The Phlebotomine sand fly fauna of Switzerland revisited

Schaffner, Francis ; Silaghi, Cornelia ; Verhulst, Niels O ; Depaquit, Jérôme ; Mathis, Alexander

Abstract: Sand flies (Diptera: Psychodidae, Phlebotominae; Newstead, 1911) are widespread in Europe, being particularly common in the Mediterranean region but rare north of the Alps. Thus, Switzerland is an opportune place to investigate the sand fly fauna on both sides of the Alpine crest, in southern sub-Mediterranean climate and northern oceanic temperate climate. We reinvestigated the Swiss sand fly fauna with the aim to assess changes in composition, altitudinal distribution, abundance and seasonality. Thirty-eight sites were investigated with light traps and/or interception sticky traps in 4 years. Ninety and 380 specimens were caught by light traps and sticky traps, respectively, at 15 collecting sites. Four species were identified. Phlebotomus mascittii (Grassi, 1908), Phlebotomus perniciosus (Newstead, 1911) and Sergentomyia minuta (Rondani, 1843) were confirmed in Ticino, and P. mascittii for the first time in neighbouring Grisons. Also, Phlebotomus neglectus (Tonnoir, 1921) is for the first time reported, though at a very low density compared to P. perniciosus at the same site. Its presence in Ticino supports the northward spread observed in Italy. Sand flies were detected north of the Alps at one site only, endorsing a historical report. Overall, the low density of P. perniciosus and very low density of P. neglectus suggest that canine leishmaniosis may not be an important disease risk in Switzerland.

DOI: https://doi.org/10.1111/mve.12690

Received: 13 January 2023 | Accepted: 12 August 2023

DOI: 10.1111/mve.12690

ORIGINAL ARTICLE

Medical and Veterinary Entomology
Royal Entomological Society

The Phlebotomine sand fly fauna of Switzerland revisited

Francis Schaffner[1] | Cornelia Silaghi[1,2] | Niels O. Verhulst[1] | Jérôme Depaquit[3] | Alexander Mathis[1]

[1]National Centre for Vector Entomology, Institute of Parasitology, Vetsuisse and Medical Faculty, University of Zürich, Zürich, Switzerland

[2]Institute of Infectology (IMED), Federal Research Institute for Animal Health, Greifswald – Insel Riems, Germany

[3]Université de Reims Champagne-Ardenne, EA7510 ESCAPE, ANSES, USC "Pathogènes-Environnement-Toxoplasme-Arthropodes-Réservoirs-bioDiversité (PETARD)", Reims, France

Correspondence
Francis Schaffner, National Centre for Vector Entomology, Institute of Parasitology, Vetsuisse and Medical Faculty, University of Zürich, Winterthurerstrasse 266a, 8057 Zürich, Switzerland.
Email: francis.schaffner@uzh.ch

Funding information
Swiss Federal Food Safety and Veterinary Office (FSVO)

Associate Editor: Emma Weeks

Abstract

Sand flies (Diptera: Psychodidae, Phlebotominae; Newstead, 1911) are widespread in Europe, being particularly common in the Mediterranean region but rare north of the Alps. Thus, Switzerland is an opportune place to investigate the sand fly fauna on both sides of the Alpine crest, in southern sub-Mediterranean climate and northern oceanic temperate climate. We reinvestigated the Swiss sand fly fauna with the aim to assess changes in composition, altitudinal distribution, abundance and seasonality. Thirty-eight sites were investigated with light traps and/or interception sticky traps in 4 years. Ninety and 380 specimens were caught by light traps and sticky traps, respectively, at 15 collecting sites. Four species were identified. *Phlebotomus mascittii* (Grassi, 1908), *Phlebotomus perniciosus* (Newstead, 1911) and *Sergentomyia minuta* (Rondani, 1843) were confirmed in Ticino, and *P. mascittii* for the first time in neighbouring Grisons. Also, *Phlebotomus neglectus* (Tonnoir, 1921) is for the first time reported, though at a very low density compared to *P. perniciosus* at the same site. Its presence in Ticino supports the northward spread observed in Italy. Sand flies were detected north of the Alps at one site only, endorsing a historical report. Overall, the low density of *P. perniciosus* and very low density of *P. neglectus* suggest that canine leishmaniosis may not be an important disease risk in Switzerland.

KEYWORDS
abundance, first record, *mascittii*, *minuta*, *neglectus*, *perniciosus*, *Phlebotomus*, presence, *Sergentomyia*, trapping

INTRODUCTION

Phlebotomine sand flies are vectors mainly of protozoa (e.g., *Leishmania* spp.) but also bacteria (e.g., *Bartonella* spp.) and arboviruses (phleboviruses; Depaquit et al., 2010).

Sand flies are widespread on the European continent with 21 reported species (Karakülah et al., 2016). They are particularly common in the Mediterranean region but rare north of the Alps (Medlock et al., 2014). Thus, Switzerland, which embraces both regions, is an opportune place to investigate the sand fly fauna in southern sub-Mediterranean temperate climate and northern oceanic temperate climate. Autochthonous cases of leishmaniosis in dogs or humans in Switzerland have admittedly been reported but were not evidenced. Earlier investigations of *Leishmania infantum* Nicolle, 1908 antibodies in dogs from southern Switzerland did not reveal infections (Deplazes et al., 1995). More recently, a low seroprevalence was determined for dogs from the same region but all had a travel history in endemic regions (Olcer, 2017).

Sand flies are known to occur in Switzerland since 1911 when two females of *Phlebotomus mascittii* (Grassi, 1908; mentioned under the name *Phlebotomus papatasi*) were caught in Orbe, western canton of Vaud (VD; Galli-Valerio & Rochaz de Jongh, 1912). Later, another individual of *P. mascittii* (also recorded as *P. papatasi*) was described from Cassarate, southern canton of Ticino (TI; Vogel, 1931), and sand flies collected at Ascona (TI) in 1944 were attributed to either *Phlebotomus perniciosus* (Newstead, 1911) or *Sergentomyia minuta* (Rondani, 1843; under the name *Phlebotomus parroti* var. *italicus*; Gaschen, 1945). In 1956, Gaschen confirmed the identity and the occurrence of *P. mascittii* in VD, based on another individual collected in La Rosiaz (Gaschen, 1956a). Besides, he also confirmed the identity of *S. minuta* and its occurrence in Lugano (TI; Gaschen, 1956b). Since then, no additional species have been reported from TI (Grimm et al., 1993; Knechtli & Jenni, 1989), and no studies were implemented in other Swiss cantons. The recent mention of the presence of sand flies in the northern Swiss region of Jura (Kasbari et al., 2012) refers to the data described in this article.

Like other insects, sand flies may change their distribution and abundance as a consequence of environmental and climatic changes. In northern Italy, canine leishmaniosis has extended northwards, and the sand fly species *Phlebotomus perniciosus* and *Phlebotomus neglectus* (Tonnoir, 1921) have increased in density and expanded their geographic range, with autochthonous foci suspected near the Swiss border (Ferroglio et al., 2010; Maroli et al., 2008). North of the Alps and during the last two decades, putative autochthonous human and canine leishmaniosis cases have been described in France, Germany and the Netherlands (Kasbari et al., 2012; Maroli et al., 2008; Naucke et al., 2008), and new foci of *P. mascittii* have been reported in Belgium, France and Germany (Depaquit et al., 2005; Oerther et al., 2020). During the same period, this species also was reported for the first time from Austria and Slovakia (Dvořák et al., 2016; Naucke et al., 2011). Overall and at the European scale, changes in the distribution of sand fly species were evidenced, and an increasing risk of establishment of sand flies has been identified especially along the Atlantic Coast but also inland in parts of Austria, Germany and Switzerland (Medlock et al., 2014).

In Switzerland, the most recent field studies date back to 1981–1989 and encompassed only a few localities in TI (Grimm et al., 1993; Knechtli & Jenni, 1989). Therefore, between 2009 and 2016, 20 years after the last studies, we reinvestigated the Swiss sand fly fauna with the aims of (1) assessing changes in fauna composition, altitudinal distribution, abundance, seasonality and possible northward spread in TI (south of the Alps) and (2) investigating sand fly presence in other cantons both south and north of the Alps.

MATERIALS AND METHODS

Study areas

Ticino and neighbouring area of Grisons

Sand fly trapping was primarily performed in the cantons Ticino (TI) and neighbouring Grisons (GR) in 2009, 2010, 2011 and 2016. These areas, south of the Alps, benefit from a warm and temperate climate influenced by the Mediterranean Sea (classified by the Köppen–Geiger system as Cfa and Cfb; Beck et al., 2018), with winters being considerably milder than north of the Alps. The study sites included the sites of former studies (Grimm et al., 1993; Knechtli & Jenni, 1989) for comparative purposes, and further sites by considering favourable environmental suitability for sand flies breeding habitats, such as the presence of old barns, shelters and house basements if possible with ground floors, stone walls, rocks and caves, preferably sun exposed. Overall, eight places were chosen for the trapping in 2009, seven in TI and one in GR, from 245 to 860 m above sea level (m a.s.l.). Twelve new places were added in 2010, while four places from 2009 were omitted (Figure 1, Tables S1 and S2), with altitudes reaching 1,010 m a.s.l. In 2011, two new sites were investigated, and in 2016, seven sites among those previously investigated were sampled again. At each site, trapping was performed at one or more stations of a specific environment (e.g., stone wall, barn, basement). The numbers and locations of sampling sites were selected according to the specific aims of the annual studies and the temporal fieldwork capacities. Generally, the environments of the places were either hilly or mountainous.

Other cantons

Additional samplings were performed in more distant areas in GR and eight other cantons, within (valleys) and north (Swiss plateau) to the Alpine crest. These areas have a climate heavily influenced by the Atlantic Ocean (classified as Cfb and Dfb; Beck et al., 2018). As above, study sites were selected either according to previous findings (VD; Galli-Valerio & Rochaz de Jongh, 1912; Gaschen, 1956a) for comparative purposes, because of suspicion of *Leishmania* parasite transmission (canton Zürich, ZH: ZH03; Lobsiger et al., 2010), and by considering favourable environment suitability for sand flies, at eight, four and five sites in 2009, 2010 and 2011/2012, respectively (Figure 1, Tables S1 and S2).

Sampling methods

Overall, 38 sites were investigated with light traps (LTs) and/or interception sticky traps (STs) between early July and September of each year (Table S2). The LTs (New Standard Miniature Light Traps; John W. Hock Company, Gainesville, USA) were run overnight once a week, for one or two successive nights. The integrated photosensor allowed an automatic switch on of the light before sunset and a switch off after sunrise. The traps were adapted to collect the caught insects directly in 70% ethanol. About 10–20 STs (white paper 80 g/m^2, 20 × 20 cm, impregnated with castor oil; Sigma-Aldrich, Buchs, Switzerland) were placed per site, depending on the availability of suitable places, and collected usually after seven nights (minimum 4, maximum 22 nights). The LTs were hung in or nearby old barns and stables or cellars with non-paved flooring, in and outside poultry houses or nearby caves, rocks and stone piles or walls, while the STs were fixed with wood sticks vertically above the ground or in drain holes or crevices of stone walls (Figure S1). For small holes, the STs were cut into two pieces.

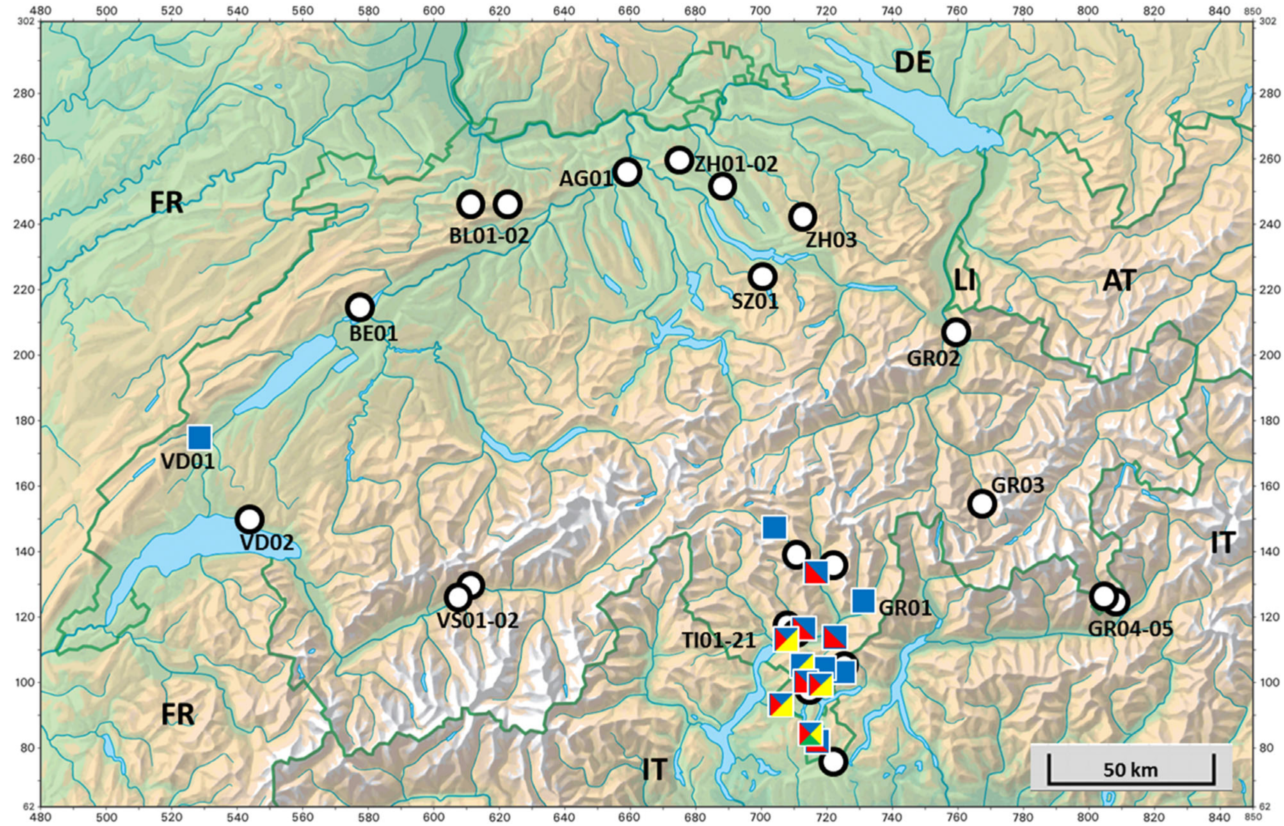

FIGURE 1 Sampling sites investigated for sand flies in Switzerland, 2009–2016. White dots: sand flies not detected; Squares: sand flies detected; Blue: *Phlebotomus mascittii*; Red: *Phlebotomus perniciosus*; Green: *Phlebotomus neglectus*; Yellow: *Sergentomyia minuta*. Swiss cantons: AG, Aargau; BE, Bern; BL, Basel-Landschaft; GR, Grisons; SO, Solothurn; SZ, Schwyz; TI, Ticino; VD, Vaud; VS, Valais; ZH, Zürich. Map source: Federal Office of Topography Swisstopo.

Sand fly identification

In the laboratory, the flies sticking on the STs were counted, and the sand flies were gently removed under a magnifying glass with the aid of forceps and absolute ethanol and then stored in 70% ethanol. The LT collections were inspected under a binocular microscope; the insects were counted, and sand flies were extracted and stored in 70% ethanol before further processing. Male specimens were mounted on slides for morphological identification; some females were similarly processed, while others were handled for molecular identification. For this, specimens were dissected under a binocular microscope to separate the head and the male genitalia or the female spermathecae from the rest of the body. To be mounted on slides, at least the head and the genitalia/spermathecae were lightened in potassium hydroxide (10%) and washed and dehydrated in several ethanol concentrations (from 70% to absolute). Then they were placed between the slide and the cover slide in Euparal balsam (Chroma, Münster, Germany). A fraction of the specimens was identified by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) as described before (Mathis et al., 2015), whereas another fraction (whole specimen or only the thorax and abdomen from dissected specimen) was submitted to DNA isolation, amplification of the mitochondrial cytochrome oxidase subunit I gene (*COI*) and sequencing as described elsewhere (Mathis et al., 2015). The morphological identification was performed based on available identification keys (Dantas-Torres et al., 2014; Fauran et al., 1998; Gatt et al., 2009; Rioux et al., 1969).

Data analysis

To allow a comparison of sand fly density between the different places and with other studies, the relative abundances were calculated for each place and species. Abundance of data are given in terms of density, as the number of sand flies collected divided by the sampling effort (Medlock et al., 2018; Muñoz et al., 2021). The sampling effort is the number of trapping nights multiplied by the trap area (m^2) for ST (=STN) and by the number of traps for LT (=LTN). Impregnated papers are usually used for a period from one to seven nights (Maroli et al., 2008; Muñoz et al., 2021; Risueño et al., 2017; Rossi et al., 2008). In our study, because of travel constraints, we chose to routinely run STs for seven nights. In the Swiss context, we observed that most of the papers were still sticky after seven nights and harboured many insects. However, when STs were placed for more than seven nights, we did consider a sticky period of (the first) seven nights only for density calculations.

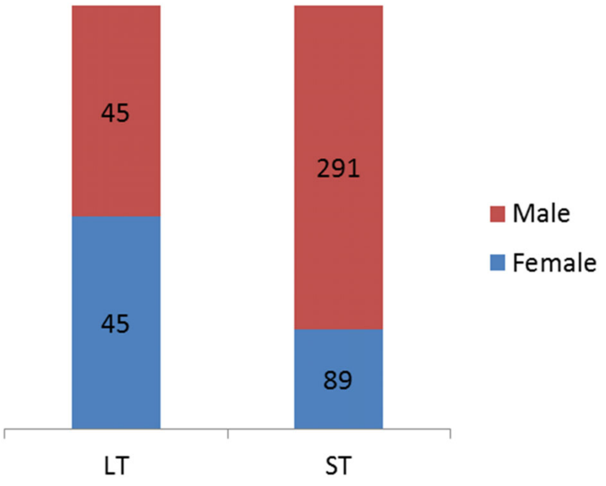

FIGURE 2 Effect of trap type on sex ratio for all sand fly species captured in Switzerland, 2009–2016. The sex ratio significantly differs between the trapping methods (generalised linear model [GLM], df = 134, $p < 0.001$). LT, light trap; ST, sticky trap; total $n = 470$.

Statistics

The effect of trap type and trap site (barn, poultry house, stable, etc.), collection week and altitude (independent variables) on the sex ratio (dependent binary variable) of the collected sand flies was investigated with a generalised linear model (GLM) with binomial distribution, logit link function and dispersion estimated. The sex ratio was expressed in the GLM as the fraction of female sand flies in a trap divided by the total number of sand flies in the trap.

The effect of trapping site, trap type, collection week and altitude (independent variables) on the collection of the three most abundant species (count as the dependent variable; *P. mascittii*, *P. perniciosus* and *S. minuta*) was investigated with a GLM with negative binomial distribution with log link and dispersion estimated. Data were weighted for sampling effort by the number of collection nights.

Effects as well as their two-way interactions were fitted in the different GLMs, and nonsignificant factors were removed. Models were compared by the corrected Akaike's information criterion. *p*-values below 0.05 were considered significant. All statistical analyses were performed using SPSS® statistics software, version 27 (IBM Corp., Armonk, NY, USA).

RESULTS

Trapping method and effort and numbers of sand flies caught

Overall, 188 LT- and 563.02 m^2 of ST-trapping nights were performed in Switzerland in 2009, 2010, 2011/2012 and 2016 (Tables S3 and S4). According to the sampling objective (see Introduction), the trapping efforts varied. It was globally more intense in TI and neighbouring GR, and in particular at 17 sites (16 in TI, 1 in GR), with averages of 10 weeks of sampling and 20 m^2 of ST-trapping nights per site. At the other sites, sampling was performed usually for 1 week (3 weeks at ZH01 and ZH02) with an average of less than 4.3 m^2 of ST-trapping nights per site.

A total of 470 sand fly specimens were caught, 90 and 380 by LTs and STs, respectively (Figure 2). After correction for the number of collection nights, the type of trap only influenced the number of collected *S. minuta*, the species being more frequently caught in STs and not the other species (GLM, df = 589, $p < 0.001$, Tables S3 and S4). The sex ratio of the sand flies collected with the LTs was significantly different from the sex ratio of sand flies caught with STs (GLM, df = 134, $p < 0.001$, Figure 2). While the sex ratio with the LTs did not differ from a 50:50 distribution (95% confidence interval [CI; 0.45, 0.71], GLM), the STs caught more males (95% CI [0.18, 0.30], GLM). The trapping site also had a significant effect on the sex ratio (GLM, df = 126, $p < 0.001$, Table S5). More males were caught in barns (building for keeping equipment), barn basements and garden shelters (shed for keeping gardening equipment), while more females were caught near stone walls (95% CI, GLM, Table S5). No effect of collection week or altitude on the sex ratio was found (GLM, $p = 0.674$ and $p = 0.059$, respectively, Table S5).

Sand fly fauna

Phlebotomine sand flies were collected at 15 of the 38 sites (Table 1) and during every year but 2011 (when only some sites, north of the Alps, were sampled). The 470 specimens collected belonged to four species: *P. (Transphlebotomus) mascittii*, *P. (Larroussius) perniciosus*, *P. (Lar.) neglectus* and *S. (Sergentomyia) minuta*.

Most of the specimens were identified by morphology, 38 by barcoding (2009: $n = 8$, 2010: $n = 26$; 2016: $n = 4$) and 35 by MALDI-TOF MS (2016). Obtained sequences are available at GenBank under the accession numbers OQ238828-OQ238844 (*P. mascittii*), OQ248080-OQ248085 (*P. neglectus*), OQ223299-OQ223300 (*P. perniciosus*) and OQ238860-OQ238867 (*S. minuta*).

Male specimens represented 71.5% of the catches. The species *P. mascittii* was found at all 15 positive sites and was the most numerous species with 306 specimens (65.1%). It was the only species found outside TI, in neighbouring GR (GR01, Figure S1E) but also north of the Alps in VD (VD01, Figure S1H). The species *S. minuta* was collected at five sites ($n = 97$; 20.6%) and *P. perniciosus* at nine sites ($n = 57$; 12.1%). The less abundant species *P. neglectus* was found at a single site (TIS01, Figure S1A,B; $n = 10$; 2.1%), which is one of the southernmost sites investigated in TI (Figure 1).

Sand fly habitat

There were differences between the sand fly species and the type of habitat where they were mostly collected (Figures 3 and S2). The type

TABLE 1 Sand flies captured in Switzerland, 2009–2016.

Site	*Phlebotomus mascittii* females				*Phlebotomus mascittii* males				*Phlebotomus neglectus* males		*Phlebotomus perniciosus* females			*Phlebotomus perniciosus* males				*Sergentomyia minuta* females			*Sergentomyia minuta* males		
	2009	2010	2016	T	2009	2010	2016	T	2009	T	2009	2010	T	2009	2010	2016	T	2010	2016	T	2009	2010	T
GR01	17			17	78			78															
TI01	10			10	3		1	4	10	10	2		2	4		5	9		3	3	4		4
TI02	8	1	1	10	13	2	1	16						2	2	2	6	12		12		6	6
TI03	4	9		13	13	5	7	25			2	2	4	10	10		20	31		31		14	14
TI04	2			2	7			7						1			1						
TI05	2			2	12		1	13						7		1	8						
TI06	1			1										1			1						
TI10		5	2	7		26	23	49				1	1		1		1						
TI14		1		1		7		7															
TI15		1		1																			
TI17						29	1	30										1		1			
TI18		2		2		2		2							1		1						
TI19		2		2		4		4							3		3	10		10		16	16
TI20			1	1																			
VD01	1			1	1			1															
Total	45	21	4	70	127	75	34	236	10	10	4	3	7	25	17	8	50	54	3	57	4	36	40

Note: T = total for each species. Overall total = 470 specimens. Swiss cantons: GR, Grisons; TI, Ticino; VD, Vaud.

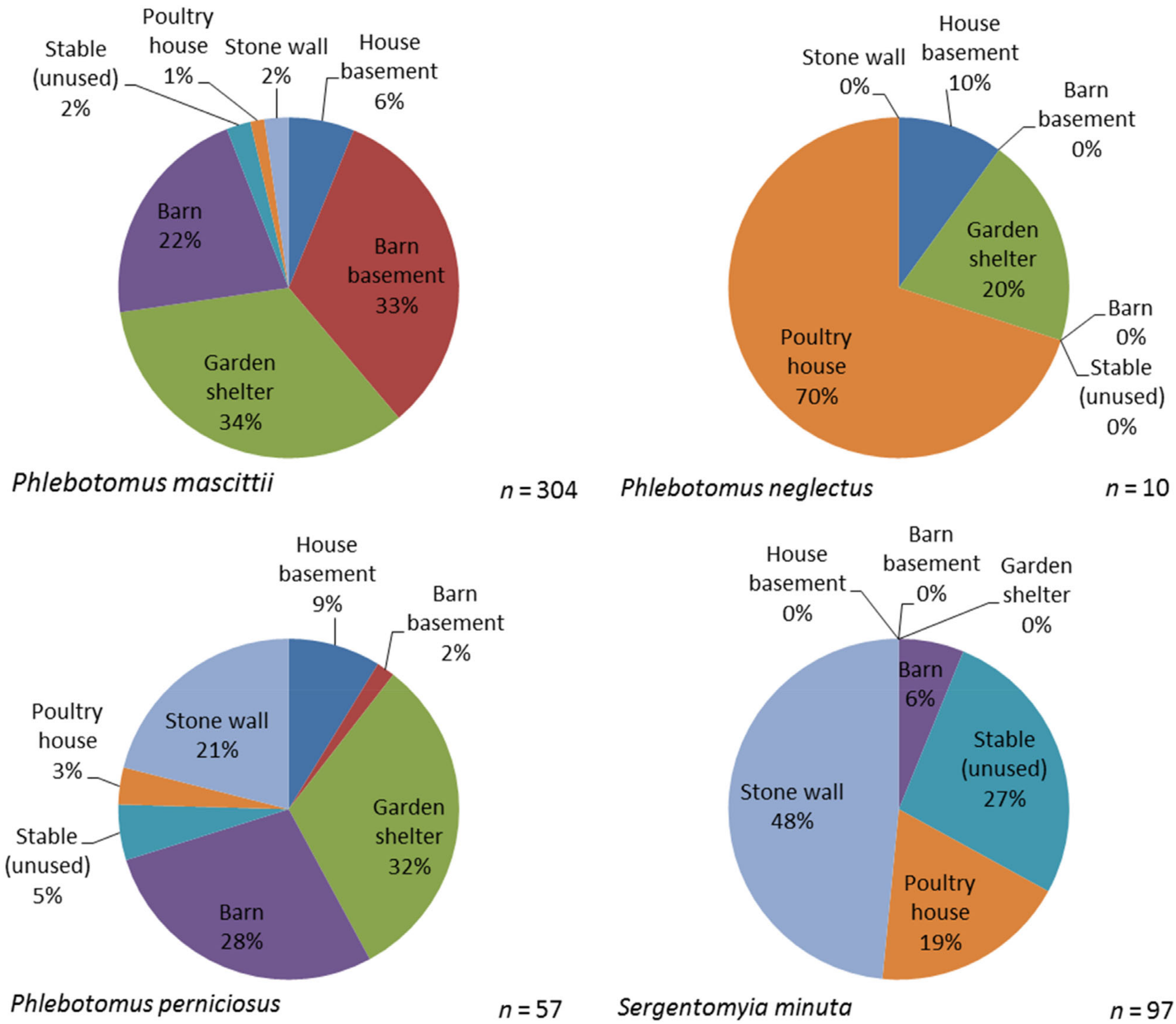

FIGURE 3 Types of sites harbouring sand flies in Ticino and neighbouring area of Grisons, 2009–2016.

of habitat had a significant effect on the number of *P. mascittii*, *P. perniciosus* and *S. minuta* caught (GLM, df = 589, all $p < 0.001$). *Phlebotomus mascittii* was found mainly in barn basements, barns and garden shelters (almost 90% of the specimens). The species was collected significantly more often in barn basements than in all other types of habitats (GLM, df = 589, $p < 0.001$). The trappings near stone walls caught significantly lower *P. mascittii* than at all other habitats (GLM, df = 589, $p < 0.05$) except at poultry houses (GLM, df = 589, $p = 0.106$). By comparing GLM back-transformed means (Figure S2), *P. perniciosus* was caught significantly more often in house basements than all other types of habitats (GLM, df = 589, $p < 0.01$). For *S. minuta*, unused stables revealed the highest number of specimens (GLM, df = 589, $p < 0.002$), and poultry houses were second. At this habitat, significantly more *P. perniciosus* were caught than at the other habitats (GLM, df = 589, $p < 0.001$) except for the unused stables (GLM, df = 589, $p < 0.002$). *Phlebotomus neglectus*, detected in small numbers at a single site and therefore not included in the statistical analysis, was mainly collected in a poultry house (70%, Figure 3).

Species' horizontal and vertical distribution

Altitude significantly influenced the trap catches and was included in the GLMs for all species (GLM, df = 589, $p < 0.001$, Figure 4). *Phlebotomus mascittii* was the most widespread species, occurring north (VS01) and south of the Alps (Figure 1). In TI and neighbouring GR, the species was detected at 14 sites, including the northernmost one (TI20) and the two at the highest altitude (TI14 and TI20, 660 and 715 m a.s.l., respectively). It occurred through the 250–750 m a.s.l. elevation range. *Phlebotomus perniciosus* and *S. minuta* were reported from seven and five sites, respectively, distributed over southern TI, through the 250–600 m a.s.l. elevation range. The single site positive for *P. neglectus* was located in the

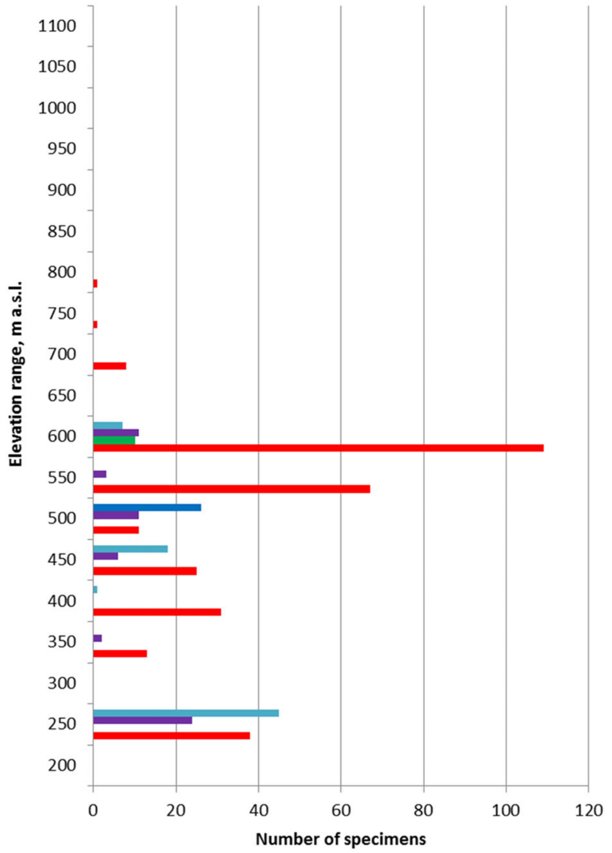

FIGURE 4 Numbers of sand fly specimens caught according to elevation in Ticino and neighbouring area of Grisons, 2009–2016. Red: *Phlebotomus mascittii*; Green: *Phlebotomus neglectus*; Violet: *Phlebotomus perniciosus*; Blue: *Sergentomyia minuta*. Numbers of specimens are grouped within 50 m altitude intervals. Altitude significantly influenced the trap catches and for all species (generalised linear model [GLM], df = 589, $p < 0.001$).

very south, at 580 m a.s.l. No sand flies were detected at the six sites located in the 750–1050 m a.s.l. elevation range (TI07, TI09, TI11, TI12, TI13 and TI16).

Species' abundance and seasonality

In TI and neighbouring GR, the overall density reached 0.76 sand fly/LT/night and 0.66 sand fly/STm^2/night. The 2-week period in which the sand flies were collected had a significant effect on *P. mascittii* and *P. perniciosus* (GLM, $p < 0.001$, Figure 5). The highest sand fly density observed by LTs was reported from TI03 in week 27 (1 LTN, 07–08 July 2010) with 9 sand fly/LT/night, including five *P. mascittii* and four *P. perniciosus*, while the highest density observed by STs was determined from GR01 in week 30 (13.33 m^2 of ST for seven trapping nights, 22–27 July 2009) with 2.22 sand fly/STm^2/night (all *P. mascittii*; Tables S3 and S4). All trappings were performed within weeks 26 and 35 (end of June to early September), and highest densities were observed in weeks 28–29 for all species except *S. minuta*, which

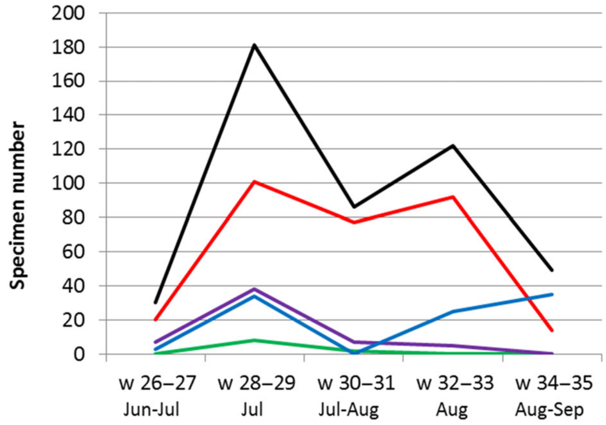

FIGURE 5 Overall numbers of sand fly specimens caught in Ticino and neighbouring area of Grisons within 2-week periods, end of June to early September, all years 2009–2016, all trapping methods. Red: *Phlebotomus mascittii*; Green: *Phlebotomus neglectus*; Violet: *Phlebotomus perniciosus*; Blue: *Sergentomyia minuta*; Black: all species. The 2-week period in which the sand flies were collected had a significant effect on *P. mascittii* and *P. perniciosus* (generalised linear model [GLM], $p < 0.001$).

showed the highest densities in weeks 34–35 (in 2010 only, but at two sites; Figure 5).

DISCUSSION

We investigated the presence and abundance of sand flies at various sites in Switzerland, between 2009 and 2016. We demonstrated the persistence of *P. mascittii*, *P. perniciosus* and *S. minuta* in TI, and we report for the first time the presence of *P. mascittii* in neighbouring GR. In TI, we found a lower density of sand flies compared to a previous study (Knechtli & Jenni, 1989), in particular when assessed with ST. Our study yielded overall sand fly/STm^2/night means between 0.02 and 2.73 compared to 0.83–84.26 sand fly/STm^2/night in 1981. Also, LT catches were lower, though only slightly (0.76 sand fly/LT/night as compared to 0.80 or 1.20 sand fly/LT/night in 1982 or 1983, respectively). Lower densities were found, compared to 1981, for *P. perniciosus* and *S. minuta* (0.04–0.27 vs. 1.04–12.50 and 0.03–0.82 vs. 0.93–75.93 sand fly/STm^2/night, respectively) but not for *P. mascittii* (0.03–4.13 vs. 0.83–3.33 sand fly/STm^2/night). The apparent decrease of *P. perniciosus* density contradicts the increase reported in neighbouring Italy (Maroli et al., 2008). The overall density of sand flies in TI is also much lower than what can be found in Mediterranean countries, for example, in Spain where a mean of 3.1 sand fly/STm^2/night was found for a sampling effort of 1471 m^2 trap nights and 7.2 sand fly/LT/night for 67 LT nights in a Spanish peri-urban residential area (Murcia; Muñoz et al., 2021). Our study reveals the occurrence of *P. perniciosus* and *S. minuta* up to 595 m a.s.l., while *P. mascittii* was found up to 755 m a.s.l.; sand flies were not detected at higher sites, which is consistent with other findings at similar latitudes (Kniha et al., 2021; Rioux et al., 1967).

We also report for the first time the presence of *P. neglectus* in TI in 2009 at a site located close to the Italian border (Meride). Its density was low (0.02 sand fly/STm^2/night) compared to *P. perniciosus* at the same site (0.18 sand fly/STm^2/night). Its presence in TI supports the northward spread observed in Italy (Biocca et al., 1977; Maroli et al., 2008). We record its occurrence at a site located 580 m a.s.l., while it was found at a maximum of 330 m a.s.l. in Piedmont, Italy (Maroli et al., 2002). We also report sand flies at more northern places within TI (*P. mascittii* at Faido, site TI20, 715 m a.s.l.) compared to previous studies. However, as this site was not surveyed in the past, and considering the observed low densities, it is not possible to conclude for a northward spread or expanding local sand fly populations, except for *P. neglectus*.

In more distant areas of GR and other Swiss cantons, sand flies were only detected in VD endorsing the historical report of *P. mascittii* at Orbe (Galli-Valerio & Rochaz de Jongh, 1912) but not of the same species at La Rosiaz (Gaschen, 1956a). Not a single sand fly was found at the location of the suspected autochthonous bovine *Leishmania* infection (Lobsiger et al., 2010) in northern Switzerland, although the local environment also did not seem suitable for sand flies (ZH03, 850–900 m a.s.l.). However, this case of infection was due to *L. (Mundinia) martiniquensis* (= *L. siamensis*), which is suggested to be vectored by biting midges (Diptera: Ceratopogonidae; Becvar et al., 2021). The sampling effort performed in VD was slightly higher than the mean effort (5.40 vs. 4.24 STN) but lower than for some other locations (e.g., 12.60 STN at ZH02), suggesting the absence of sand flies at these places or presence at very low densities. The density of *P. mascittii* observed in VD (0.42 sand fly/STm^2/night) was lower than the average density observed at positive sites in TI (0.54 sand fly/STm^2/night). Despite the fact that the species is considered strongly attracted by LTs (Depaquit et al., 2005), our traps remained negative in VD (but two trap nights only), while LTs were successfully used to detect the species in Styria, Austria, in 2018–2019 (up to 0.90 sand fly/LT/night) and in southwest Germany (up to 0.30 sand fly/LT/night; Oerther et al., 2020). The species was detected in Northern France, Belgium and Germany in the last two decades (Depaquit et al., 2005; Naucke et al., 2008; Oerther et al., 2020).

Phlebotomus perniciosus originating from TI was a successful laboratory vector for *L. infantum* (Knechtli & Jenni, 1990), and *P. neglectus* is considered as an important vector of *Leishmania* parasites in Southeast Europe and Anatolia (Trájer, 2021), while *P. mascittii* is not considered as an efficient vector (Maroli et al., 2013; Ready, 2010). However, single cases of assumed autochthonous leishmaniosis have been reported north of the Alps (Maroli et al., 2008; Naucke et al., 2008), suggesting the occurrence of *P. perniciosus* at these latitudes, sufficient vectorial capacity of *P. mascittii* populations or existence of nonvector associated transmission. In a recent investigation (2016–2017) of 545 dogs from TI, seroprevalence for *L. infantum* was 2.9%, but all positive dogs had a travel history in endemic regions (Olcer, 2017). This seroprevalence is similar to recent observations in neighbouring Italy (2.6% and 3.6%, animals from nine pre-Alpine or seven pre-Apennine/Po valley territories, respectively, 2003–2005; Maroli et al., 2008). However, dog seroprevalences were considerably higher in a panel of published studies from Italy (39.8%, 377 studies, 1971–2006; Franco et al., 2011) and also from France (17.8%; 169 studies, 1971–2006). Overall, the low density of *P. perniciosus* and very low density of *P. neglectus* suggest that canine leishmaniosis may not be an important disease risk in Switzerland.

AUTHOR CONTRIBUTIONS

Francis Schaffner: Conceptualization; investigation; writing – original draft; data curation; formal analysis. **Cornelia Silaghi:** Methodology. **Niels O. Verhulst:** Validation; writing – review and editing; data curation. **Jérôme Depaquit:** Writing – review and editing; data curation. **Alexander Mathis:** Supervision; resources; writing – review and editing; methodology; validation.

ACKNOWLEDGEMENTS

We thank the Istituto Cantonale di Microbiologia (renamed as University of Applied Sciences of Southern Switzerland—SUPSI) and in particular its former director Orlando Petrini for the efficient collaboration in implementing the field studies. We highly acknowledge Claudia Bremen and Meltem Olcer who have contributed to this study with their veterinary master theses. We particularly thank Saul Rusconi, Simona Casati and Evelyn Casati (Istituto Cantonale di Microbiologia/SUPSI) for their active contribution to the fieldwork. We are also grateful to Felix Grimm (Institute of Parasitology, University of Zürich) for helping to identify study sites and to Jeannine Hauri for technical support. We highly acknowledge the Swiss Federal Food Safety and Veterinary Office (FSVO) as a sponsor of the National Centre for Vector Entomology. Open access funding provided by Universitat Zurich.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at http://doi.org/10.6084/m9.figshare.22262428.

ORCID

Francis Schaffner https://orcid.org/0000-0001-9166-7617
Niels O. Verhulst https://orcid.org/0000-0002-1106-9711
Jérôme Depaquit https://orcid.org/0000-0001-6995-0088
Alexander Mathis https://orcid.org/0000-0002-5499-531X

REFERENCES

Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A. & Wood, E.F. (2018) Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Scientific Data*, 5, 180214.

Becvar, T., Vojtkova, B., Siriyasatien, P., Votypka, J., Modry, D., Jahn, P. et al. (2021) Experimental transmission of *Leishmania (Mundinia)* parasites by biting midges (Diptera: Ceratopogonidae). *PLoS Pathogens*, 17, e1009654.

Biocca, E., Coluzzi, A. & Costantini, R. (1977) Osservazioni sulla attuale distribuzione dei flebotomi italiani e su alcuni caratteri morfologici differenziali tra le specie del sottogenere *Phlebotomus* (*Larroussius*). *Parassitologia*, 19, 19–32.

Dantas-Torres, F., Tarallo, V.D. & Otranto, D. (2014) Morphological keys for the identification of Italian phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae). *Parasites & Vectors*, 7, 479.

Depaquit, J., Grandadam, M., Fouque, F., Andry, P.E. & Peyrefitte, C. (2010) Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review. *Eurosurveillance*, 15, pii=19507.

Depaquit, J., Naucke, T.J., Schmitt, C., Ferté, H. & Léger, N. (2005) A molecular analysis of the subgenus *Transphlebotomus* Artemiev, 1984 (Phlebotomus, Diptera, Psychodidae) inferred from ND4 mtDNA with new northern records of *Phlebotomus mascittii* Grassi, 1908. *Parasitology Research*, 95, 113–116.

Deplazes, P., Guscetti, F., Wunderlin, E., Bucklar, H., Skaggs, J. & Wolff, K. (1995) Endoparasitenbefall bei Findel- und Verzicht-Hunden in der Südschweiz. *Schweizer Archiv für Tierheilkunde*, 137, 172–179.

Dvořák, V., Hlavackova, K., Kocisova, A. & Volf, P. (2016) First record of *Phlebotomus (Transphlebotomus) mascittii* in Slovakia. *Parasite*, 23, 48.

Fauran, P., Izri, A., Delaunay, P. & Marty, P. (1998) Les phlébotomes (Diptera, Phlebotominae) des Alpes-Maritimes et de Monaco. *Riviéra Scientifique*, 82, 41–48.

Ferroglio, E., Romano, A., Dettoni, F. & Trisciuoglio, A. (2010) Distribution of *Phlebotomus perniciosus* in North-Italy: a study on 18S rDNA of phlebotomine sand flies. *Veterinary Parasitology*, 170, 127–130.

Franco, A.O., Davies, C.R., Mylne, A., Dedet, J.-P., Gállego, M., Ballart, C. et al. (2011) Predicting the distribution of canine leishmaniasis in western Europe based on environmental variables. *Parasitology*, 138, 1878–1891.

Galli-Valerio, B. & Rochaz de Jongh, J. (1912) Beobachtungen über Culiciden und Mitteilung über das Vorkommen von *Phlebotomus papatasi* Scop. im Kanton Waadt (Schweiz). *Centralblatt für Bakteriologie. Parasitenkunde und Infektionskrankheiten. I. Abt. Originale*, 63, 222–227.

Gaschen, H. (1945) Phlébotomes de Suisse. *Acta Tropica*, 2, 137–154.

Gaschen, H. (1956a) Présence de *Phlebotomus mascittii* Grassi 1908 dans le canton de Vaud. *Bulletin de la Société Entomologique Suisse*, 29, 223–225.

Gaschen, H. (1956b) Captures de Phlébotomes dans le canton du Tessin. *Bulletin de la Société Entomologique Suisse*, 29, 226–228.

Gatt, P., Williams, J. & Mifsud, D. (2009) New distributional data on sandflies from rubble walls in the Maltese islands with an illustrated key to the Maltese species (Diptera: Phlebotominae). *Bulletin of the Entomological Society of Malta*, 2, 95–110.

Grimm, F., Gessler, M. & Jenni, L. (1993) Aspects of sandfly biology in southern Switzerland. *Medical and Veterinary Entomology*, 7, 170–176.

Karakülah, G., Karakuş, M., Suner, A., Demir, S., Arserim, S.K., Töz, S. et al. (2016) sandflyDST: a dynamic web-based decision support tool for the morphological identification of sandflies present in Anatolia and mainland Europe, and user study. *Medical and Veterinary Entomology*, 30, 321–329.

Kasbari, M., Ravel, C., Harold, N., Pesson, B., Schaffner, F. & Depaquit, J. (2012) Possibility of leishmaniasis transmission in Jura, France. *Emerging Infectious Diseases*, 18, 1030.

Knechtli, R. & Jenni, L. (1989) Distribution and relative density of three sandfly (Diptera:Phlebotominae) species in southern Switzerland. *Annales de Parasitologie Humaine et Comparée*, 64, 53–63.

Knechtli, R. & Jenni, L. (1990) Experimental transmission of *Leishmania infantum* by the bite of *Phlebotomus perniciosus* from Switzerland. *Acta Tropica*, 47, 213–216.

Kniha, E., Milchram, M., Dvořák, V., Halada, P., Obwaller, A.G., Poeppl, W. et al. (2021) Ecology, seasonality and host preferences of Austrian *Phlebotomus (Transphlebotomus) mascittii* Grassi, 1908, populations. *Parasites & Vectors*, 14, 291.

Lobsiger, L., Müller, N., Schweizer, T., Frey, C.F., Wiederkehr, D., Zumkehr, B. et al. (2010) An autochthonous case of cutaneous bovine leishmaniasis in Switzerland. *Veterinary Parasitology*, 169, 408–414.

Maroli, M., Feliciangeli, M.D., Bichaud, L., Charrel, R.N. & Gradoni, L. (2013) Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. *Medical & Veterinary Entomology*, 27, 123–147.

Maroli, M., Khoury, C., Bianchi, R., Ferroglio, E. & Natale, A. (2002) Recent findings of *Phlebotomus neglectus* Tonnoir, 1921 in Italy and its western limit of distribution. *Parassitologia*, 44, 103–109.

Maroli, M., Rossi, L., Baldelli, R., Capelli, G., Ferroglio, E., Genchi, C. et al. (2008) The northward spread of leishmaniasis in Italy: evidence from retrospective and ongoing studies on the canine reservoir and phlebotomine vectors. *Tropical Medicine & International Health*, 13, 256–264.

Mathis, A., Depaquit, J., Dvorak, V., Tuten, H., Banuls, A.-L., Halada, P. et al. (2015) Identification of phlebotomine sand flies using one MALDI-TOF MS reference database and two mass spectrometer systems. *Parasites & Vectors*, 8, 266.

Medlock, J., Balenghien, T., Alten, B., Versteirt, V. & Schaffner, F. (2018) *Field sampling methods for mosquitoes, sandflies, biting midges and ticks – VectorNet project 2014–2018*. Parma: European Food Safety Authority, pp. 1–37.

Medlock, J.M., Hansford, K.M., Van Bortel, W., Zeller, H. & Alten, B. (2014) A summary of the evidence for the change in European distribution of phlebotomine sand flies (Diptera: Psychodidae) of public health importance. *Journal of Vector Ecology*, 39, 72–77.

Muñoz, C., Risueño, J., Pérez-Cutillas, P., Bernal, L.J., Ortiz, J.M., Ruiz de Ybáñez, R. et al. (2021) Density assessment and reporting for *Phlebotomus perniciosus* and other sand fly species in periurban residential estates in Spain. *Parasitology Research*, 120, 3091–3103.

Naucke, T.J., Lorentz, S., Rauchenwald, F. & Aspock, H. (2011) *Phlebotomus (Transphlebotomus) mascittii* Grassi, 1908, in Carinthia: first record of the occurrence of sandflies in Austria (Diptera: Psychodidae: Phlebotominae). *Parasitology Research*, 109, 1161–1164.

Naucke, T.J., Menn, B., Massberg, D. & Lorentz, S. (2008) Sandflies and leishmaniasis in Germany. *Parasitology Research*, 103, 65–68.

Oerther, S., Jöst, H., Heitmann, A., Lühken, R., Krüger, A., Steinhausen, I. et al. (2020) Phlebotomine sand flies in Southwest Germany: an update with records in new locations. *Parasites & Vectors*, 13, 173.

Olcer, M. (2017) *Vorkommen der caninen Leishmaniose und deren Überträger in der Schweiz*. Master thesis, University of Zürich.

Ready, P.D. (2010) Leishmaniasis emergence in Europe. *Eurosurveillance*, 15, pii=19505.

Rioux, J.-A., Golvan, Y.-J., Croset, H., Houin, R., Juminer, B., Bain, O. et al. (1967) Ecologie des Leishmanioses dans le sud de la France. 1. Les phlébotomes. *Annales de Parasitologie Humaine et Comparée*, 42, 561–603.

Rioux, J.-A., Golvan, Y.-J., Croset, H., Tour, S., Hovin, R., Emile, A. et al. (1969) Epidémiologie des leishmanioses dans le sud de la France. In: *Monographies de l'Institut National de la Santé et de la Recherche Médicale*. Paris: Institut National de la Santé et de la Recherche Médicale, pp. 1–223.

Risueño, J., Muñoz, C., Pérez-Cutillas, P., Goyena, E., Gonzálvez, M., Ortuño, M. et al. (2017) Understanding *Phlebotomus perniciosus* abundance in south-east Spain: assessing the role of environmental and anthropic factors. *Parasites & Vectors*, 10, 189.

Rossi, E., Bongiorno, G., Ciolli, E., Di Muccio, T., Scalone, A., Gramiccia, M. et al. (2008) Seasonal phenology, host-blood feeding preferences and natural *Leishmania* infection of *Phlebotomus perniciosus* (Diptera, Psychodidae) in a high-endemic focus of canine leishmaniasis in Rome province, Italy. *Acta Tropica*, 105, 158–165.

Trájer, A.J. (2021) The potential impact of climate change on the seasonality of *Phlebotomus neglectus*, the vector of visceral leishmaniasis in the East Mediterranean region. *International Journal of Environmental Health Research*, 31, 932–950.

Vogel, R. (1931) Beobachtungen über blutsaugende Zweiflügler im Kanton Tessin. *Zoologischer Anzeiger*, 93, 1–3.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Examples of sampling sites for sand flies, Switzerland, 2009–2016. (A, B) Open barn at Meride, canton Ticino (site TI01E: *Phlebotomus neglectus*, *Phlebotomus mascittii*), with a light trap (A) and interception sticky traps (B); (C, D) Cellar openings/windows at Sessa, Canton Ticino, with interception traps (site TI02B: *Phlebotomus mascittii*, *Phlebotomus perniciosus*); (E) Old barn at Gaggiole, canton Ticino, with a light trap (site TI03A: *Phlebotomus mascittii*, *Phlebotomus perniciosus*, *Sergentomyia minuta*); (F) Stone wall at Biasca, Canton Ticino, with interception sticky traps in the holes (site TI04C: *Phlebotomus mascittii*); (G) Old barn at Verdabbio, canton Grisons, with interception sticky traps (site GR01e: *Phlebotomus mascittii*); (H) Stone walls at Montcherand, canton Vaud, north to the Alps, with interception sticky traps (site VD01: *Phlebotomus mascittii*).

Figure S2. Statistical representation of the types of habitats harbouring sand flies, all sites, Switzerland, 2009–2016. The distribution over the different sites was based on the back-transformed means of the generalised linear model with the effect of altitude (all species), collection week (*Phlebotomus mascittii* and *Phlebotomus perniciosus*) and trap type included (*Sergentomyia minuta*). Because of the low number of specimens caught, *Phlebotomus neglectus* was not included in the statistical analysis.

Table S1. Characteristics of sampling sites and stations investigated for sand flies in Switzerland, 2009–2016. Swiss cantons: AG, Aargau; BE, Bern; BL, Basel-Landschaft; GR, Grisons; SO, Solothurn; SZ, Schwyz; TI, Ticino; VD, Vaud; VS, Valais; ZH, Zürich.

Table S2. Sand fly sampling effort performed per method and year in Switzerland, 2009–2016. LTN: number of trapping nights multiplied by the number of light traps; STN: number of trapping nights multiplied by the sticky trap area (m^2); w: week. Swiss cantons: AG, Aargau; BE, Bern; BL, Basel-Landschaft; GR, Grisons; SO, Solothurn; SZ, Schwyz; TI, Ticino; VD, Vaud; VS, Valais; ZH, Zürich.

Table S3. Sand fly sampling effort performed with light traps in Switzerland, 2009–2016, and results in sand fly numbers and density by species. Swiss cantons: AG, Aargau; BL, Basel-Landschaft; GR, Grisons; TI, Ticino; VS, Valais; ZH, Zürich.

Table S4. Sand fly sampling effort performed with sticky traps in Switzerland, 2009–2016, and results in sand fly numbers and density by species. Swiss cantons: AG, Aargau; BE, Bern; BL, Basel-Landschaft; GR, Grisons; SO, Solothurn; SZ, Schwyz; TI, Ticino; VD, Vaud; ZH, Zürich.

Table S5. Sex ratio generalised linear model (GLM) effects overview and back-transformed estimates for the different types of trapping sites. No effect of collection week or altitude on the sex ratio was found and therefore not included in the model (GLM, $p = 0.674$ and $p = 0.059$, respectively).

How to cite this article: Schaffner, F., Silaghi, C., Verhulst, N.O., Depaquit, J. & Mathis, A. (2023) The Phlebotomine sand fly fauna of Switzerland revisited. *Medical and Veterinary Entomology*, 1–10. Available from: https://doi.org/10.1111/mve.12690