mountainous area of altitude 1,300 to 1,400 meters, and Konya (Central Anatolia), a flat area and altitude 1,000 to 1,030 meters. Although it was found in a very low ratio (1.6%) in Konya province, *P. kandelakii* is the dominant *Larroussius* species in several visceral leishmaniasis endemic areas in Kars province, where it might act as vector (Ozbel Y, unpublished). However there are no recent studies on biology, ecology and vectorial competence of this species, which should be done in the near future.

***Phlebotomus (Paraphlebotomus) alexandri* Sinton**

Phlebotomus alexandri is an anthropophilic species and proven vector of *L. donovani* and a suspected vector of *L. infantum* and also probable vector of *L. major* in Turkmenistan (Killick-Kendrick, 1999). Even though this species is important disease vector, little is known about its ecology and distribution. *Phlebotomus alexandri* is recorded from Spain and Morocco, East to Northwestern China and as far south as Southern Ethiopia. Its altitudinal distribution goes from sealevel up to 1,500 meters.

The role of *P. alexandri* for transmitting kala azar caused by *L. donovani* was demonstrated first time, in the Old World, in Meiyaogou, (Turfan County, Xinjiang Uygur Autonomous Region, China) where kala azar has been found sporadically since 1968 (Guan *et al.*, 1986). The authors also emphasized that *P. alexandri* was the predominant species up to 81.1% of the collected sand flies and it was the only anthropophilic species in that region. Furthermore, they found *Leishmania* promastigotes in 13 specimens out of 643 collected in nature and indoors (Guan *et al.*, 1986). An entomological survey carried out in Sanliurfa province (Turkey), showed that *P. alexandri* was present in the more urban areas of the province, but not in the rural areas (Toprak & Ozer, 2007). Because the species is anthropophilic, it is

possible that its feeding preferences would drive the species to more urban environments where humans are more readily available. More ecological studies should be conducted to determine if there is a relationship between the species and urban areas or if this is just a reflection of sampling bias due to the presence of human leishmaniasis cases (Colacicco-Mayhugh *et al.*, 2010). Even though this species is not a dominant species in Europe, the colonization of *P. alexandri* and the experimental studies for demonstrating the interaction between *P. alexandri* and *L. infantum* are very noteworthy.

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5. Sandfly bites, saliva and gut secretions: Could it contribute to disease control?

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Leishmaniasis is a group of diseases, caused by *Leishmania* parasites, protozoan microorganisms belonging to the family Trypanosomatidae (order Kinetoplastida). These parasites are transmitted between humans, dogs, rodents and other mammals by females of some species of phlebotomine sand flies. The life cycle of the parasite begins when a sand fly female bites an infected human or other mammalian host and ingests *Leishmania* amastigote. Inside the alimentary tract of the sand fly, the amastigote transforms into promastigotes that undergo a cycle of multiplication, maturation, and migration to the stomodeal valve region where they secrete a “Promastigote Secretory Gel” (PSG) made of filamentous proteophosphoglycan (fPPGS) (Rogers *et al.*, 2002, 2004) and also transforms into the infective metacyclic stage. When the infected sand fly takes a subsequent blood meal from human or another mammalian host, it deposits the infective stage of the parasite accompanied by the PSG plug material and its own saliva. Despite this apparently simple life cycle of their causative agents, leishmaniasis has a complex epidemiology driven by a large diversity of *Leishmania* species, sand fly vectors, reservoir hosts and a number of clinical manifestations that result from the infection. Depending on the species of *Leishmania* parasite and a number of other factors, the infection may remain asymptomatic; cause a cutaneous lesion, mucocutaneous disfiguring ulcers or the fatal disease known as visceral leishmaniasis. Despite intensive research and progress in understanding the immunopathology of leishmaniasis, no protective vaccines are available against these diseases.

The transmission of *Leishmania* parasites remained a scientific enigma for several decades following the discovery of the parasite, at the beginning of the 20th century. There have been outstanding problems in replicating transmission experiments in the laboratory, incriminating natural vectors and understanding the mechanism by which an infected sand fly deposits the parasites in the skin of their mammalian host. However, over the past few years it has become widely accepted that sand flies are responsible not only for transmission of *Leishmania* parasites, but also for influencing the outcome of the infection. Several studies showed that *Leishmania* parasites transmitted through sand fly bites results in more exacerbated infection than parasites injected by syringes. Initially, it was thought that this might be mainly due to difficulties in producing virulent infective parasites under culture conditions, or mimicking the natural way by which sand flies injects the parasites in a specific site of the skin. However, recent findings showed that the role of sand flies in helping establish the parasite and exacerbating its infection in the mammalian host may also be related to a number of additional factors, including the co-injection of saliva and PSG as well neutrophil recruitment following injury caused by the bite (Titus & Ribeiro, 1988; Belkaid *et al.*, 1998; Kamhawi *et al.*, 2000; Valenzuela *et al.*, 2001; Rogers *et al.*, 2002; 2004; Peters *et al.*, 2008).

It has been shown that, in addition to their anti-hemostatic effects, sand fly salivary molecules have strong immuno-modulatory properties that may assist in establishment and exacerbation of *Leishmania* infection in their mammalian host. It has also been demonstrated that pre-exposure to sand fly bites or immunization with some sand fly salivary molecules can confer strong protection against cutaneous and visceral leishmaniasis (Belkaid, 1998; Kamhawi *et al.*, 2001; Valenzuela *et al.*, 2001; Gomes *et al.*, 2008). Recently, Peters *et al.* (2008) demonstrated that tissue damage caused by sand fly bites induces the formation of a

plug of neutrophils that phagocytose 80-90% of deposited *Leishmania* parasites. On failure of these neutrophils to cure the infection, *Leishmania* parasites ended up in macrophages and dendritic cells where they maintained long lasting infections. Interestingly the exacerbation effect of the sand fly bite was abolished when the neutrophils were depleted. Thus neutrophils recruited by the sand fly bite provided a temporary home for the parasite until macrophages and dendritic cells picked them up. Although both needle injection and natural inoculation of *Leishmania* parasites by sand flies induced acute neutrophil recruitment, persistence of neutrophils occurred only at the sand fly biting site. In a subsequent report Peters *et al.* (2009) demonstrated that neutrophil recruitment at the biting site of sand flies abrogated protective effects of vaccines that otherwise provided high level of immunity against needle injected *Leishmania* parasites (Peters *et al.*, 2009). This finding indicated that natural transmission by sand fly bite might be needed for testing efficacy of anti-*Leishmania* vaccines.

In addition to the effects of their bites and salivary molecules, sand flies may also influence the establishment of *Leishmania* parasites in the mammalian host by stimulating production and delivery of some parasite factors in the skin of the mammalian host. Rogers *et al.* (2004, 2010) showed that fPPG secreted by *Leishmania* parasites in the stomodeal valve region of the alimentary tract of their sand fly vectors exacerbates the infection of *Leishmania* parasites in their mammalian host. It was also demonstrated that vaccination by this material provides strong anti-*Leishmania* immunity (Rogers *et al.*, 2006). Although PSG is produced by the parasites, it is likely that the gel also contains sand fly material and its formation and delivery are assisted by the structure of the alimentary tract and the feeding mechanisms of infected flies (Bates, 2007).

The recent findings on the immuno-modulatory effects of sand fly bite, saliva and *Leishmania* plug material on pathogenesis of *Leishmania* parasites are offering a great promise for control of leishmaniasis. These findings indicate that challenge by infective sand fly bites is essential for proper laboratory testing of anti-*Leishmania* vaccines (Rogers *et al.*, 2006; Peters *et al.*, 2009). Furthermore, sand fly salivary proteins and the PSG plug materials are becoming strong candidates for future anti-*Leishmania* vaccines. It is probable that anti-*Leishmania* future vaccines will employ these molecules either independently or in combination with parasite antigens.

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6. Transmission trends for *Leishmania*: what we know and what we can learn from *Phlebotomus papatasi* populations in endemic and non-endemic areas

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In the Mediterranean basin, visceral leishmaniasis (VL) caused by *Leishmania infantum* is endemic. The different cutaneous forms of the disease, caused by *Leishmania major* (Zoonotic Cutaneous Leishmaniasis or ZCL), *Leishmania tropica* (Anthroponotic Cutaneous Leishmaniasis or ACL) or less frequently *Leishmania infantum*, are restricted to the oriental and meridional part of the basin. *Leishmania* is transmitted by the bite of phlebotomine sand flies that are dipteran insects within the Psychodidae family. Approximately 700 species were described to date; of these 10% have been incriminated as *Leishmania* vectors. The distribution of the proven vectors for the various forms of leishmaniasis varies greatly between the oriental, meridional and the occidental part of the Mediterranean basin.

In Morocco, all forms of the disease constitute an increasingly public health problem. Dogs are the common reservoir host for VL. The vector has not yet been identified, but, from studies performed in other Mediterranean countries, it is suspected to be one or more of the three dominant endemic species of the subgenus *Larrousius*: *Phlebotomus perniciosus*, *P. longicuspis*, or *P. ariasi*. *Phlebotomus sergenti* is the proven vector of *L. tropica* and the transmission cycle is considered to be anthroponotic, even if some dogs have been found to be infected (Dereure *et al.*, 1991). The ZCL caused by *L. major* is transmitted by *P. papatasi* with *Meriones shawi grandis* (Rodentia, Gerbillidae) as the main reservoir host (for an exhaustive review see Rioux *et al.*, 1986). Additionally, some CL cases are caused by a dermatropic variant of *L. infantum* but no information on the vector and transmission cycle, are available (Rioux *et al.*, 1996). Besides the already suspected *Leishmania* vectors, other endemic sand fly species are present. Within species of the subgenus *Paraphlebotomus*, *P. sergenti* is sympatric, according to the region, with *P. alexandri*, *P. chabaudi*, *P. kezeruni* and/or *P. riouxi*. Within the subgenus *Larrousius*, little is known about the distribution of *P. mariae*, *P. chadlii*, *P. perfilliewi* or *P. ariasi*. Furthermore, the taxonomic status of the *P. perniciosus* complex is not clear (*P. perniciosus*, *P. longicuspis* and a potential cryptic species called PerniX by authors) (Pesson *et al.*, 2004). In all cases, the vectorial status of all these species in *L. tropica* and *L. infantum* transmission is currently not solved. Morocco represents a crossroad between Europe and Africa, and is therefore an important area where leishmaniasis risk, sand flies diversity and their potential displacement, as well as vectorial competence and capacity required further studies. Obviously, leishmaniasis risk will depend on the capacity of sand fly populations (newly established or not) to readily and efficiently transmit *Leishmania*. In fact, in many if not all cases, sand fly distribution does not correlates well with the leishmaniasis distribution, for reasons that seem not to be purely linked to the presence of the different hosts involved in *Leishmania* transmission. These areas might thus represent a paradigm, where underlying factors that influence the vectorial capacity of sand fly populations in natural conditions can be studied. To collect such information, experimental data have to be analysed in the light of the ecological context that *Leishmania* encounters during their intravectorial development.

Phlebotomus sergenti has an extensive geographical distribution, wider than that of ACL. It is suggested that the presence of *P. sergenti* in a *L. tropica* free areas and thus trends in

transmission patterns observed for ACL is related to the existence of some cryptic vector species (Depaquit *et al.*, 2002). The distribution of *P. papatasi* extends from southern Europe and eastern regions to the Indian subcontinent. It thus largely exceeds those of *L. major* and its reservoir hosts. Locally, in Egypt for example, ZCL is primarily present in northern Sinai while the vector distribution is more extensive. Indeed, despite the wide distribution of both *P. papatasi* and *Meriones shawi* in Morocco, ZCL and *L. major* are restricted to the pre-Saharan areas. Among factors that might be informative for *L. major* and ZCL distribution, those linked to sand fly genetics and environmental conditions has to be taken into account. In fact, their potential interference with the vectorial competence and/or capacity of natural sand fly populations remains currently poorly studied. In fact, *Leishmania* resides continuously in the sand fly gut, which represents therefore an important interface for vector-parasite interaction. The developmental life cycle of *Leishmania* in their insect host includes a first initial step; the attachment of *Leishmania* to gut epithelial cells and then several rounds of proliferation and differentiation that ends with the appearance of a transmissible *Leishmania* population. The first epidemiological and genetic studies we have engaged in Morocco suggested that populations structures of *P. papatasi* that colonize regions where ZCL is endemic are to some extent different from those of non-endemic foci (Guernaoui *et al.*, unpublished). However, to what extent such differences have some influence on the capacity of *P. papatasi* populations to efficiently transmit *Leishmania* is unknown and await further studies. The attachment of *L. major* to their specific sand fly vector has been extensively studied on colony-reared sand flies. A galectin expressed at the luminal part of the gut is essential for *Leishmania* attachment (Kamhawi *et al.*, 2004). However, the molecular diversity of this gene, in particular at the galactose recognition site of the insect galectin, has never been investigated in natural sand fly populations. At local geographical areas, environmental conditions encountered by sand flies could also interfere with the *Leishmania* intravectorial development. In sand fly gut, *Leishmania* have to coexist with other microorganisms whose composition and diversity depend on the feeding behaviour of the adults and the terrestrial stages of sand flies, but also on the transtidial transmission of gut associated microbiota. Thus, the relationships that *Leishmania* have with other microorganisms resident in the sand flies gut are currently unknown but can play important role in the determinism of sand fly vectorial capacity in the field. Indeed, resident bacteria have been recently shown to directly influence the capacity of the anopheline vector to sustain *Plasmodium* development. This occurs throughout interactions with the immune status of the anopheles (Pumpuni *et al.*, 1996; Dong *et al.*, 2009). The feeding behaviour of sand fly, nature and origin of sugar meal taken by sand flies, are poorly studied but can substantially interfere with the *Leishmania* attachment and survival in their gut (Schlein & Jacobson, 1994). It is thus important to dissect step by step the influence of these factors on the capacity of sand fly to sustain *Leishmania* development and transmission. Such analysis of the interplay between genetical and ecological factors that modulate vectorial capacity of natural sand fly populations will give information on the leishmaniasis risk associated with sand fly displacement. It could also help to delineate new original and more ecological approaches aimed to tackle the parasite in its insect vector.

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7. Leishmania antimony resistance in the Mediterranean basin

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Leishmaniasis constitutes a diseases' complex that involves morbidity and mortality in 88 countries. The clinical manifestations range from self-healing cutaneous and mucocutaneous skin ulcers to a fatal visceral form, named visceral leishmaniasis or kala-azar. These pathogeneses are due to at least 17 different *Leishmania* species that can be globally considered as pathotypes. In the absence of any effective vaccine, the only mean to treat and control leishmaniasis is affordable medication.

Chemotherapeutic arsenal is restricted to four families of drugs: pentavalent antimony compounds (Pentostam, Glucantime) that were developed in the 1950's, different formulations of Amphotericin B, Pentamidine, and more recently Miltefosine. Cost associated with treatment depends mainly on the drug. In most of south countries where leishmaniasis are endemic, the chemotherapy always relies on the use of antimonial compounds that are cheaper than Pentamidine, or liposomal formulation of Amphotericin B currently used in developed countries. During the 10 latest years, the emergence of high rate of antimonial therapy failures in India has stressed questions about processes that govern the selection and the transmission of chemoresistant parasites. The spreading of such strains has consequences in public health.

Failures of treatments using antimonials are not always related to a resistant phenotype toward antimony for the parasite. They can be a consequence of drug misuse or the host immune status. The resistant phenotype of parasites involves a modification of the target or a decrease of intracellular drug concentration that can result from the diminution of drug uptake, the increase of efflux, the inhibition of the pro-drug activation or the inactivation of drug after its metabolisation. These mechanisms can be driven by mutations or by the modulation of the expression of some metabolic pathways under the influence of environmental factors (Leprohon *et al.*, 2009). Whatever the determinism involved in the resistant phenotype, some efforts must be done to relate biological mechanisms to clinical resistance. But estimating risks of antimony resistance for *Leishmania* needs to take in account the structure of natural population of the parasites, the cost on their fitness and environmental factors.

Structure of *Leishmania* natural populations is basically clonal with rare genetic exchanges taking place in the invertebrate host (Tibayrenc *et al.*, 1991; Akopyants *et al.*, 2009). The probability of such an event is likely very low. Consequently the spread of a chemoresistant phenotype in the field will depend mainly on the capacity of such parasites to be readily transmitted from host to host rather than on genetic exchanges between susceptible and resistant clones. Thus, risk for antimony-resistant leishmaniasis epidemic might be mainly driven by the fitness cost of drug resistance. Experimental *in vitro* studies of generated antimony-resistant *Leishmania* pinpoint that antimony resistance is associated with modifications in the expression of proteophosphoglycans (PPG) that play a role in *Leishmania* attachment and survival to both its vertebrate and invertebrate host (Samant *et al.*, 2007). We have previously documented that antimony resistance impacts the fitness of mutants selected *in vitro* (Sereno *et al.*, 2001) and provided further evidences that *in vitro* selection for resistance is associated with competitive cost (Agnew *et al.*, 2001). In natural conditions we have recently found that some *Leishmania infantum* strains isolated from dogs

are highly tolerant toward antimony. The recurrent sampling of these strains in an active focus supports the notion that they are at least as well transmitted as other strains (Aït-Oudhia *et al.*, personal communication). Zoonotic forms of leishmaniasis primarily target feral or domestic mammalian hosts and occasionally humans. So the quantity of drug used and the time that a parasite is exposed to drug probably need to be dramatically reduced. However environmental factors, yet unidentified, could favour the selection of *Leishmania* parasites bearing modified antimony susceptibility. SbV resistance would not be a consequence of a previous contact with the drug during chemotherapy but could result from another phenomenon. This hypothesis has already been proposed by Yardley *et al.* (2006) in the context of Neotropical leishmaniasis.

In the Mediterranean basin anthroponotic cutaneous leishmaniasis caused by *L. tropica* (ACL), zoonotic cutaneous leishmaniasis (ZCL) caused by *L. major* or less frequently by *L. infantum* and zoonotic visceral leishmaniasis (VL) caused by *L. infantum* is endemic. VL is widely distributed around the Mediterranean basin unlike ACL or ZCL which are restricted to the Southern and Eastern parts. ACL is a threatening problem in many developing and emerging countries. In Iran therapeutic failures are observed in 12% of patients treated with antimonials (Hadighi *et al.*, 2006). However, in the Mediterranean basin, treatment efficiency as high as 95% is reported for the treatment of VL (Gradoni *et al.*, 2008) and reports on antimonial susceptibility of *Leishmania* strains that are circulating in this area are scarce. The sole data on antimony susceptibility concern Algeria where treatment failures of ZCL were reported from 1986 as related with the circulation of antimony tolerant isolates (Belazzoug & Neal, 1986). Given that the various forms of leishmaniasis constitute an emerging and/or a re-emerging problem in the Mediterranean region, care should be taken in the survey of antimonial susceptibility of strains that are transmitted in the Mediterranean basin. However for the occidental part of the Mediterranean where antimony is a clinical use to treat canine leishmaniasis, care should be taken in the follow of amphotericin susceptibility that can diminish simultaneously with antimony susceptibility. This close relation between both susceptible traits has been proved to be experimentally feasible but till now no *Leishmania* isolates resistant to both antimony and amphotericin B has been isolated. The situation is clearly more acute for countries from the Southern and Eastern parts of the Mediterranean where ACL, ZCL and VL occur. Because antimony is the only available drug, the selection and transmission of antimony resistant strains have to be taken into account.

Estimating antimony resistance risk for *Leishmania* in the Mediterranean basin remains a prerequisite to establish antimonials prescribing policy. This aim supposes (i) to identify key factors and associated co-factors driving selection and spread of antimony resistant strains of *Leishmania* and (ii) to understand their role in order to minimize the evolution and spread of chemoresistance. Obviously, these factors might be different for anthroponotic or anthrozoonotic forms of leishmaniasis. At these aims, it will be crucial (i) to gather more information on the frequency of therapy failure linked to proven parasite resistance, (ii) to search for co-factors present in the natural environment that play role in shaping the antimony susceptibility of *Leishmania* populations, and (iii) to further delineate potential fitness cost associated with antimony resistance in order to point the risk of antimony resistance spreading. The latest item is of particular importance when knowing that drug resistance spread could depend greatly on the capacity of parasites to readily accomplish their life cycle.

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8. Intraspecific variability of *Phlebotomus sergenti*, a vector of human cutaneous leishmaniasis, and its possible epidemiological implications

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The phlebotomine sand fly *Phlebotomus sergenti* Parrot, 1917 is a main vector of *Leishmania tropica*, a causative agent of cutaneous leishmaniasis and thus a sand fly species of great medical importance. It was originally described from Algeria, although the species has a broad range of distribution which covers vast areas of Southern Mediterranean (Morocco, Algeria, and Tunisia), Northern Mediterranean (Portugal, Spain, and Sicily), Middle East, Arabia, Afghanistan, Pakistan and northern parts of India. Such a broad distribution suggests a notable intraspecific variability and since the distribution of the vector is broader than the distribution of the transmitted parasite (Depaquit *et al.*, 2002), it may play important role in epidemiology of the disease.

The results of the study on variability of the internal transcribed spacer 2 (ITS2) on populations of *P. sergenti* from 10 different countries outlined two distinct branches: one geographically related to the north-eastern Mediterranean area (Cyprus, Pakistan, Syria and Turkey), the other in south and west of the first one (Egypt, Morocco and Israel). These branches are in accord with postulated migration routes of *P. sergenti* along the Thetys Sea at the Miocene time. Different *P. sergenti* populations may differ in ecology, host preferences and possibly also in vectorial capacity (Depaquit *et al.*, 2002). Potential existence of sibling species within *P. sergenti* should therefore be considered, as proven by means of molecular biology in *Lutzomyia longipalpis*, where different cryptic species were established (Arrivillaga *et al.*, 2003; Maingon *et al.*, 2003).

We studied intraspecific variability of *P. sergenti* by several methods, including molecular approaches (RAPD, sequencing), geometric morphometrics and cross-mating study. RAPD analysis has been repeatedly used to evaluate intraspecific variability in sand flies. It proved to be useful to reveal heterogeneity among different laboratory populations of *L. longipalpis sensu lato* (Dias *et al.*, 1998) and different geographical populations of *L. whitmani* (Margonari *et al.*, 2004). Both taxa are considered to be complexes of morphologically hardly distinguishable sibling species. We have shown by RAPD analysis based on twelve random decamer primers that two laboratory colonies originating from Turkey and Israel form two clearly distinguishable groups (Dvorak *et al.*, 2006). Similar grouping was also observed for wild-caught specimens and sand flies from Turkey, Syria, Israel and Uzbekistan (representing a very distant population) respectively formed clades according to their geographical origin. There was no distinct grouping within the Turkish clade, although studied localities in Eastern Anatolia are separated not only by geographical distance but also by a natural barrier formed by the Amanos Mountain range. This range runs roughly parallel to the Gulf of Iskenderun and divides the coastal region of Cilicia from inland provinces of Turkey and Syria. Reaching

heights of over 2000 m, it is open by several passes which descent to an altitude of approximately 700 meters above sea level. Sand flies are generally considered to be poor flyers, not traveling from their breeding and resting sites (Killick-Kendrick, 1990). However, *P. papatasi* was reported to disperse in the open desert when carried by air currents (Perfiliev, 1968). According to the obtained results of RAPD analysis the Amanos Mountains are not a barrier for sand fly dispersion as the passes play a role of transitional gaps. This is in accord with previously published results based on geometric morphometrics (Aytekin *et al.*, 2007). RAPD marker had been proposed to distinguish *P. papatasi* and *P. duboscqi*, two closely related and morphologically similar vectors of cutaneous leishmaniasis (Mudhopadhyay *et al.*, 2000). Based on results of presented RAPD analysis, we could not find a marker that would have appropriate discriminatory power to distinguish clearly and reproducibly *P. sergenti* specimens from two hypothetical sibling species.

Insect wings are the most appropriate structures for geometric morphometric studies (Pavlinov, 2001). The results obtained from the wing shape and size supported those obtained from the RAPD. Both PCA and UPGMA results showed no shape difference between sexes but the deformation of the wing shape among the Israeli and Turkish populations is significant. The means of the centroid sizes are also supported by the same scheme obtained from the configuration of the landmarks. The sizes are also significantly different in both populations. Similar grouping obtained by RAPD and geometric morphometrics suggests that these two methods are complementary.

Our cross-mating study was possible to perform due to maintenance of two laboratory colonies originating from Turkey and Israel, respectively, areas corresponding to two different branches described by Depaquit *et al.* (2002). The results demonstrate that crossing is possible between *P. sergenti* specimens from Turkey and Israel. We observed successful mating and insemination and obtained viable hybrid F1 and F2 offsprings from both Turkish male/Israeli female and Israeli male/Turkish female combinations. We tested possible differences in egg production and batches descended from Turkish male / Israeli female were compared with egg production from Israeli male/Israeli female mating. Although preliminary results showed a reduction of egg production in females from Israel inseminated by males from Turkish colony, this effect was not statistically significant. According to our results there is no reproductive barrier among sand flies from Turkish and Israeli colonies. The examples of *P. duboscqi* / *P. papatasi* (Ghosh *et al.*, 1999) and *P. bergeroti* / *P. papatasi* (Fryauff and Hanafi, 1991) crossings shows that reproductive isolation may be incomplete even among closely related, but well established species. If we consider *P. sergenti* populations from two above mentioned branches being in the state of speciation or representing sibling species within *P. sergenti* complex, we may expect an incomplete reproductive barrier (Dvorak *et al.*, 2006).

For the sequencing analysis, two markers were chosen: nuclear ITS2 sequence and mitochondrial cytochrome B gene sequence. ITS2 rDNA sequencing is widely used in numerous studies of molecular evolution and phylogeny of various organisms, including dipterans. In Old World phlebotomine sand flies, it was successfully deployed in phylogenetic analyses of subgenus *Larroussius* (Di Muccio *et al.*, 2000) and *Paraphlebotomus* (Depaquit *et al.*, 2000). On intraspecific level, it revealed a notable variability of *P. sergenti* (Depaquit *et al.*, 2002), while there was a significant homogeneity in diverse populations of *P. papatasi* (Depaquit *et al.*, 2008). Our results of ITS2 rDNA sequencing corroborated the previously published intraspecific division of *P. sergenti* into two branches, north-eastern and south-western. Mitochondrial genes are also very popular and useful molecular markers as the lack of recombination helps to elucidate the evolutionary history even better. Mitochondrial cytochrome B (cyt B) genes were successfully used to evaluate intraspecific variability of *P. papatasi* populations (Hamarshah *et al.*, 2007). In our study, mtDNA haplotypes from Turkey, Israel, Syria, and Uzbekistan formed three lineages. All but one remaining specimens from Turkey formed a clade with samples from Israel.

Combination of several approaches and the use of different markers give more appropriate and relevant idea about the intraspecific variability of *P. sergenti*. Results presented here together with cytochrome B sequencing analysis of specimens from Morocco (Yahia *et al.*, 2004) favor this marker as more discriminative than ITS2. Similarities between cytochrome B

sequences of specimens from Turkey and Israel supported by the fact that males and females originating from Turkey and Israel mated readily and produced viable and fertile progeny under experimental laboratory conditions put in doubt the concept of a *P. sergenti* species complex (Dvorak *et al.*, submitted). Nevertheless, further analysis with more molecular markers is worth testing to resolve the taxonomy status of *P. sergenti* and closely related species of subgenus *Paraphlebotomus* as it is required for relevant assessment of their role in *Leishmania tropica* transmission.

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9. Updates on distribution of new sand fly fever viruses

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Sand fly fever (SF) first became a major problem to the Western world among New Zealand troops in Egypt in June 1941 and then for American and British troops in North Africa and Sicily. Sand fly fever Sicilian virus (SFSV) and sand fly fever Naples virus (SFNV) were isolated during follow up studies (Duncan & Stout, 1954; Coates, 1963; Sabin *et al.*, 1944).

Clinical reports and seroprevalence studies of SFSV infection have since been reported from Croatia (Punda-Polic *et al.*, 1990) Cyprus (Niklasson & Eitrem, 1985; Eitrem *et al.*, 1991), Egypt (Feinsod *et al.*, 1987), Pakistan (Bryan *et al.*, 1996), Iran (Tesh *et al.*, 1977; Saidi *et al.*, 1977; Javadian *et al.*, 1977), Iraq (Ellis *et al.*, 2008) and India (Goverdhan *et al.*, 1976). Seroepidemiological studies have shown that the prevalence of antibodies to SFSV and SFNV in indigenous populations follows the distribution of *Phlebotomus papatasi* around the Mediterranean region, the Middle East, North Africa and Western Asia (Tesh *et al.*, 1977; Saidi *et al.*, 1977; Javadian *et al.*, 1977; Tesh *et al.*, 1976; Cohen *et al.*, 1999; Batieha *et al.*, 2000; Dionisio *et al.*, 2003).

Since 2006, SFV epidemics were reported from Cyprus (Papa *et al.*, 2006; Konstantinou *et al.*, 2007) and from troops in Iraq (Ellis *et al.*, 2008). A new isolate termed sand fly fever Cyprus virus (SFCV) was isolated and characterized. An outbreak in 2007 in Mediterranean Turkey in Kozan and Izmir as well as in Ankara in central Turkey was clearly attributed to an isolate from a case in Izmir termed sand fly fever Turkey virus (SFTV). This virus was completely sequenced and showed highest homologies to SFCV (Carhan *et al.*, 2010). Additional seroprevalence studies seem to indicate activity of SFTV, SFNV and for Toscana Virus TOSV in central Turkey. For TOSV partial sequences were determined from patient material (Ergünay *et al.*, 2010). Phlebotomus survey studies are ongoing and so far only the vector of TOSV *P. perfilliewi* has been found close to Ankara (Özbel, 2009). A review of the clinical cases of SF in Turkey describes severe leucopenia, thrombocytopenia and elevated liver enzymes, a surprising finding which has not yet been reported from other outbreaks (Tufan *et al.*, submitted). In a recent study from Greece, a new SFS like virus was isolated from the cerebrospinal fluid of a patient suffering from acute encephalitis. This virus appears to belong to the Salehabad serocomplex of Rift Valley fever virus just like Arbia isolated from from *P. perniciosus* in Italy in 1988 (Papa, 2010; Verani *et al.*, 1988) for which however involvement in human infection has not yet been shown.

Recently, several studies on Phlebotomes have resulted in the discovery of new *Phlebovirus* isolates. One SFSV like virus designated SFSV Corfou virus was isolated from a pool of *P. major* in Greece in 1985 (Rodhain *et al.*, 1985). In Algeria seroprevalence studies in humans also pointed to SFSV activity. Studies revealed a partial SFSV like L-segment sequence from a pool of *P. ariasi* (SFSV Algeria) and a pool of *P. papatasi* (SFSV Kabylia) and SFNV-like sequences from *P. longicuspis* (Izri *et al.*, 2008, Moureau *et al.*, 2010). Studies in neighbouring Tunisia described more SFSV-like sequences from *P. perniciosus*, *P. longicuspis* and *Sergentomyia minuta*, and SFNV-like sequences from *P. perniciosus* and *P. longicuspis*. Furthermore a new SFNV-like virus termed Punique virus was isolated and characterised from a pool of *P. perniciosus* during the same study (Zhioua *et al.*, 2010). Another virus belonging to the SFNV complex was also isolated from a pool of *P. perniciosus* collected in Marseille, France. This virus was termed Massilia virus (MASV) (Charrel *et al.*, 2009). Some of these studies showed clinical involvement indirectly by detecting IgG in human sera, but none detected viruses in clinical material.

A study in the upper Rhine valley of Germany identified IgG against TOSV in 7% of a healthy control group and 10 IgM and IgG positive cases in a group of acute aseptic meningo-encephalitis cases. Additionally partial TOSV S-segment sequences were determined from one cerebrospinal fluid and one serum. These results seem to indicate an emergence of TOSV north of the Alps, since the last serostudy in 1995 had found a seroprevalence for TOSV infection of only 1% presumed due to travel activities. *Phlebotomus perniciosus* has

been described in a neighbouring region in 2001 and currently *Phlebotomus* studies are ongoing in the upper Rhine valley (Meyer-König *et al.*, submitted; Naucke *et al.*, 2008).

In conclusion, recent studies have described many new SFSV- and SFNV-like viruses and new sand fly species have been implicated in their carriage. Much more sand fly species appear to be competent for particular phleboviruses than hitherto thought (Table 1). It remains to investigate if the emergence of sand fly fever in central Turkey and of Toscana encephalitis in southern Germany is due to the ingression of sand fly populations into more northern regions due to climate change.

Table 1 – *Phlebotomus* species and isolated Sand fly viruses

	SFSV	SFNV	TOSV	Arbia V	MASV	Punique V
<i>P. ariasi</i>	X					
<i>P. longicuspis</i>	X	X				
<i>P. major</i>	X					
<i>P. neglectus</i>	X					
<i>P. papatasi</i>	X	X				
<i>P. perfilliewi</i>		X	X			
<i>P. perniciosus</i>	X	X	X	X	X	X
<i>S. minuta</i>	X		X			

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10. Surveillance of Toscana virus in Europe: Studies on sand flies and clinicoepidemiological features of human TOSV infection

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Toscana virus (TOSV) has been isolated from the phlebotomines *Phlebotomus perniciosus* (Verani *et al.*, 1980), *P. perfiliewi* (Verani *et al.*, 1988), and recently, it has been detected in *Sergentomyia minuta* (Charrel *et al.*, 2006). Studies in pools of phlebotomines have shown 0.05-0.2% infection rates (Verani *et al.*, 1988; Sanbonmatsu-Gámez *et al.*, 2005). TOSV is transmitted to humans by the bite of *P. perniciosus* and *P. perfiliewi*, since *S. minuta* feeds on reptiles but not on humans.

Viral culture has demonstrated to be less sensitivity than RT (reversetranscription)-PCR assays for TOSV detection in sand flies (Sanbonmatsu *et al.*, 2005; Charrel *et al.*, 2006). The design of RT-PCR methods have to take into account the genetic variability of TOSV strains from different geographical areas, since two TOSV genotypes have been described. Thus, conserved regions of TOSV genome need to be targeted to obtain reliable PCR results. Five PCR methods are available for specific amplification of TOSV genome. Three systems use RT-nested-PCR protocols, followed by visualization of the PCR products by agarose gel

electrophoresis (Schwarz *et al.*, 1995a; Valassina *et al.*, 1996; Sánchez-Seco *et al.*, 2003). The other two assays use real-time PCR systems and Taqman® probes for detection of amplicons (Pérez-Ruiz *et al.*, 2007; Weidmann *et al.*, 2008).

Phlebotomines are active during the warm months of the year (from May to October), coinciding with the peak of incidence of human cases of TOSV infection. Other mechanisms should occur to maintain the virus throughout fall and winter. Several studies have demonstrated transovarial and sexual transmission of TOSV among phlebotomines (Verani *et al.*, 1988; Tesh *et al.*, 1982; Tesh & Modi, 1987) that might contribute to maintain the virus all the year.

Although reservoir vertebrates have been documented for other arboviruses, to date, there is no known reservoir for TOSV. TOSV has only been isolated from the brain of a bat, although no TOSV antibodies were detected in these animals (Verani *et al.*, 1988). A recent serosurvey study conducted on 1,186 domestic animals within Granada province (southern Spain) has shown an overall 36.2% seroprevalence rate of TOSV antibodies in cats, dogs, pigs, cows, horses and sheep (Navarro-Marí *et al.*, 2010). However, the virus could not be isolated or detected from any animal serum except from one positive PCR result (low-level viremia) in a goat from a village where TOSV meningitis had taken place in the past few years and where TOSV had been isolated from phlebotomine pools (Sanbonmatsu-Gámez *et al.*, 2005). This seems to be a TOSV hyperendemic area. Even though humans and animals may play a role in the life cycle of TOSV by infecting naïve sand flies, the vector must ingest large quantity of virus in order to become infected. No high-level viremia has been demonstrated in humans or animals. Thus, the reservoir of TOSV is probably its vector.

Since the isolation of TOSV in phlebotomines in 1971, the presence of the virus was first suspected in Italian patients (Nicoletti *et al.*, 1991). Autochthonous human cases of TOSV infection have been described in Portugal, Spain, France, Slovenia, Greece, Cyprus, and Turkey, on the basis of viral culture, reverse transcription (RT)-PCR and serology (Charrel *et al.*, 2005). Travel-related TOSV infection has been reported in patients from other countries (US, Germany, Sweden, and Switzerland), that returned from endemic areas of TOSV (Calisher *et al.*, 1987; Eitrem *et al.*, 1991a; Schwarz *et al.*, 1993; Sonderegger *et al.*, 2009; Tappe *et al.*, 2010).

Serosurvey studies have demonstrated high TOSV seroprevalence rates in these areas. In Italy, TOSV seroprevalence rates from 16 to 22% have been reported in different areas (Braito *et al.*, 1998; Valassina *et al.*, 2003). An occupational risk study conducted on forest workers in the region of Siena, Italy, showed anti-TOSV IgG positivity in 77% of individuals (Valassina *et al.*, 2003). In Spain, two serosurvey studies have reported 26% and 24.9% of seroprevalence rates (Echevarría *et al.*, 2003; Sanbonmatsu-Gámez *et al.*, 2005). In Cyprus, a seroprevalence rate of 20% was demonstrated in healthy population (Eitrem *et al.*, 1991b). A significant age-dependent increase in TOSV seroprevalence rate has been documented in Spain and Italy (Sanbonmatsu-Gámez *et al.*, 2005; de Ory-Manchón *et al.*, 2007; Terrosi *et al.*, 2009). Apart from the Mediterranean countries, seroprevalence rates reported in other areas are low (Schwarz *et al.*, 1995b).

The high seroprevalence rates reported in several studies suggest that an important proportion of TOSV infections may be asymptomatic or produce a mild disease. Aside from TOSV, other sand fly fever viruses cause a brief, self-limiting febrile illness (Nicoletti *et al.*, 1996). However, few cases of mild disease due to TOSV infection have been documented. To date only three studies have reported mild TOSV infections, i.e. two cases of exanthema without neurological involvement in Italy and Spain (Portolani *et al.*, 2002; Sanbonmatsu-Gámez *et al.*, 2009), and one case of febrile illness in southern France (Hemmersbach-Miller *et al.*, 2004).

TOSV infection is associated with neurological disease. The most common clinical feature is aseptic meningitis, which is usually mild and self-limited, and patients affected recover in a few days without sequelae. Few cases of severe disease (encephalitis and meningoencephalitis) have been reported, some of them related to predisposing immunosuppressed conditions. Clinical signs related to these severe cases were ischemic

complications, stiff neck, deep coma, maculopapular rash, hepatosplenomegaly, diffuse lymphadenopathy, and diffuse intravascular coagulopathy. As well, sequelae (impaired speech, paresis, ataxia, aphasia and deafness) have been documented in 4 cases of severe TOSV neurological infection (Dionisio *et al.*, 2001; Baldelli *et al.*, 2004; Kuhn *et al.*, 2005; Martínez-García *et al.*, 2008; Sanbonmatsu-Gámez *et al.*, 2009; Anagnostou *et al.*, 2010).

The geographical distribution of the two TOSV genotypes varies throughout Europe. TOSV A genotype includes Italian strains (although it has also been detected in patients from France and Portugal), whereas TOSV B genotype circulates in Spain, France and Portugal (Collao *et al.*, 2009). These differences in geographic distribution of TOSV genotypes might be explained in part by vector characteristics. Two lineages of the main vector of TOSV, *P. perniciosus*, have been reported, the typical lineage found in Morocco, Tunisia, Malta, and Italy, and the Iberian lineage. These lineages remain isolated because sand flies move in short hops, flying no more than a few hundred meters from their resting places (Pesson *et al.*, 2004).

New members of the Naples serocomplex have recently been described, as a result of surveillance of TOSV and other phleboviruses in sand flies, Massilia virus in France (Charrel *et al.*, 2009) and Granada virus in Spain (Collao *et al.*, 2010). The latter was isolated from a phlebotomine pool during the study conducted in 2005 by Sanbonmatsu-Gámez *et al.* Interestingly Granada virus was reported from the same area where TOSV RNA could be detected in a serum sample from a goat. Genetic analysis of Granada virus isolate suggests that this may constitute a reassortant of Massilia virus (donor of L and S genomic segments) and a yet undefined phlebovirus (donor of M segment). The detection of specific neutralizing antibodies in human serum collected in Granada demonstrates that Granada virus infects humans.

Continuous surveillance, especially in these hyperendemic areas, is important for a deeper knowledge of TOSV and other phleboviruses ecology, and to define a possible pathogenic role in humans of new undefined phleboviruses.

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11. Effects of settlement and construction on transmission of cutaneous leishmaniasis in dry habitats of the Old World

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Human activities and human induced changes are considered as the major factors that increase the risk for leishmaniasis (e.g. Desjeux, 2001; Ready, 2010). Global warming models predict the geographical spread of leishmaniasis into current disease free areas (e.g. Schönian *et al.*, 2008). Movement of people, rise of tourism, traveling and trade increase the risk of exposure of people from non-endemic areas. The risk of introducing parasites into new areas where suitable vector and reservoir animals are present is an additional concern (e.g. Ready, 2010). In endemic areas, urbanization, deforestation, settlement of non-immune human population, agro-industrial projects and military activities are considered to be the major causes of the increase of the leishmaniasis incidence (e.g. Desjeux, 2001; Reithinger *et al.*, 2007).

This review focuses on the effects of local ecological changes related to human activities on transmission of the parasites causing cutaneous leishmaniasis in endemic areas of the Middle East and neighbouring countries. Natural transmission in the region occurs mostly in arid or semi-arid areas. Typical habitats are characterized by sparse native vegetation cover, a hot / dry season, lasting six or more months, and high ground surface temperatures. Two species of *Leishmania* parasites, *L. major* and *L. tropica* are responsible for the majority of endemic cutaneous leishmaniasis cases in the area. The transmission cycle of *L. major* is zoonotic (ZCL) involving the sand fly vector *Phlebotomus papatasi* and rodent reservoir animals (*Psammomys obesus*, *Rhombomys opimus*, and several species of *Meriones*). In urban foci *L. tropica* is considered anthroponotic (ACL) and is transmitted by *P. sergenti* (Ashford & Bettini, 1987). In Israel *L. tropica* is transmitted by *P. sergenti* and *P. arabicus*. The transmission is zoonotic and the rock hyrax (*Procapra capensis*) is the major reservoir animal (Jacobson *et al.*, 2003; Jaffe *et al.*, 2004; Talmi-Frank *et al.*, 2010). Reservoir animals in natural habitats depend on availability of food plants, suitable hiding places or burrowing conditions (Ashford & Bettini, 1987). Sand flies are found in close association with rodent burrows and are limited by the availability of moist soil rich in organic matter for the development of the immature stages, cool and humid hiding places for the small fragile adults, sugar sources and blood meals for the females (Killick-Kendrick, 1999).

Enhanced leishmaniasis transmission risk due to man-made local ecological changes has been observed in several locations (Table 1). In general, these observations indicate that environmental modifications favor the multiplication of sand flies and reservoir animals, thus enhancing the risk of an increase in rates of bites from infected sand flies and subsequent increase in leishmaniasis transmission. The major constraint limiting development of sand flies in desert areas is soil moisture, so the addition of water to the soil is a very important causative factor that enhances sand fly breeding.

In rural areas, the construction of dams and irrigation systems not only increases soil moisture, but they also contribute to rich vegetative growth. The soft, moist soil and improved growth of vegetation increase the land's carrying capacity of reservoir rodents. Dense rodent populations and the abundance of burrow systems in the soil favor an increase in sand fly breeding sites. The simultaneous favorable conditions for the sand fly vector and reservoir host enhance the risk for zoonotic leishmaniasis transmission. A similar phenomenon on a smaller scale has been observed along the road margins in desert areas. Richer vegetation

develops in shallow road drainage ditches, and rodent burrows are more abundant in the loose soil along the road edges compared to nearby fallow land.

In urban environments where the human population lives in poverty, the effect of increased soil moisture is an outcome of poor infrastructure, lack of organized sewage and drainage systems. In addition, urban environments are often characterized by the close proximity of domestic animals to human inhabitants. The organic waste matter from domestic animals attracts breeding sand flies and increases the number of resting sites, thus increasing the density of biting sand flies near humans and the risk of anthroponotic transmission.

In modern urban neighborhoods, conditions are less favorable for the development of sand flies. However, improved biotopes for both sand fly vectors and rodent reservoir animals may develop in the interface between human settlements and uncultivated (wild) habitats. The creations of regularly irrigated ornamental gardens provide rich food sources for herbivores, which in turn create suitable conditions for sand flies and rodents. The construction of supportive walls on hills and the accumulation of debris from boulders and dirt create superb breeding habitats and hiding/resting sites for various small mammals. The transmission of zoonotic leishmaniasis in the periphery of modern neighbourhoods is the result of thriving reservoir animals and sand fly vectors in this man-made ecological niche.

While increased soil moisture increases the number of potential breeding sites for sand flies and rodents, the construction of new housing developments as a sole factor has been related to an increase in sand fly populations. In Jordan and in Morocco, demolition and reconstruction of human habitations initially decreased sand fly populations, but the populations later recovered and expanded beyond the pre-development levels. In the Judean Desert in Israel, a greater than 10-fold increase in sand fly densities was observed in the year following area wide construction of new housing developments. One explanation for the increased densities might be that flattening the area and covering it with loose dirt rockfalls increased the absorption capacity of the area.

It seems impractical to prevent leakage of water in traditional agriculture systems, old urban quarters and existing settlements. However, when new human settlements or military camps are established, the use of modern technologies could reduce or prevent the risk for leishmaniasis transmission. The use of trickle irrigation systems that allow a slow dripping of water directly to the roots of plants could prevent the rise of the water table and increased soil moistening in large agricultural areas. Simplified hydroponics, controlled environment and soilless gardening and agriculture in water sensitive areas might also help to reduce unwanted soil moisture along with additional benefits, including water conservation. Soil water absorption and suitable habitats for rodents can also be reduced by compacting the soil during and after digging and construction, and eliminating loose soil humps, rockfalls and boulder piles in the margins of irrigation canals, roads and settlements. A recent report describes a significant reduction in leishmaniasis incidence following environmental modifications (Faulde *et al.*, 2009). Appropriate planning and implementation of deliberate environmental modifications during urban and rural development are imperative to prevent increased risk of *L. major* and *L. tropica* transmission in endemic areas.

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Table 1. Effect of human ecological modifications on the transmission of *Leishmania tropica* and *L. major* causing anthroponotic cutaneous leishmaniasis (ACL) or ZCL (zoonotic cutaneous leishmaniasis) in the Middle East and surrounding countries (cities or regions are indicated in parentheses). Ecological modifications causing an increase in sand fly biting nuisance in areas with unknown ACL or ZCL transmission are also shown.

Eco transmission type / <i>Leishmania</i> sp.	Location	Type of modification	Effect of modification	Possible explanation	Reference
ACL / <i>L. tropica</i>	Afghanistan (Kabul)	Unplanned city expansion, without proper infrastructure	Outbreak of cutaneous leishmaniasis	Environment supporting development of sand flies	Nadim and Rostami, 1974
ACL / <i>L. tropica</i>	Iran (Neishabur)	Unplanned city extension, scattered houses with between open areas	Outbreak of cutaneous leishmaniasis	Environment supporting development of sand flies	Nadim and Tahvildary, 1977
ACL / <i>L. tropica</i>	Iran (Kerman)	Proximity to medieval adobe castle and mud-brick city	High incidence rate in certain areas of the city	Suitable breeding and resting sites for <i>Phlebotomus</i> spp.	Seyedi-Rashti <i>et al.</i> , 1984
ACL / <i>L. tropica</i>	Iran (Isdahan)	Construction of new housing and proximity to old mud houses	<i>Leishmania</i> cases in new houses and old quarters	Creation of suitable breeding conditions for sand flies in new developments and proximity to existing breeding sites in old houses	Zahraei-Ramazani <i>et al.</i> , 2007
ACL / <i>L. tropica</i>	Turkey (Sanliurfa)	Lack of infrastructure and animals kept in cellars	High densities of sand flies and increased <i>Leishmania</i> incidence	Organic matter and moisture and moderate temperature	Svobodová <i>et al.</i> , 2003
ZCL / <i>L. major</i>	Former U.S.S.R.	Agricultural development	Outbreaks of cutaneous leishmaniasis and sharp increase in <i>P. papatasi</i>	Irrigation increasing soil moisture	Kellina, 1981 (a review, summarizing several reports)
ZCL / <i>L. major</i>	Israel (Jordan Valley)	Agriculture and irrigation	High densities of <i>P. papatasi</i> sand flies and <i>Psammomyz obesus</i> with higher proportion of infected sand flies in irrigated areas compared with non-irrigated natural habitats	Soil conditions favoring high humidity in <i>P. obesus</i> burrows	Schlein <i>et al.</i> , 1982
ZCL / <i>L. major</i>	Tunisia, (Centre-Sud)	Construction of dam and planting of <i>Atriplex</i> spp. (salt bush)	Increase in gerbil populations and ZCL epidemic	Increased soil moisture and surplus of vegetative food sources (e.g. <i>Atriplex</i> sp.) for rodent reservoir hosts	Ben-Ismaïl & Ben Rached, 1989
ZCL / <i>L. major</i>	Jordan (Sweima & Karama)	Cultivation and canal irrigation	Increase of leishmaniasis incidence, higher densities of chenopods plants, <i>P. obesus</i> colonies and sand flies in cultivated area compared with natural biotope	Increased soil moisture	Kamhawi <i>et al.</i> , 1993; Janini <i>et al.</i> , 1995

Table 1 – Human ecological modifications on transmission of cutaneous leishmaniasis in the Middle East – (Continued).

Eco transmission type / <i>Leishmania</i> sp.	Location	Type of modification	Effect of modification	Possible explanation	Reference
ZCL / <i>L. major</i>	Syria (Dei res Sor)	Expansion of irrigation	Increase in rodent populations and ZCL epidemic in the city and nearby villages	Increased soil moisture	Desjeux, 2001
ZCL / <i>L. major</i>	Israel (Negev Desert)	Anthropogenic disturbances	Increased soil moisture, vegetation lush, <i>P. obesus</i> and sand fly abundance and <i>Leishmania</i> infection rates in <i>P. obesus</i> in disturbed areas compared with natural habitats.	Addition of water and increase in soil moisture	Wasserberg <i>et al.</i> , 2003
ZCL / <i>L. major</i>	Afghanistan (Mazar-e Sharif)	Extensive farming	Rodent infestation and high densities of sand flies following irrigation and embankments of earth deposits	Loose soil and increased soil humidity	Faulde <i>et al.</i> , 2008
ZCL / <i>L. tropica</i>	Israel (Judean Desert)	Planned modern towns interfering with natural habitat	Outbreak of ZCL, with high densities of sand flies and rock hyraxes (rodent host) in town perimeters.	Increased soil moisture, continuous supply of vegetation and creation of new hiding places for rock hyraxes	Singer <i>et al.</i> , 2008; Orshan <i>et al.</i> , 2010
ZCL / <i>L. tropica</i>	Israel, (Judean Desert)	Construction	Ten-fold increase in sand fly densities, no increase in rate of ZCL incidence	Increase water seepage into ground causing increased soil moisture	Orshan, unpublished
Transmission unknown	Jordan (Irbid)	Building activities	Increase in <i>Phlebotomus</i> numbers after human inhabitation of new houses	Creation of new sand fly breeding and resting sites	Kamhawi <i>et al.</i> , 1991
Transmission unknown	Morocco (Marrakech)	Modern and planned urbanization versus unplanned communities	Poor sanitation and animals kept in and around houses resulting in higher densities of sand flies in the unplanned communities	Suitable habitat for sand flies	Boussaa <i>et al.</i> , 2007

12. Distribution of Phlebotominae sand flies and visceral leishmaniasis in Albania

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Phlebotominae sand flies in Albania are vectors for cutaneous and visceral leishmaniasis. The etiologic agent of visceral leishmaniasis in Albania is *L. infantum* (Cani *et al.*, 2001).

Studies on the distribution of Phlebotominae in Albania were started in 1958 and conducted through 1989 by Dr. Jorgji Adhami, and restarted after a 10-year hiatus, in the year 2000, by E. Velo in collaboration with M. Maroli and G. Buongiorno, ISS.

To monitor sand flies the following techniques have been used:

- Sticky non-attraction traps
- CDC light-attraction traps
- Mechanical aspirator
- Mouth aspirator, also known as manual capturer (Maroli & Fausto, 1986)

Sticky traps have been placed in the field, about twenty sticky traps on average per site, in cattle stables, hen houses, inside and outside dwellings, and in wall crevices in castles and houses. After two to three days the trap content was collected into plastic bags, labeled with the district, date, and station number, and brought to the lab. This method of capture was used during the studies of Dr. Adhami in the period 1958-1989 and during 2002 at least once a month for all stations. CDC light traps were first successfully used in Albania by the Laboratory of Entomology in 2002, inside and outside animal shelter, in chicken-pen, around human dwellings, etc. Mouth aspirators have been used by Dr. Adhami in the period 1958-1989 to capture sand flies inside human dwellings and animal shelters from June to September and in July 2002 by the Laboratory of Entomology, IPH, Albania.

Identification of sandfly species was based on morphological characteristics described by Theodor (1958) and Léger & Pesson (1987). Classification is based on Lewis (1982).

Sand fly species present in Albania and their distribution

In Albania eight sand fly species are present, belonging to two genera, *Phlebotomus* and *Sergentomyia*. The latter is represented by *S. minuta* and *S. dentata*, while the species of genus *Phlebotomus* present in Albania are: *P. neglectus*, *P. perfiliewi*, *P. papatasi*, *P. similis*, *P. simici* and *P. tobbi* (Adhami & Murati, 2000; Velo *et al.*, 2003).

Phlebotomus papatasi (Scopoli, 1786), was historically reported from 9 out of 36 districts and made up 12.5% of the Phlebotominae captured in human habitation centers during 1958-1989 (Adhami & Murati, 2000). Recently it was found in 6 districts only: Krujë, Laç, Lezhë, Tiranë, Shkodër and Vlorë. It is believed that distribution limits of this species restricted to dwellings (Adhami, 1986) were reduced due to the extensive use of DDT in dwellings to combat malaria. Since these treatments have ceased now, it may expand again. The highest elevation where it has been found in Albania was 674 m in Krujë, while in Italy it has been found as high as 775 m in the province of Aquila (Maroli *et al.*, 1991b).

Phlebotomus perfiliewi (Parrot, 1930), was found in 19 of 36 districts of Albania, most often in habitation centers of the lower districts. It prefers sandy clay soil as found in the hills of Durrës, Elbasan, Lushnje, Fier, Berat and Sarandë. The highest point where it was found in Albania was Hlomo at 800 m (district of Gjirokastrë). Thus, unlike in the neighbouring countries, *P. perfiliewi* is present only in the western area of plains and hills in Albania. It is thought that the main influencing factor is the soil composition of the area.

Phlebotomus neglectus (Tonnoir, 1921) was the most widespread and the most frequent species in habitation centers. This species prefers rocky ground with a "solid structure", limestone in particular. In habitation centers built upon limestone the density reached up to

28.4/CDC/night or 34.6 insects per 100 sticky traps, while in a sandy-clay environment only 2.4 insects per 100 sticky traps were trapped (Adhami & Murati, 2000). This demonstrates the importance of the soil structure. Nevertheless, other factors like climate, vegetation, and the impact of human activities should not be excluded. The highest elevation where this species has been found in Albania is 1,300 m.

Phlebotomus tobbi Adler, Theodor & Lourier, 1930 was reported from 14 of 36 districts. Its highest densities were observed in the elevation range 0-300 m. In 73% of the habitation centers it was associated to *P. perfiliewi* (Adhami & Murati, 2000).

Phlebotomus similis (Perfiliew, 1963) was reported in 9 of 36 districts. It was found more frequently in barns than in human dwellings and mainly in limestone terrain.

Phlebotomus simici (Nitzulescu, 1931) was found in 5 of 36 districts at elevations from 300 to 900 m, mainly in broken and rough terrain (Adhami & Murati, 2000).

Sergentomyia minuta (Rondani, 1843) was reported in 18 of 36 districts and captures reached 52% of the total sand flies caught in natural biotopes. It is found mainly in the elevation range 0-300 m.

Sergentomyia dentata (Parrot, 1935) was very rare in Albania, both in habitation centers, as in natural biotopes. It was encountered by Adhami & Murati (2000) in only 6 of 36 districts. Both *Sergentomyia* species are herpetophilic and do not have any epidemiological importance.

From the retrospective analysis of the results of the research by Adhami for the years 1958-1989 in the whole country and the research made by us in the main source areas of leishmaniasis for the years 2001-2003, it can be concluded that the most widespread and dominant species is *P. neglectus*, followed by *P. perfiliewi* and *P. papatasi* (Adhami & Murati, 2000; Velo *et al.*, 2003; Velo *et al.*, 2005).

Zoonotic visceral leishmaniasis in Albania

Zoonotic visceral leishmaniasis (ZVL) is known to occur in Albania since 1938 as a typical childhood disease (Adhami *et al.*, 1983). Epidemiological figures of the disease have been reported by reports referring to four periods:

- (i) 1947-1962: 133 cases (8 cases/year) recorded (Todhe, 1963)
- (ii) 1960-1984: 1,264 cases (50 cases/year) (Adhami & Murati, 1986)
- (iii) 1984-1996: 1,136 cases (87 cases/year) (Kero & Xinxo, 1998)
- (iv) 1997-2001: 867 cases (173 cases/year) (Velo *et al.*, 2003).
- (v) 2004-2008: 572 cases have been reported among humans, with an incidence rate of 4.3 (cases/100,000 people) in 2004 and 3.4 in 2007. About 89% percent of the districts are infected with VL. The most affected areas are: Shkodra 1.4, Lezha 1.6, Berat, 1.1, Elbasan 0.7, Tirana 0.3 and Vlora 0.4 cases/10,000 population (Unpublished). It is believed that during the last years many cases are not reported to IPH.

The increase in VL morbidity, 20 to 40 folds higher than in other southern European countries, could be attributed to increased susceptibility of infants to clinical disease, or to variations in the force of *Leishmania* infection associated to changes in canine reservoir and/or vector populations. (Velo *et al.*, 2003). Although the incidence is high in Albania no control program is applied in the country.

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