New distributional data on sandflies from rubble walls in the Maltese Islands with an illustrated key to the Maltese species (Diptera: Phlebotominae)

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ABSTRACT. The results of a survey which involved collecting phlebotomine sandflies in rubble walls from 20 different localities on the islands of Malta and Gozo in the period from August to October 2008 are reported. A total of 402 specimens were collected, all belonging to two species: *Sergentomyia minuta* and *Phlebotomus perniciosus*. A short diagnosis, and notes on the biology of the six species of sandflies recorded from the islands is given, together with an illustrated key to their identification.

KEY WORDS. Malta, rubble walls, leishmaniasis, sandflies, distribution, identification key.

INTRODUCTION

Phlebotomine sandflies (Diptera: Phlebotominae) are small (1.5 - 3.0 mm) greyish coloured hairy flies (Fig. 1) currently generally assigned to the family Psychodidae (Diptera: Nematocera). The classification of the Psychodidae at the subfamily level, however, is still debated and the flies have also been treated as a separate family (Phlebotomidae) by some authors. The subfamily is of great medical and veterinary importance because some species are vectors of both cutaneous and visceral leishmaniasis in humans and animals. The terrestrial immature stages are very poorly known.

More than 530 species have been described, and the subfamily is widely distributed in the tropics and subtropics northwards to latitudes up to 50°N. They occur in a wide range of habitats ranging from hot deserts, savannas, tropical rainforests, open woodland and more urban environments. They have been found in soil from animal burrows, in tree buttresses and tree holes, forest floors, as well as in railway tunnels and in rubbish taken from the streets (Feliciangeli, 2004; Naucke *et al.*, 2008). The Maltese archipelago in the central Mediterranean consists of the inhabited islands of Malta, Gozo and Comino and a number of uninhabited islets and rocks, occupying an area of about 316 km^2. The climate is typically Mediterranean with characteristic mild, wet winters and hot, dry summers. Air temperatures are moderate with a mean monthly temperature range of 12 - 26°C. The average annual precipitation is 530 mm but this is highly variable. Relative humidity is consistently high throughout the year, being mostly in the range 65-80% (Schembri, 1993). These conditions make the islands a suitable environment for sandflies which require warmth and high humidity to develop and survive. The flies are mostly active from dusk to dawn mainly between May and October when temperatures are still relatively high.

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Rubble walls (Fig. 2) (commonly known in Maltese as "Ħitan tas-Sejjieħ") are a common and characteristic habitat of phlebotomine sandflies in the Maltese Islands. They provide a safe haven both for the adults and the immature stages. They are more numerous in Gozo, and in the north of Malta. The conditions are ideal being dark, humid and protected from winds. Rubble walls along with other locations such as cellars, latrine drains, house tanks, and crevices in caves and stables have all been quoted in the literature as breeding grounds for sandflies in the Maltese Islands (Marret, 1910; Newstead, 1911; Adler & Theodor, 1935; Léger *et al.,* 1991).

Twenty-two species of sandflies are known to occur in Europe (Wagner, 2004), of which six have been recorded from the Maltese Islands: *Phlebotomus perniciosus*, *P. neglectus*, *P. perfiliewi*, *P. papatasi*, *P. similis* and *Sergentomyia minuta* (Léger *et al.,* 1991; Depaquit *et al*., 2002). This compares favourably with the fauna of neighbouring Italy (8 species) and Tunisia (9 species). *P. perniciosus* and *S. minuta* are by far the most abundant, while *P. neglectus*, *P. perfiliewi* and *P. similis* are rare. *P. papatasi* has not been recorded in Malta since 1935 (Adler & Theodor, 1935). Of the six species present, *P. perniciosus*, *P. neglectus* and *P. perfiliewi* are known vectors of *Leishmania infantum*, the intracellular protozoan which causes leishmaniasis in dogs and humans. Adult female *P. perniciosus*, however, are the only significant vector in the Maltese Islands, as *P. neglectus* and *P. perfiliewi* are rare.

Leishmaniasis can be of two types in humans; visceral or cutaneous. Visceral leishmaniasis (kala-azar) is caused by the intracellular protozoa multiplying in macrophages in internal organs. Symptoms include fever, hepatosplenomegaly, lymphadenopathy, anaemia, leukopenia, thrombocytopenia, progressive emaciation and general weakness. It is a chronic disease which, left untreated, is often fatal, especially in children aged between one and four (Amato-Gauci, 1992). Cutaneous leishmaniasis in Malta causes skin papule development which is usually single but may be multiple. It may resolve spontaneously within weeks or months. Humans are only incidental hosts of leishmaniasis, the main reservoir animals being dogs. Canine leishmaniasis is always visceral but signs may be visceral only or both visceral and cutaneous. Cats may also be hosts of the disease and 18% of a population selected randomly irrespective of their state of health, breed, and status (pet or stray) tested locally were found to be seropositive for *L. infantum* by PCR and IFAT testing (Williams, 2009). Transmission rates of *L. infantum* from cat to sandflies has been found to be 21% (Maroli *et al*., 2006) and therefore cats could play also play a role in the transmission of the disease.

Although a significant body of literature exists on various aspects of leishmaniasis and its vectors in the Maltese islands, practically no distributional data of the species known to occur on the islands has been published apart from that given by Gradoni & Maroli (1987). The aim of this work was to rectify this by collecting and identifying as many adult specimens as possible collected from 20 localities on the islands, and to provide an illustrated key to the Maltese species, thus facilitating their identification by future workers.

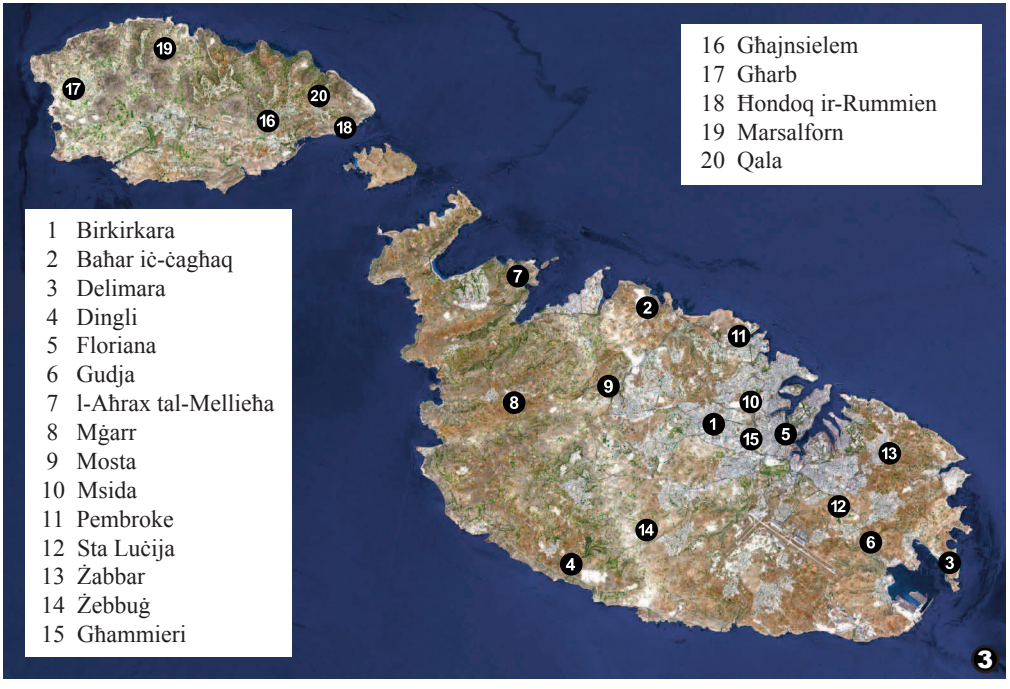
MATERIAL AND METHODS

All material was collected from the Maltese Islands between August and October 2008. Material was collected from 25 different locations in 20 localities (15 in Malta and 5 in Gozo) (Fig. 3). Material was collected using castor oil sticky traps, placed in crevices in rubble walls and drainage holes in conventional walls. Specimens attached to the sticky traps were removed with a fine brush dipped in absolute alcohol and specimens were stored in 75% ethanol. The genera *Sergentomyia* and *Phlebotomus* were readily separated using external characters under a high power stereomicroscope. Most male specimens of *P. perniciosus* could also be segregated under

Figure 1. Adult female *Phlebotomus* feeding on human blood

Figure 2. Typical rubble wall in Malta

Figure 3. Map of the Maltese Islands showing localities sampled



the stereomicroscope when the bifid aedeagus of this species was readily visible. Semi permanent slides of representative males and females were prepared using modified Berlese medium as described in Lewis (1973) and studied under a compound microscope. Unless otherwise stated drawing of morphological parts of sandflies were done from slide mounted specimens using a Zeiss Axioscope 2 plus with a drawing tube attachment.

The main morphological characters used in the identification of specimens were in the male and female terminalia. The main distinguishing features in the male is the shape of the aedeagus, the shape of the style, the number of spines and their location on the style, the presence or absence of basal outgrowths on the coxite and the shape of the parameres. In the female the arrangement and number of segments in the spermatheca is the most useful characteristic. Specimens were identified with the aid of Theodor (1948), Perfiliev (1969), Lane (1986), Léger *et al.* (1983), Lewis (1973, 1978), Nadim & Javadian (1976), Romi *et al.* (1994) and Seyedi & Nadim (1992).

KEY TO SPECIES OF MALTESE SANDFLIES

1. Cibarium with well developed posterior cibarial teeth and with pigment patch (Fig. 4a); wings narrow and apically pointed (Fig. 4c); hair sockets distinct on abdominal tergite one (AT1), hairs recumbent (Fig. 4e). Male terminalia as in figure 4g and female spermatheca as in figure 4h ..... ***Sergentomyia minuta***

– Cibarium without cibarial teeth and without pigment patch (Fig. 4b); wings broad and apically rounded (Fig. 4d); hair sockets distinct on abdominal tergites one to six, hairs erect (Fig. 4f). Male terminalia and female spermatheca different ..... ***Phlebotomus* spp.** **2**

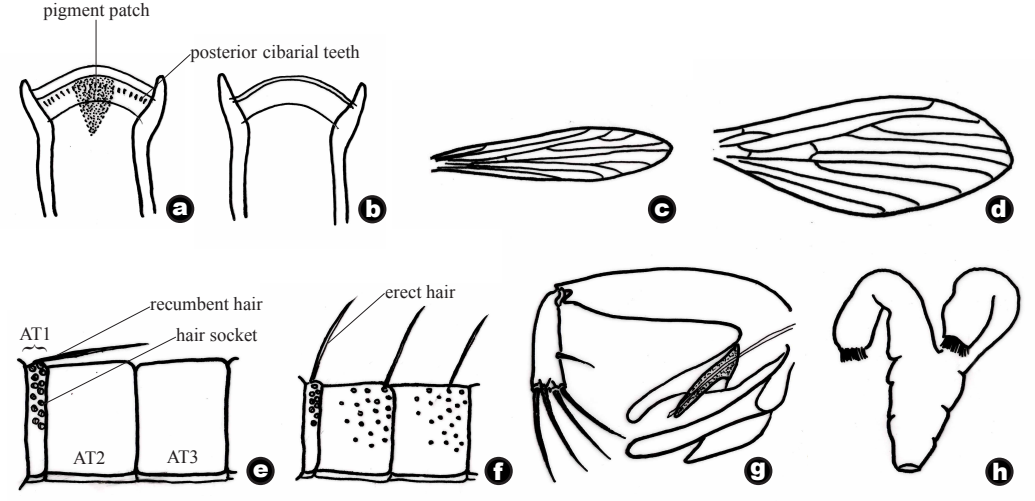


Figure 4. **a**, **c**, **e**, **g**, **h**: *Sergentomyia*; **b**, **d**, **f**: *Phlebotomus*; **a-b**, cibarium; **c-d**, wings; **e-f**, abdominal tergites; **g**, male terminalia of *S. minuta* (modified after Romi *et al*., 1994); **h**, spermatheca of *S. minuta.*

2. Tip of abdomen with distinct parameres, aedeagus and lateral appendages ..... (males) **3**

– Tip of abdomen lacking such structures ..... (females) **7**

3. Male terminalia with four spines present on style, two of which are terminal; large basal outgrowth present on coxite; aedeagus short and thick, curving outwards and slightly backwards and apically rounded (Fig. 5a)……..……..…….. ***P. similis***

– Male terminalia with five spines present on style, two or three of which are terminal (Fig. 5b, c); basal outgrowth on coxite absent or reduced (Fig. 5b); aedeagus straight either apically rounded or bent at tip (Fig. 5d, e) or bifid (Fig. 5f) or with translucent membrane at tip (Fig. 5g) **4**

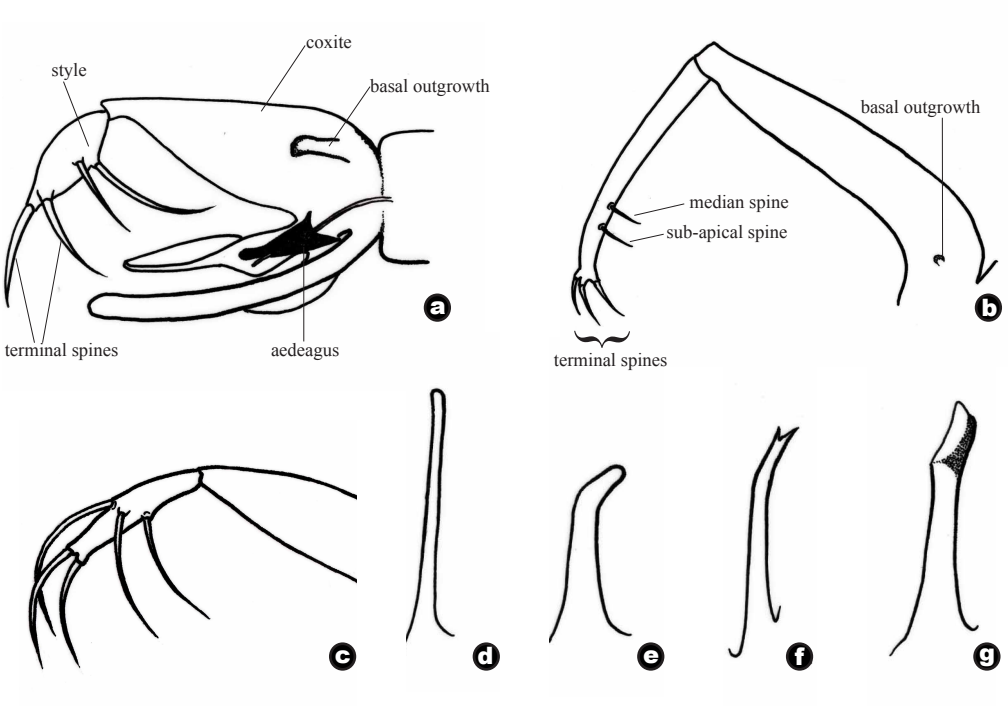


Figure 5. **a**, male terminalia of *P. similis*; **b-c**, coxite and style; b, *Phlebotomus papatasi*; **c**, *P. perfiliewi*; **d-g**, *aedeagus*; **d**, *P. neglectus*; **e**, *P. papatasi*; **f**, *P. perniciosus*; **g**, *P. perfiliewi* (**a-c**: modified after Perfiliev, 1969; **d-g**; redrawn after Romi *et al*., 1994).

4. Male terminalia with three terminal spines on style; style long and narrow; small basal outgrowth present on coxite (Fig. 5b); aedeagus apically rounded, short and curved (Fig. 5e) ……..…….. ***P. papatasi***

– Male terminalia with two terminal spines on style; style broad and short (Fig. 5c); no basal outgrowth present on coxite; aedeagus long, apically rounded and slightly bent at tip (Fig. 5d) or bifid (Fig. 5f) or with translucent membrane at tip (Fig. 5g) **5**

5. Aedeagus long and thin and apically rounded (Fig. 5d) ..... ***P. neglectus***

– Aedeagus bifid (Fig. 5f) or with translucent membrane at tip (Fig. 5g) **6**

6. Aedeagus bifid (Fig. 5f) ..... ***P. perniciosus***

– Aedeagus with apical ventral translucent membrane (Fig. 5g) ..... ***P. perfiliewi***

7. Spermatheca without neck connecting body to head (Fig. 6a) **8**

– Neck of spermatheca connecting body to head present (Fig. 6b) **9**

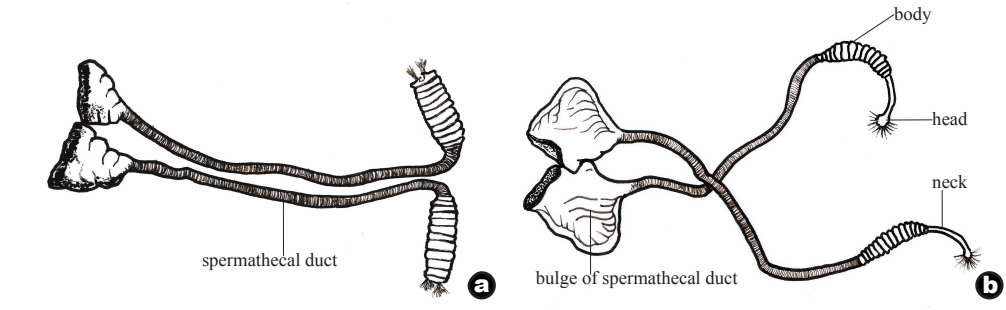


Figure 6. **a-b**: spermathecae; **a**, *Phlebotomus papatasi*; **b**, *P. perfiliewi* (redrawn after Romi *et al*., 1994)

8. Body of spermatheca with 3 - 7 segments (Fig. 7a); terminal segment larger than posterior segments ..... ***P. similis***

– Body of spermatheca with 8 - 10 segments and all of equal size (Fig. 7b) ..... ***P. papatasi***

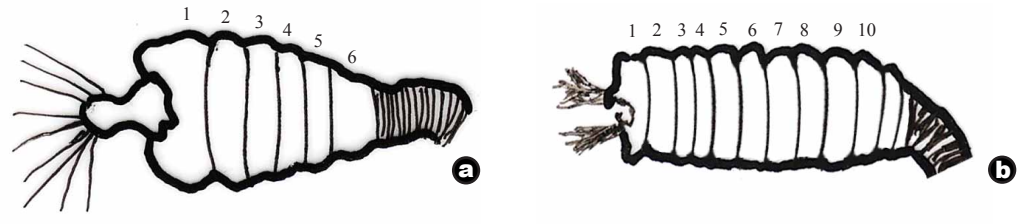


Figure 7. **a-b**: detail of spermathecal body; **a**, *Phlebotomus similis*; **b**, *P. papapatasi*. (**b**: redrawn after Romi *et al*., 1994)

9. Body of spermatheca with 12 - 16 segments and spermathecal ducts forming a common duct (Fig. 8a); no bulges at base of spermathecal ducts ..... ***P. neglectus***

– Body of spermatheca with 10 - 14 segments; spermathecal ducts not forming common duct but opening individually into genital opening (Fig. 8b); bulge present at base of spermathecal ducts **10**

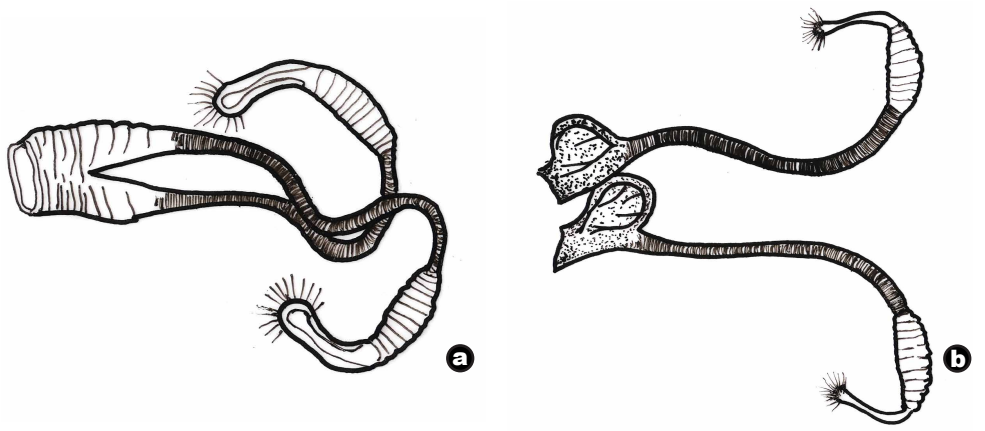


Figure 8. **a-b**: spermathecae; **a**, *Phlebotomus neglectus*; **b**, *P. perniciosus*. (**a-b**: redrawn after Romi *et al*., 1994)

10. Body of spermatheca with 10 segments; small bulge present at base of spermathecal ducts; ducts about three times the length of spermathecal body (Fig. 8b) ..... ***P. perniciosus***

– Body of spermatheca with about 14 segments; prominent large bulges at base of spermathecal ducts; ducts about four times the length of spermathecal body (Fig. 6b) ..... ***P. perfiliewi***

SYSTEMATIC LIST OF THE SANDFLIES OF THE MALTESE ISLANDS

Family PSYCHODIDAE
Sub-family PHLEBOTOMINAE

Genus: *Sergentomyia* França & Parrot, 1920

Currently the genus accommodates nine well defined subgenera but some species remain ungrouped (Davidson, 1987). Some members of this genus share intermediate characters with *Phlebotomus* and *Lutzomyia*, and this makes these species difficult to identify. The only species of *Sergentomyia* present in the Maltese islands belongs to the subgenus *Sergentomyia* França & Parrot, 1920. This subgenus is characterised by tubular spermathecae in the females with smooth sides and a wide duct. In males the aedeagus is thick. The genus *Sergentomyia* is mainly distributed in the Old World tropics (Lewis, 1973) and is particularly rich in areas where *Phlebotomus* are scarce (Lane, 1993).

Sergentomyia (*Sergentomyia*) *minuta* (Rondani, 1843)

Material examined: **MALTA:** Baħar iċ-Ċagħaq, 12-13.ix.2008, 1 ♂; Delimara, 29-30.viii.2008, 10 ♂♂, 34 ♀♀; Floriana, 11-12.viii.2008, 1 ♀; Ġudja, 11-12. ix.2008, 5 ♂♂ , 5 ♀♀; Mġarr, 4-5.x.2008, 1 ♂; Mosta, 12-13.ix.2008, 5 ♂♂ , 5 ♀♀; Msida, 11-12.viii.2008, 1 ♂, 2 ♀♀; Santa Luċia, 1-2. ix.2008, 2 ♂♂, 18 ♀♀, 29-30.viii.2008, 5 ♂♂ , 6 ♀♀; Żabbar, 11-12.ix.2008; 1 ♀; Żebbuġ, 24-25.ix.2008, 3 ♂♂ , 10 ♀♀. **GOZO:** Għajnsielem, 5-6.vii.2008, 1 ♂; Marsalforn, 6-7.vii.2008, 9 ♀♀; Qala, 6-7. ix.2008, 1 ♀; Qala (beach) 2 ♂♂, 2 ♀♀; Qala (centre), 26-27.ix.2008, 1 ♂; Qala (farmhouses), 26-27.ix.2008, 6 ♂♂, 4 ♀♀; Qala (playingfield), 26-27.ix.2008, 3 ♂♂, 10 ♀♀.

Diagnosis: Wings narrow and pointed. Recumbent hairs present on abdominal tergites one to six, arising from narrow oval sockets. Sockets clearly visible on tergite one but much smaller on tergites two to six. Cibarium with a posterior transverse row of long, fine parallel teeth. Pigment patch dark and easily observable. Pharynx narrowing markedly after posterior bulge. Male coxite simple. Style with four terminal spines, aedeagus thick and tapering to a point. Female spermathecae characteristically long, simple, broad and tubular, usually doubled back on themselves. Spermathecal walls smooth with small folds or creases.

Distribution: Widely distributed in the Mediterranean basin, including North Africa.

Biology: *S. minuta* feeds on reptilian blood namely on the gecko *Tarentola mauretanica* (Linnaeus). Its role as a vector of *Trypanosoma platydactyli* Catouillard in reptiles was studied by Adler & Theodor (1935). Feeding may take up to two hours or more, after which the female is capable of laying a full batch of eggs. *Sergentomyia* species are not known to act as vectors of human pathogens. Recent studies however have shown that Toscana virus, which is infectious to humans causing acute meningitis and meningoencephalitis, has been found in *S. minuta* specimens from France. The fact that *S. minuta* feeds on reptiles may prevent it from infecting humans (Charrel *et al*., 2006).

Notes: Newstead (1911) was the first to record this species from the Maltese Islands under the name *Phlebotomus minutus*. It was stated as being quite rare in Malta at that time. Later, Adler & Theodor (1935) cited the species (as *P. parroti*) as being far more numerous in Malta in 1931 than in 1932. No reference to its distribution or the locations sampled was made. Gradoni & Maroli (1987) collected ten specimens of *S. minuta* (4 ♂♂ and 6 ♀♀) in June 1987. In a study on sandflies in Gozo (Léger *et al*., 1991), *S. minuta* was found to be the second most abundant species in the various biotopes sampled. In a survey carried out during the last week of August of 1988 by Killick-Kendrick (1988), 3,627 specimens were *S. minuta* (relative abundance = 53.28%) out of a total of 6,808 sandflies. Another, follow-up study carried out the following year revealed that 57.8% of 2,788 sandflies caught were *S. minuta* (Léger *et al*., 1991). Between August and September of 1988, 141 female *S. minuta* were tested for flagellates (Léger *et al*., 1991). One specimen was found to be infected with a flagellate of the family Trypanosomatidae which infects only reptiles. Similarly in 1989, 433 female *S. minuta* were tested, of which 4 were found to contain Trypanosomatidae (Léger *et al*., 1991). During the present study, a total of 153 specimens of *S. minuta* (46 ♂♂ and 107 ♀♀), were caught from 17 locations in 13 different localities. The overall relative abundance of *S. minuta* was 37.59%.

Genus: *Phlebotomus* Rondani, 1840

This genus comprises almost all of the man-biting sandflies and is the only genus whose members act as vectors of pathogens to man. It is widely distributed in the Old World with most species occurring in the north (Lewis, 1978). Their altitude of distribution ranges from below sea level near the Dead Sea to 3,300 m above sea level in Afghanistan (Lane, 1993). The genus is characterised by species having broad and rounded wings; presence of erect hairs on all tergites, arising from large round sockets clearly visible on all tergites; cibarial armature and pigment patch absent; males with four or five spines on style. The five species of *Phlebotomus* recorded from the Maltese Islands belong to three different subgenera.

Subgenus: *Larroussius* Nitzulescu, 1931

This subgenus is characterised by species having the following combination of morphological characters. Male coxite with no basal outgrowth, style with five terminal spines, paramere simple. Female spermatheca with a neck between the head and the spermathecal body, segments of which are rounded. Pharyngeal teeth punctiform. Several species accommodated in this genus are vectors of leishmaniasis in the Mediterranean basin and mountainous areas from the Middle East to Northern Pakistan (Lane, 1993).

Phlebotomus (*Larroussius*) *perniciosus* Newstead, 1911

Material examined: MALTA: Baħar iċ-Ċagħaq, 12-13.ix.2008, 4 ♂♂; Delimara, 29-30.viii.2008, 1 ♂; Dingli, 22-23.viii.2008, 13 ♂♂, 16 ♀♀; Mgarr, 4-5.10.2008, 4 ♂♂, 1 ♀; Mosta, 12-13.ix.2008, 2 ♂♂; Żabbar, 11-12.ix.2008, 3 ♂♂; Żebbug, 24-25.ix.2008, 7 ♂♂, 2 ♀♀. **GOZO:** Kerċem, 17.viii.2008, 1 ♂, 2 ♀♀; Marsalforn, 6-7.vii.2008, 4 ♂♂, 7 ♀♀; Qala (beach), 37 ♂♂, 14 ♀♀; Qala (centre), 26-27.ix.2008, 7 ♂♂, 1♀; Qala (Ħondoq ir-Rummien fields), 26-27.viii.2008, 40 ♂♂, 30 ♀♀; Qala (playing-field), 26-27.ix.2008, 6 ♂♂, 32 ♀♀; Qala (farmhouse), 26-27.ix.2008, 2 ♂♂, 1 ♀; Qala, 6-7.ix.2008, 7 ♂♂, 5 ♀♀.

Diagnosis: Male terminalia distinctive, with bifid aedeagus. Style with 5 spines, two of which are terminal. Female spermatheca with head, neck and body. Head with spiny outgrowths. Body with ten segments. Spermathecal ducts separated, with a bulge on each at base.

Distribution: A Mediterranean species known from the Balearic islands, Corsica, Malta, Spain, Portugal, Italy (and its islands) and North Africa.

Biology: *P. perniciosus* females feed on both endoderms such as dogs, humans, horses, cats, rats etc. as well as on fruits and nectars (Rossi *et al*., 2008). They require blood meals for development of eggs. Males only feed on fruits and nectars. *P. perniciosus* is of medical importance as a vector of *Leishmania infantum,* which causes visceral and cutaneous leishmaniasis in humans and dogs. It is also known as a vector of Toscana & Arbia phleboviruses in the Northern and Western Mediterranean (Balducci, 1988).

Notes: *P. perniciosus* was first described by Newstead (1911) from material collected in Malta. It was regarded as being widely distributed over the island and especially abundant in the months of July, August and beginning of September. Newstead (1911) also described *P. nigerrimus*, from two females. These two specimens were previously cited by Marret (1910) from Gozo. According to Leger *et al.* (1991), *P. nigerrimus* is synonymous with *P. perniciosus*. Investigations carried out in the 1930's showed that *P. perniciosus* was by far the commonest sandfly species in Malta, occurring at least 20 times more abundantly than *P. papatasi* (Adler & Theodor, 1935). In June 1987, Gradoni & Maroli (1987) collected 120 specimens (49 ♂♂ and 71 ♀♀) of *P. perniciosus* out of a total of 133 sandflies (relative abundance = 90.2%). Léger *et al.* (1991) found *P. perniciosus* to be the most abundant species in a number of different biotopes during a study carried out in 1988. During the same period a quantitative survey was carried out using sticky traps (Killick-Kendrick, 1988). A total of 6,808 sandflies were caught, of which 2,990 were *P. perniciosus*, making it the second most abundant species caught (relative abundance = 43.92%). In a follow-up study carried out in 1989 the relative abundance was very similar with a value of 41.89%. Out of a total of 2,788 sandflies collected, 1,167 were *P. perniciosus* (Léger *et al*., 1991). In both 1988 and 1989, dissection and testing of *P. perniciosus* was carried out for

flagellates. In 1988 out of 1,463 sandflies, six were found to be positive for *Leishmania infantum* while in 1989 seven out of 2,063 were positive (Léger *et al*., 1991). During the present study, 249 (139 ♂♂ and 110 ♀♀), specimens of *P. perniciosus* were collected from rubble walls, from 16 locations in 13 different localities in Malta and Gozo. The overall relative abundance of *P. perniciosus* was 63.18%.

Phlebotomus (*Larroussius*) *perfiliewi* Parrot, 1930

Diagnosis: Aedeagus with distal ventral translucent extremity. Style with 5 spines, two or which are terminal. Spermatheca with head, neck and body; body with an average of 14 segments, spermathecal ducts separated, with a very large bulge at the base.

Distribution: Widely distributed in the Mediterranean, and extending eastwards to Turkey and the Ukraine. Recorded also from North Africa.

Biology: Females feed on sheep, goats, humans and possibly dogs (Naucke, 2002). They require a blood meal for development of eggs. Males feed on fruits and nectars. *P. perfiliewi* has been found naturally infected with *Leishmania infantum* (Maroli *et al*., 1987). Its role in the transmission of visceral leishmaniasis has been suspected but not proven (Killick-Kendrick *et al*., 1977; Maroli & Bettini, 1977).

Notes: *P. perfiliewi* was first mentioned from the Maltese Islands by Adler & Theodor (1935). It was however misinterpreted as *P. macedonicus* (Léger *et al*., 1991). The species was found in small numbers from limited areas in Gozo (exact location not given) and was quite rare compared to the other species recorded at the time. No specimens of *P. perfiliewi* were found during a sampling effort in July 1987 (Gradoni & Maroli, 1987). Léger *et al.* (1991) in August 1988, set up CDC light traps in areas surrounding house, stables and caves. Relative abundances of this species were reported as 17.4%, 2.26% and 0.75% respectively. Sticky traps set up by Léger *et al.* (1991) in August 1988 and 1989, caught 135 and 6 specimens respectively. There was a decrease in relative abundance from 1.98% in 1988 to 0.21% in 1989. Léger *et al.* (1991) examined females for parasites but none were found positive for flagellates. During the present study, no specimens of *P. perfiliewi* were found.

Phlebotomus (*Larroussius*) *neglectus* Tonnoir, 1921

Diagnosis: Males with a very long straight aedeagus, the tip of which forms a bulge rather than tapering to a point. Female spermatheca with head, neck and body; head with projections emerging from it, body with 12 - 16 segments and spermathecal ducts joining to form common duct leading to genital opening.

Distribution: The species is known from Albania, Austria, Corfu, Crete, Italy (including Sicily), Malta, Romania and ex-Yugoslavia.

Biology: Females feed on humans, horses, rodents and possibly dogs. Females require a blood feed for development of eggs. Males feed on fruits and nectars. *P. neglectus* was found to act as a vector for *L. infantum* in specimens obtained from Corfu in Greece (Léger *et al*., 1988).

Notes: Adler & Theodor (1935) were the first to mention the presence of *P. neglectus* (as *P. major*) in the Maltese Islands after examining specimens sampled in Gozo. It was said to be

more common than *P. similis* but still much rarer than the two most abundant species at that time. Gradoni & Maroli (1987) recorded the presence of *P. neglectus* (as *P. major*) in Malta. Two males were present out of a total of 133 sandflies captured. In 1988 and 1989 sampling efforts were carried out in Gozo by Léger *et al.* (1991). A survey using sticky traps spanned 16 different locations over August 1988 and July 1989. Totals of 6,808 and 2,788 sandflies were collected respectively. In both years *P. neglectus* was one of the least abundant species caught, with 53 specimens collected in 1988 (relative abundance = 0.78%) and only one specimen caught in 1989 (relative abundance = 0.04%). CDC light traps were set up by Léger *et al.* (1991) in areas surrounding houses, in stables and in a cave. Nine specimens were caught in total, 8 from the cave (4 ♂♂ and 4 ♀♀) and 1 male from an area surrounding houses. No specimens were caught from the stables. Out of 13 specimens of *P. neglectus* tested for parasites by Léger *et al.* (1991) none were found positive for flagellates. During the present study, no specimens of *P. neglectus* were found.

Subgenus: *Paraphlebotomus* Theodor, 1948

This subgenus is characterised by the following combination of morphological characters. Inner surface of male coxite with a fleshy sub-basal outgrowth which is medium sized to large and bears long hairs. Style with only four spines. Female spermatheca usually with a much larger terminal segment than the posterior segments. Pharyngeal teeth large and scale like, with smooth margins which appear like a network. This subgenus occurs mainly in the Palaearctic Region, and many species are associated with rodent burrows (Lane, 1993).

Phlebotomus (*Paraphlebotomus*) *similis* Perfiliev, 1963

Diagnosis: Aedeagus thick and curved outwards and slightly backwards. Style short and thick with four spines two of which are terminal. Coxite twice as long as style, with a prominent, hairy basal outgrowth. Female with strong prominent pharyngeal teeth. Body of spermatheca with 3 - 7 segments and with the terminal segment larger than the others.

Distribution: Azerbaijan, Russia, Ukraine, Romania, ex-Yugoslavia, Albania, Greece, Turkey and Malta.

Biology: Owing to its similarities to *P. sergenti,* questions have been raised as to whether *P. similis* can also act as a vector for *L. tropica*. This however has not been proven (Depaquit *et al*., 2002).

Notes: Throughout the entire literature of sandflies in Malta, *P. similis* has been misidentified as *P. sergenti* (Depaquit *et al*., 1998). All previous continental or insular records of *P. sergenti* should in fact refer to *P. similis* (Depaquit *et al*., 2002). *P. similis* was first recorded in the Maltese Islands from the island of Gozo by Adler & Theodor (1935). They stated that it was rare, irregularly distributed and of no great local importance. It was later recorded in 1987 (Gradoni & Maroli, 1987) this time from the Island of Malta. Similar to the situation in Gozo, *P. similis* was found to be a very rare species. Out of 133 sandflies caught, only one was identified as *P. similis* (relative abundance = 0.76%). Sticky traps set up by Léger *et al.* (1991) in various locations around Gozo gave very low relative abundances of *P. similis*. In 1988, it was the least abundant species collected, with only 3 specimens out of 6,808 sandflies caught (relative abundance = 0.04%). In 1989, five specimens were found out of a total of 2,788 sandflies (relative abundance = 0.21%). Léger *et al.* (1991) also set up CDC light traps in three different biotopes. Relative abundances

were very low ranging from 0% - 0.9%. Out of 28 specimens of *P. similis* tested for flagellates by Léger *et al*. (1991), none were found to contain parasites. During the present study, no specimens of *P. similis* were found.

Subgenus: *Phlebotomus* Rondani & Berté, 1840

This subgenus is characterised by the following combination of morphological characters. Male paramere with two processes. Coxite long with a small basal outgrowth. Style long with short spines. Female spermatheca long with equal segmentation. Pharyngeal teeth large and fringed with minute denticles (microtrichia). Many species of this subgenus are found in arid areas along the margins of the Sahara desert (Sahel and Mediterranean littoral) through the Middle East to Northern Iran (Lane, 1993).

***Phlebotomus* (*Phlebotomus*) *papatasi* (Scopoli, 1786)**

Diagnosis: Male terminalia distinct. Paramere with three lobes, the dorsal lobe much longer than the broad median lobe. Coxite with small tufts of hairs on basal outgrowth, and another clump of long hairs distally. Style long, slender with 5 short pointed spines, three of which are terminal. Distance between median and sub-apical spine less than between sub-apical and terminal spines. Aedeagus apically rounded and slightly bent at tip. **S**permatheca composed of 7 - 8 segments. No neck present. Spermathecal ducts long and thin and not forming common duct. Base of spermathecal ducts somewhat thicker than rest of duct.

Distribution: This is the most widespread species of sandfly, ranging from Portugal and Morocco in the west to Bangladesh in the east, and from ex-USSR in the north to Sudan in the south.

Biology: An anthropophilic, peridomestic species the females of which feed on man, but also on dogs. It is the main vector of cutaneous leishmaniasis in man. Males feed on fruits and nectars. *P. papatasi* acts as a vector of *L. turanica* (Strelkova, 1996), *L. major* (Naucke, 2002) and sandfly fever (Marret, 1910). Natural infection rates of sandflies with sandfly fever are thought to range between 0.015% and 0.5% (Lane, 1993).

Notes: *P. papatasi* was first documented as being present in Malta by Marret (1910). It drew a lot of attention due to its role as a vector of sandfly fever. Newstead (1911) stated that *P. papatasi* was one of the two most common sandflies in Malta along with *P. perniciosus*. Both were thought to be approximately equally abundant. Adler & Theodor (1935) stated that in 1931/1932 the ratio of *P. papatasi* to *P. perniciosus* was 1:20. This was the last time *P. papatasi* was recorded in the Maltese Islands as sampling efforts carried out in the late 1980's (Gradoni & Maroli, 1987; Léger *et al*., 1991), as well as the present study failed to capture any specimens.

DISCUSSION

During August to October of 2008, rubble walls in 25 locations from 20 different localities in Malta and Gozo were sampled for sandflies, using sticky traps. Sixteen of these localities (80%) were found to be positive indicating that sandflies are common and widely distributed on the islands. Of the 16 positive localities, 11 were positive for both *P. perniciosus* and *S. minuta*. Five locations were positive for *S. minuta* only, and three for *P. perniciosus* only (Fig. 9). *P. perniciosus* had a relative abundance of 62% and *S. minuta* of 38%. The four species not

caught during the present study (*P. neglectus*, *P. perfiliewi*, *P. papatasi* and *P. similis*) have in the past been reported as very rare, especially when sticky traps were used (Gradoni & Maroli, 1987; Léger *et al*., 1991). The localities which yielded the largest number of specimens were in rural areas with uncultivated fields, such as the slope in Ħondoq ir-Rummien (70 specimens), Delimara (54 specimens) and Dingli (29 specimens). Sandflies were less abundant in urban areas like Birkirkara, Floriana and Msida indicating that urbanization drastically reduces the sandfly populations, probably through habitat destruction. Nonetheless, it does not eradicate them completely. Rural areas which were cultivated had quite a low abundance of sandflies, comparable to that of urban areas. There are a number of possible explanations for this, the most likely being the widespread use of plant protection products on crops. Also, uncultivated fields tend to provide a more permanent shelter for rodents and other small mammals on which female sandflies feed, than cultivated land. The results from semi-rural areas, which constituted the majority of localities sampled, varied from one another (6 - 22 sandflies per location). The only exception was in Qala (playing field) where 51 sandflies were caught, 32 of which were female *P. perniciosus*. It would seem that children making use of this playing field are potentially at significant risk of exposure to the leishmania parasite.

There is a common belief that coastal areas are free from sandflies because the coastal breeze inhibits them from flying around. Three localities on the coast were sampled for sandflies: Baħar iċ-Ċagħaq and Pembroke in Malta, and Ħondoq ir-Rummien in Gozo. The results obtained from these three localities varied. No sandflies were collected from Pembroke. Ħondoq ir-Rummien, on the other hand, was the overall second most infested locality (with 4 *S. minuta* and 51 *P. perniciosus*). Baħar iċ-Ċagħaq, which is only just north of Pembroke, was positive for 7 sandflies (1 *S. minuta*

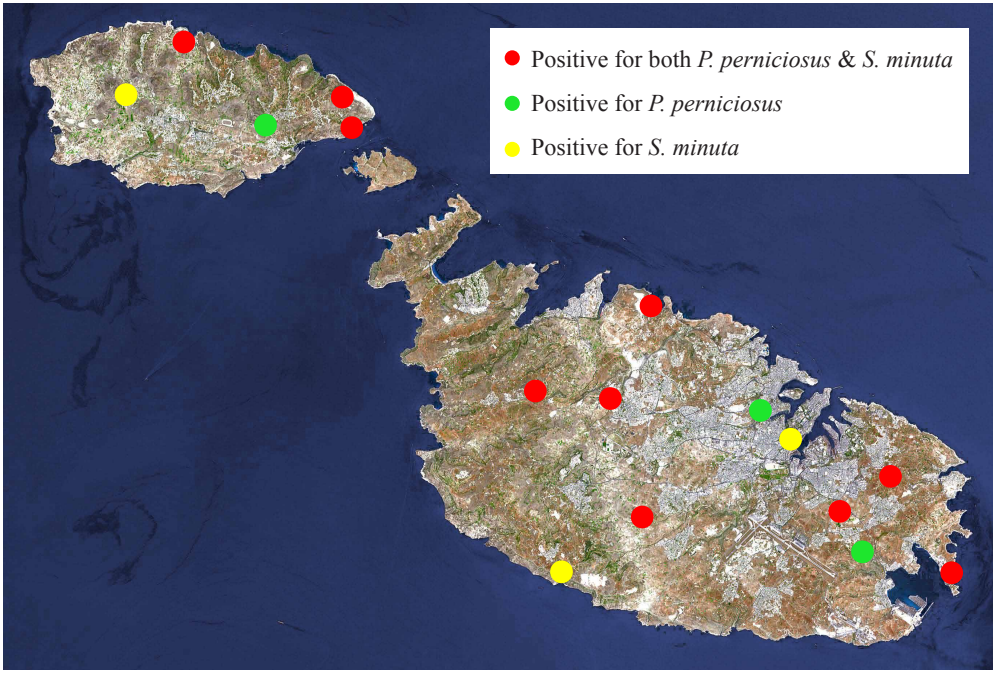


Figure 9. Map showing localities where *P. perniciosus, S. minuta,* or both were found.

and 6 *P. perniciosus*). The high infestation in Ħondoq ir-Rummien proves that sandflies, can in fact, survive in coastal areas. The locality is protected from the north by a steep embankment which may shield sandflies to some extent from exposure to wind. Pembroke and Baħar iċ-Ċagħaq, on the other hand, are open and exposed. Other factors such as host availability and insecticide use may also account in part for the different results obtained in these three similar habitats. One of the aims of this survey was to carry out sampling in Malta and Gozo simultaneously. The latest records of samples taken in both Malta and Gozo date back to 1910 and 1911 (Marret, 1910; Newstead, 1911) and, although vague references are made to locations, any type of quantitative data is lacking. Studies carried out since 1911 all either refer to Malta, or to Gozo (Adler & Theodor, 1935; Gradoni & Maroli, 1987; Léger *et al*., 1991). The relative abundance of *S. minuta* and *P. perniciosus* differed significantly at 95% confidence levels. There were significantly more *P. perniciosus* in Gozo (83.19%) than in Malta (32.94%). The opposite was observed for *S. minuta* where Malta had a higher abundance (67.05%) than Gozo (16.8%).

P. perniciosus is of medical importance because it is a vector of leishmaniasis. The fact that there are significantly more *P. perniciosus* in Gozo than in Malta probably explains why there are significantly more cases of cutaneous leishmaniasis originating from Gozo (Vella Briffa, 1985; Gradoni *et al.,* 1991). A number of cases over the years have been reported from a relatively small coastal area covering Qala, Għajnsielem and Nadur (Vella Briffa, 1985; Fenech, 1997; Mamo & Gauci, 2004). Sampling was carried out in Għajnsielem where only one male of *S. minuta* was found, a species of no importance with respect to leishmaniasis. Qala, however proved to be one of the most densely populated areas in terms of sandfly specimens. The highest number of sandflies were caught from the Ħonqod ir-Rummien area, along the slope and near the coast. Almost half (48.59%) of the total number of *P. perniciosus* caught in this survey were collected from these two locations alone.

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