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RESEARCH ARTICLE

A survey of Phlebotomine sand flies across their northern distribution range limit in Western Europe

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Abstract

Sand flies are vectors of *Leishmania* spp. protozoa, phleboviruses and *Bartonella bacilliformis*. In this study we surveyed areas of central-western Europe, encompassing the northern limit of the known sand fly distribution, and investigated the relationship between their presence and environmental variables. In this area, very limited occurrence data exists, none of them recent. Sampling was performed in July and August 2023 using CDC-light traps, sticky traps and human landing captures, at 179 selected sites in 48 municipalities. A total of 55 sand fly specimens were collected at 11% (20/179) of the sites sampled – including 16 sites in France, 2 in Luxembourg and 1 in Germany – comprising the first published records for Luxembourg and the Trier-Saarburg County of Germany. No sand flies were detected in Belgium or in the Netherlands. Two species were captured: *Phlebotomus mascittii* (37 females, 2 males) and *Ph. perniciosus* (1 female, 15 males). The latter species was only found in Savoie, the southernmost region sampled in France, while the former species was detected as far north as latitude 50°N. Logistic regression modelling indicated that the probability of sand fly presence gradually decreased with increasing latitude and altitude ($P < 0.05$), and it was not associated to other analysed landscape features in the proximity of the traps. The study confirms and provides new evidence for the presence of sand flies in areas of Western Europe and highlights the need to be aware of potential autochthonous transmission of sand fly-borne pathogens in these areas.

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Phlebotominae – first record – Luxembourg – *Phlebotomus mascittii* – *Phlebotomus perniciosus*

1 Introduction

Phlebotomine sand flies (Diptera, Psychodidae, Phlebotominae) are hematophagous vectors of protozoan *Leishmania* spp. parasites (Trypanosomatidae), phleboviruses (Phenuiviridae) in tropical and subtropical regions, including the Mediterranean Basin, and the bacterium *Bartonella bacilliformis* in the Andes. Sand fly-borne phleboviruses are a genetically diverse group, that includes Toscana virus (TOSV) as the most clinically relevant, causing aseptic meningitis and encephalitis in humans (Lamberth and Hughes, 2021). *Leishmania* parasites are also a complex taxonomic group and, of the 20 species pathogenic to humans and animals, autochthonous infections in Europe are caused by *Leishmania infantum* only (Maia *et al.*, 2023). Dogs are highly vulnerable to infection by this parasite and are its primary reservoir, and canine leishmaniasis (CanL) is a deadly canine disease with a high prevalence in southern European countries (Moreno and Alvar, 2002). While humans are dead-end hosts of *L. infantum,* they may also develop leishmaniasis, which may be a life-threatening condition (Gradoni *et al.*, 2017). Sand flies in Europe include 23 species belonging to the genera *Phlebotomus* and *Sergentomyia* (Galati *et al.*, 2023). Confirmed vectors of *L. infantum* in Europe, meeting conventional vector incrimination criteria (Killick-Kendrick, 1990), are *Phlebotomus ariasi* Tonnoir, 1921, *Ph. balcanicus* Theodor, 1958*, Ph. langeroni* Nitzulescu, 1930*, Ph. neglectus* Tonnoir, 1921*, Ph. perfiliewi* Parrot, 1930*, Ph. perniciosus* Newstead, 1911 and *Ph. tobbi* Adler, Theodor and Lourie, 1930 (Alten *et al.*, 2016). Additionally, *Ph. alexandri* Sinton, 1928 and *Ph. mascittii* Grassi, 1908 are suspected vectors, but further studies are required to evaluate some of the vectorial capacity criteria of these species (Alten *et al.*, 2016). Vector species associated with TOSV infections include *Ph. perfiliewi, Ph. perniciosus, Ph. longicuspis* Nitzulescu, 1930*, Ph. papatasi* (Scopoli, 1786)*, Ph. sergenti* Parrot, 1917 and *Se. minuta* (Rondani, 1843) (Ayhan and Charrel, 2017).

Unlike mosquitoes, sand flies breed in terrestrial sites protected from desiccation and containing decomposing organic matter for their larvae to feed on. Adults, measuring 1 to 3 mm, are weak fliers and are not capable of surviving a long passive transportation to colonise new habitats (ECDC, 2020). All species show a markedly seasonal and nocturnal activity. Both sexes feed on plant juices and females also require blood to produce eggs, which they may take from a wide range of domestic and wild warm and cold-blooded animals (Muñoz *et al.*, 2019). Sand fly populations are strongly aggregated and concentrate in domestic and open countryside environments such as animal sheds and burrows, abandoned buildings, cellars, caves, stone walls, and sheltered rocky emplacements (Feliciangeli, 2004; Risueño *et al.*, 2017). Species distributions are also heterogeneous on a large geographical scale (ECDC, 2023). *Phlebotomus mascittii* is the species with the northernmost distribution in Europe, reaching latitude 49–50°N in southern Belgium and Germany, albeit at a very low density (Depaquit *et al.*, 2005; Naucke *et al.*, 2008; Oerther *et al.*, 2020). *Phlebotomus perniciosus* has also been occasionally found in southern Germany as well as in central, north and northeast France (Kasbari *et al.*, 2012; Naucke and Schmitt, 2004; Perrotey *et al.*, 2005).

Distributions of sand flies and their transmitted diseases are spatially and temporarily dynamic and susceptible to environmental and non-environmental human-made changes (Gradoni, 2018). Predicted habitat suitability studies for *Ph. mascittii* suggest most of Western Europe towards as far north as southern Scandinavia as suitable. Discrepancy with its more restricted currently known distribution was considered to be possibly related to the species' breeding sites preference for urban and periurban habitats (Kniha *et al.*, 2023). Ongoing global climate warming is likely to expand sand flies beyond their present distribution areas (Medlock *et al.*, 2014). Indeed, there is already evidence of sand fly presence and autochthonous endemic foci of CanL in pre-alpine north Italian regions that were free of vectors and CanL before 1990 (Gradoni *et al.*, 2022; Maroli *et al.*, 2008). The establishment of new endemic areas is facilitated by the frequent movement of infected dogs between endemic and non-endemic regions in Europe (Maia and Cardoso, 2015; Wright *et al.*, 2020). Experts suggest that CanL may continue to spread into areas where *Leishmania* spp. are not currently present but vectors are found, and highlight the need to improve our understanding of vector competence and distribution (EFSA, 2015).

In order to increase knowledge of vector competence and distribution, a consolidated entomological network is needed. VectorNet (https://www.ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and

-laboratory-networks/vector-net) is an entomological network funded by the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) (Braks *et al.*, 2022), whose main objective is to map vector distributions to facilitate preparedness and response for vector-borne diseases. The present study, commissioned by VectorNet, had the double objective of capacity building and sand fly surveillance in France, Luxembourg, Germany, Belgium and the Netherlands, at the northern limit of the known sand fly distribution range, where very limited or no recent occurrence data exists. Here we report on the results of the entomological survey, together with an investigation of the relationship between sand fly distribution and environmental factors.

2 Materials and methods

Sampling strategy

The survey covered localities situated in a narrow south to north transect from Savoie (Lat: 45.8219°N, Lon: 5.8492°E) in France to Zuid-Limburg (Lat: 50.8421°N, Lon: 5.7690°E) in the Netherlands, also encompassing sampling sites in Luxembourg, Germany and Belgium (Figure 1). It targeted the five northernmost latitudinal degrees where sand flies have been reported, and, according to the VectorNet database, most NUTS3 geographical administrative units in the transect had not been surveyed before the present study, as indicated in the February 2023 *Ph. mascittii* distribution map (Supplementary Figure S1; https://www.ecdc.europa.eu

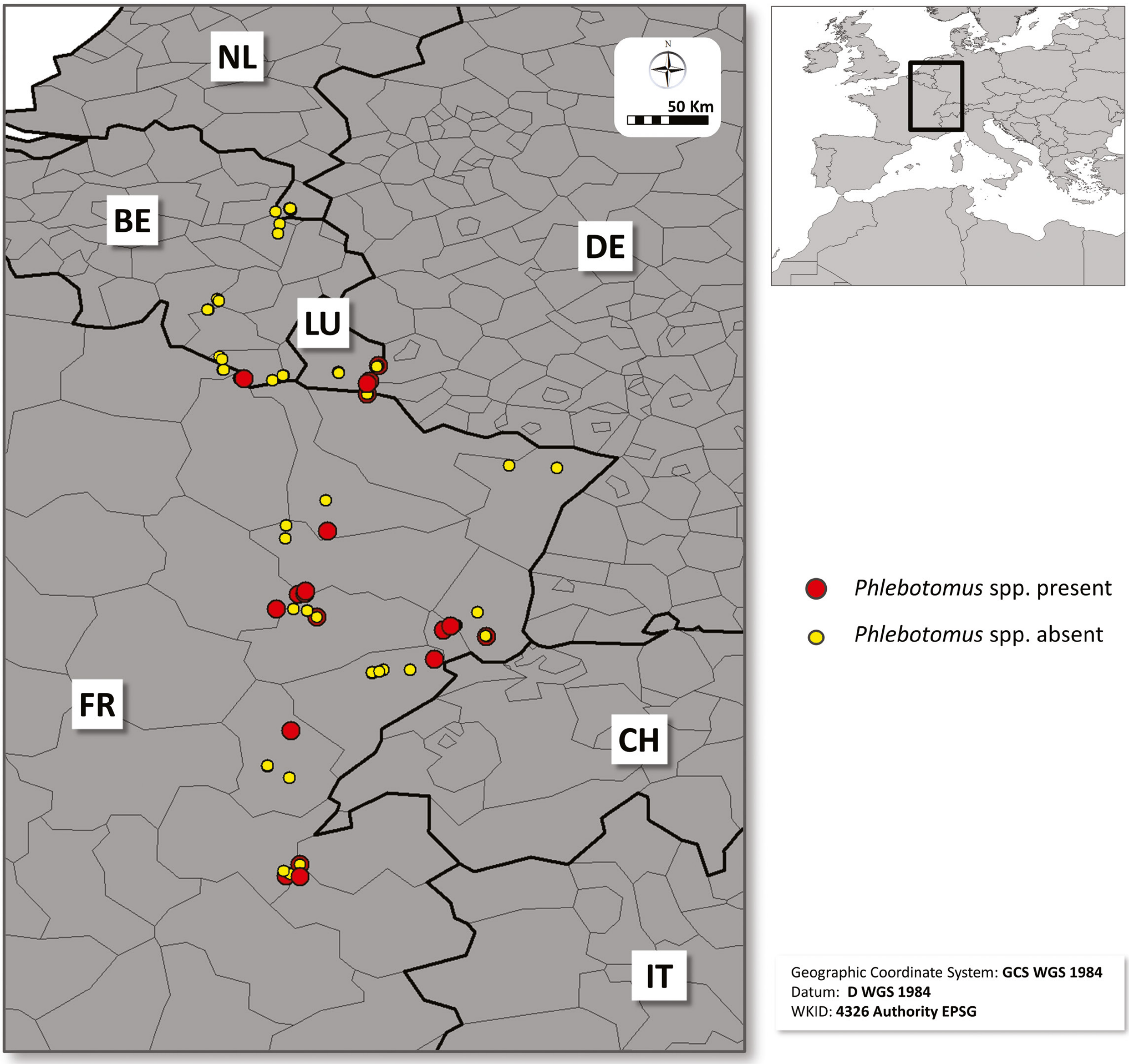

FIGURE 1 Sampling sites in the Netherlands, Luxembourg, Germany and Belgium used in the survey of Phlebotomine sand flies

/en/publications-data/phlebotomus-mascittii-current-known-distribution-february-2023).

The survey included 176 sites (with unique point geographical coordinates) in rural and peri-domestic environments at 48 localities (municipalities), and was carried out during 24 nights, between 3 July and 22 August 2023. Sampling in France was performed in July and August, and limited to July in the other countries. The number of sites (and localities) by country were 107 (35) in France, 31 (7) in Belgium, 24 (4) in Luxembourg, eight (1) in the Netherlands and six (1) in Germany. Sampling locations were selected in a two-step process. First, two or three locations were selected in the territorial NUTS3 units along our transect based on an environment suitability modelling run for *Ph. mascittii* (updated from Alten *et al.*, 2016). Satellite images (Google Earth™) were used to identify villages with old houses/barns and cliffs located in areas showing a high environment suitability on the modelling map output. We also included locations where sand flies had been previously detected (Depaquit *et al.*, 2005). In the field, precise trapping locations were chosen based on environmental features considered suitable for sand flies (Feliciangeli, 2004; Risueño *et al.*, 2017), such as protected gardens, stone walls, abandoned houses, cellars, old barns possibly hosting animals inside or nearby, rock piles, base of cliffs and caves (Supplementary Figures S2–S8).

Each site was sampled for one night using one trapping method, except three sites in Luxembourg that were sampled twice in two consecutive nights with two methods. Seven sites were sampled using human sand fly landing catches, performed with mouth aspirators during 20 minutes/site, whilst the remaining 169 sites (172 trap-nights) were sampled using miniature CDC light traps (John W. Hock, Gainesville, FL, USA), of which three traps were baited with CO_2 (BG-CO_2 Generator (Biogents AG, Regensburg, Germany)), and three interception sticky traps (for a total of 2.0 m^2 of castor oil impregnated A4 tracing paper sheets hung with clothes pegs). Light and sticky traps were placed indoors or outdoors, close to the floor and to vertical surfaces (Muñoz *et al.*, 2021), between 4:00–10:00 pm and collected between 19:00–23:00 am the following day.

Environmental data

Features of the sample site and its immediate surroundings were recorded, including trap location inside (e.g. building or a cave) or outside (e.g. garden), orientation of traps placed outside, proximity to a stone wall or a cliff, and presence of domestic animals (dogs, cats and farm animals) within visible range from the trap. In addition, the geographical coordinates were obtained with a global positioning system (GPS) device, and used to map sites and to determine site altitude, using the geographical information system ArcGIS® (ESRI).

Sand fly collection and identification

Upon recovery of traps, collection cups of light traps were transported to the lab in a cool box and then stored in a freezer for at least 30 min to kill the insects, and sticky traps were transported and stored between A4 sheets of white paper until analysis. Catches were carefully examined for sand flies. When present, their gender was determined and specimens cleared with Marc-André solution, mounted on glass slides with Hoyer solution and identified morphologically. This was done according to features of the parameral sheaths, gonostyle and gonocoxite in males and the pharynx, cibarium and spermatheca in females (Galati *et al.*, 2017), using the entomological keys of Fauran *et al.* (1998), Gállego Berenguer *et al.* (1992), Lawyer *et al.* (2011) and Lewis (1982).

Statistical analysis

The frequency distributions of sand fly species and of possible environmental explanatory variables were examined, and latitude and altitude were categorised into respectively four and three approximately equal bands. The proportion of traps with sand flies (positive traps) was then compared across levels of explanatory variables, using Yates-corrected chi-square test or when necessary, Fisher exact test. The probability of sand fly presence was then investigated using multivariable logistic regression modelling (Kleinbaum and Klein, 2010). Model building used a backward strategy beginning with the saturated model incorporating as many variables as possible, and the final model included only variables significantly associated with sand fly presence. The model estimation method was the maximum likelihood, and significance was taken for $P < 0.05$ for a double-sided test.

3 Results

Sand fly species presence and abundance

A total of 55 sand flies were collected, 52 with CDC traps and three with mouth aspirators (human landing). They comprised 39 *Ph. mascittii* (37 females, two males) and 16 *Ph. perniciosus* (one female, 15 males). In France, 33 *Ph. mascittii* and 16 *Ph. perniciosus* were collected, in Luxembourg five *Ph. mascittii* and in Germany one *Ph. mascittii* (Table 1; Figures S2–S8). No sand flies were detected in Belgium and the Netherlands. Sand flies were found in 14 out of the 22 NUTS3 units sampled and

TABLE 1 Frequency of phlebotomine sand fly species by locality (listed by country from lowest to highest latitude), trapping method and date in our field study, July-August 2023.

Country	NUTS3[1]	Locality	Latitude	Longitude	Altitude (m)	Trapping Method[2]	Sampling start date	*Ph. mascittii*			*Ph. perniciosus*			All
								F	M	all	F	M	all	
France	Savoie*	Chindrieux	45.82188	5.84917	336	LT	03/07	0	0	0	0	4	4	4
	Ain*	Ceyzérieu	45.83269	5.74420	231	LT+CO_2	04/07	1	0	1	0	0	0	1
	Ain	Ceyzérieu	45.83270	5.74424	231	LT	04/07	3	0	3	0	0	0	3
	Savoie	Motz	45.91852	5.84824	715	LT	03/07	1	0	1	1	11	12	13
	Jura*	Montigny-lès-Arsures	46.92100	5.77970	423	LT	07/07	3	0	3	0	0	0	3
	Jura	Montigny-lès-Arsures	46.92317	5.78049	423	HL	07/07	2	0	2	0	0	0	2
	Doubs*	Seloncourt	47.45940	6.85920	386	HL	03/08	1	0	1	0	0	0	1
	Haut-Rhin	Altkirch	47.62623	7.24643	316	LT	31/07	1	1	2	0	0	0	2
	Territoire-de-Belfort*	Roppe	47.67317	6.92495	365	LT	17/08	1	0	1	0	0	0	1
	Territoire-de-Belfort	Felon	47.70846	6.97781	391	LT	17/08	2	0	2	0	0	0	2
	Haute-Saône*	Aboncourt-Gesincourt	47.77078	5.97146	230	LT	10/08	0	1	1	0	0	0	1
	Haute-Marne	Anrosey	47.83658	5.67247	243	LT	11/08	2	0	2	0	0	0	2
	Haute-Marne	Fresne-sur-Apance	47.94105	5.83442	265	LT	11/08	6	0	6	0	0	0	6
	Vosges*	Châtillon-sur-Saône	47.94720	5.88472	252	LT	11/08	1	0	1	0	0	0	1
	Vosges	Lironcourt	47.96930	5.89194	249	LT	11/08	1	0	1	0	0	0	1
	Vosges	Lironcourt	47.97040	5.89116	237	LT	11/08	2	0	2	0	0	0	2
	Meurthe-et-Moselle*	Vaudemont	48.41637	6.05549	495	LT	20/07	2	0	2	0	0	0	2
	Moselle*	Contz-les-Bains	49.44994	6.35437	160	LT	17/07	1	0	1	0	0	0	1
	Meuse*	Thonne-la-Long	49.56258	5.42662	237	LT	16/07	1	0	1	0	0	0	1
Germany	Trier-Saarburg*	Nittel	49.66330	6.43935	273	LT+CO_2	12/07	1	0	1	0	0	0	1
Luxembourg	Luxembourg	Bech-Kleinmacher	49.53006	6.35499	142	LT	12/07	1	0	1	0	0	0	1
	Luxembourg*	Remich	49.54751	6.37206	148	LT	11/07	1	0	1	0	0	0	1
	Luxembourg	Remich	49.54751	6.37206	148	LT	12/07	1	0	1	0	0	0	1
	Luxembourg	Remich	49.54801	6.37241	148	LT	11/07	2	0	2	0	0	0	2
All								37	2	39	1	15	16	55

[1] NUTS3 units where sand flies were reported for the first time.
[2] LT: CDC light trap, HL: human landing.

in 12 of them sand flies were detected for the first time (Table 1). *Phlebotomus mascittii* was present in most sand fly-positive NUTS3 units and localities between latitudes 45.83°N and 49.66°N. In contrast, *Ph. perniciosus* was only found in Chindrieux and Motz (Savoie, France), at latitudes of 45.82°N and 45.92°N, respectively. The highest trapping result was obtained in Motz, with 13 sand fly specimens in one trap-night, followed by those in Fresne-sur-Apance (Haute-Marne, France) with six specimens and Chindrieux with four specimens. The number of sand flies in other sand fly-positive sites ranged from one to three specimens per trap-night (Table 1 and Supplementary Table S1).

Relationship between sand fly presence and environmental variables in trapping sites

In the bivariate analysis, sand fly presence was significantly and negatively associated with increasing latitude, but not to altitude, orientation or other characteristics of the immediate trap surroundings (Table 2).

TABLE 2 Percentage of traps with sand flies (positive traps) according to characteristics of the environment where the traps were placed in our field study, July-August 2023.

Variable	Level	Traps			*P*-value
		No.	% positive	95% CI	
Latitude	45.82–46.92	23	26	22–30	0.0009
	47.35–47.97	46	22	20–24	
	48.36–49.57	48	15	13–16	
	49.59–50.84	62	2	1–2	
Altitude (m)	59–275	95	16	15–17	0.6852
	284–495	72	11	10–12	
	525–715	12	8	4–13	
Orientation	North	11	0	0	0.5720
	East	23	17	14–21	
	South	95	16	15–17	
	West	14	7	4–11	
Land use (visually assessed)	Agriculture	24	8	6–11	0.8456
	Forest and shrub	8	0	0	
	Nature, other	9	11	4–18	
	Rural habitat	96	17	16–17	
	Suburban habitat	35	11	10–13	
	Urban habitat	7	14	5–24	
Location	Indoor	26	15	13–18	0.9014
	Outdoor	113	13	13–14	
	Indoor/Outdoor[1]	40	13	11–14	
Next to a cliff	No	151	15	14–15	0.3781
	Yes	28	7	5–9	
Next to a stone wall	No	123	15	14–15	0.6371
	Yes	56	11	10–12	
In a garden	No	134	13	13–14	1.0000
	Yes	45	13	12–15	
Inside a building	No	143	14	14–15	0.7889
	Yes	36	11	9–13	
Inside a cave	No	171	14	14–14	0.6000
	Yes	8	0	0	
Close to domestic animals	No	160	14	13–14	1.0000
	Yes	19	11	7–14	

[1] Semi-open barns.

TABLE 3 Results from the logistic regression analysis examining the relationship between sand fly presence, latitude and altitude in the site where sand fly traps were placed in our field study, July–August 2023.

Variable	Level	Estimate	Std. error	*P*-value
Intercept		0.60	0.71	0.4024
Latitude (°N)	45.82–46.92	0.00		
	47.35–47.97	–0.87	0.75	0.2456
	48.36–49.57	–2.00	0.80	0.0120
	49.59–50.84	–4.51	1.23	0.0002
Altitude (m)	59–275	0.00		
	284–495	–1.46	0.57	0.0104
	525–715	–2.99	1.26	0.0178

However, in the multivariable logistic model sand fly presence was independently and negatively associated to both latitude and altitude, with the predicted probability of sand fly presence gradually decreasing with increasing latitude and altitude ($p < 0.05$) (Table 3).

4 Discussion

Investigating sand fly vector distribution is a prerequisite for assessing the risk of infection with the pathogens they transmit, and present knowledge of sand fly distributions in their northern range limit in Europe is comparatively scarce. This study made a contribution by investigating for the first time the presence of sand fly vectors in 12 out of 22 NUTS3 surveyed, including 10 in France as well as in Luxembourg and in neighbouring Trier-Saarburg district in Germany. Species found included *Ph. perniciosus* in Savoie, the southern-most region surveyed, at 45.92°N latitude, and *Ph. mascittii* in all 12 areas, and as far north as 49.63°N in Germany. The absence of sand flies above latitude 50°N, and *Ph. mascittii* being the only species found in the northernmost latitudes, is in agreement with other surveys in Belgium (Depaquit *et al.*, 2005) and northern France (Depaquit *et al.*, 2005; Schaffner, 2023) and further east, in Germany (Naucke *et al.*, 2008; Oerther *et al.*, 2020; Steinhausen, 2005) and Austria (Kniha *et al.*, 2021; Obwaller *et al.*, 2016). Still, the species' latitudinal limits may change beyond the longitudes explored as it will depend on other environmental factors affecting sand fly distributions such as altitude and climatic variables, as shown in the present study. Also, similarly to the above cited studies, the density of sand flies was low, in particular for *Ph. mascittii,* of which not more than six specimens per CDC light trap-night were collected in the present study. Generally, sand flies thrive in warm climates but *Ph. mascittii* is not abundant even in southern *L. infantum*-endemic Mediterranean areas, and the reasons for this are not clear and warrant further investigation (Depaquit *et al.*, 2005; Prudhomme *et al.*, 2015; Veronesi *et al.*, 2007). The highest density of *P. mascittii* ever reported was in an old, abandoned railway tunnel in Corsica, and some specimens were collected in early February when average temperatures and relative humidity inside the tunnel ranged between 16.1–16.6 °C and 61–67%, respectively (Naucke *et al.*, 2008b). Optimal rearing temperature and RH for other sand fly species in the laboratory typically range between 22–28 °C and 70–80%, respectively, depending on the species (Lawyer *et al.*, 2017; Volf and Volfova, 2011). Other essential factors for sand fly colonies to thrive include availability of adequate food resources for larvae and adult stages, photoperiod, breeding space and avoiding mold growth and pathogen infections (Lawyer *et al.*, 2017). Deviations from ideal conditions result in slow larval development, diapause and increased mortality, and low adult fertility, leading to longer life cycles and lower population densities (Lawyer *et al.*, 2017). In the present study, sand fly presence decreased with increasing latitude and altitude, strongly suggesting a temperature effect. In contrast, sand fly presence was not associated with other environmental factors close to where the trap was placed, like proximity to animals or stone walls, or being inside or outside a building, cave or garden. This may not be surprising given that sampling places were purposely selected with the aim of finding sand flies, meeting various environmental settings deemed ideal for sand fly breeding and resting (Feliciangeli, 2004; Risueño *et al.*, 2017). No sand flies were captured in the few sticky traps used in the study, although they were placed in the same sites where sand flies were collected with light traps.

The latter attract phototropic insects (including sand flies) located within a few meters of the trap, whilst the former are interception traps for sand flies passing by, and tend to produce comparatively lower sand fly yields (Muñoz *et al.*, 2017).

Despite the presence of sand fly vectors in such northern latitudes, autochthonous cases of leishmaniasis have only rarely been reported so far north (ECDC, 2023; Kasbari *et al.*, 2012; Naucke *et al.*, 2008). Whilst there is no evidence so far that *Ph. mascittii* transmits phleboviruses, its potential role in the transmission and establishment of *L. infantum* in a dog population in the areas investigated is critical. A key aspect of vectorial competence is the parasite´s ability to survive blood digestion and remain in the fly´s gut after it defecates bloodmeal remnants (Kamhawi, 2006). Therefore, detecting infection in sand flies with no blood in their abdomen suggests a potential role as vector of this sand fly, and this was demonstrated in a *Ph. mascittii* specimen captured in a farm in Austria, where a dog was also infected with the same strain (Obwaller *et al.*, 2016). Successful transmission and subsequent infection establishment in the population depends on several other factors related to the vector, such as the infective dose acquired by the vector and delivered to the host, the host´s susceptibility of infection and the density of infected and non-infected hosts and vectors in the area (Courtenay *et al.*, 2017). The typically low density of *Ph. mascittii* and its ability for autogeny i.e. females not requiring blood to produce eggs, were considered limiting epidemiological factors (Ready, 2010). There is a need for further research on *Ph. mascittii* biology and vectorial potential in central western European countries, where a large number of imported *L. infantum* infected dogs are present (Naucke *et al.*, 2008).

5 Conclusions

The study corroborates that sand flies in Western Europe are found as far north as latitude 50°N and that they are present in Luxembourg and Trier-Saarburg district in Germany, where no previous information was available. The dominant species beyond latitude 45°N is *Ph. mascittii*, while the latitudinal limit of *Ph. perniciosus* is probably around 45°N. The density of sand flies at these latitudes is very low, and presence was negatively associated with increasing latitude and altitude. The low density of *Ph. mascittii* and its known ability for autogeny are limiting factors for the establishment of foci of *L. infantum* in this area, but a better assessment of this important issue must include an evaluation of its as yet, unconfirmed vectorial capacity.

Supplementary material

Supplementary material can be found online at https://doi.org/10.6084/m9.figshare.25690455

Figure S1. Distribution of *Phlebotomus mascittii* in Europe and neighbouring countries.

Figure S2. Sand fly sampling site in Ain (Ceyzérieu), France (henhouse and garden; SF01-001; 04/07).

Figure S3. Sand fly sampling site in Jura (Montigny-lès-Arsures), France (uninhabited house; SF39-003; 08/07).

Figure S4. Sand fly sampling site in Vosges (Lironcourt), France (old barn with ground floor; SF88-004; 11/08).

Figure S5. Sand fly sampling site in Haut-Marne (Fresne-sur-Apance), France (semi-open old barn with ground floor; SF52-002; 11/08).

Figure S6. Sand fly sampling site in Savoie (Motz), France (unused stable; SF73-002; 04/07).

Figure S7. Sand fly sampling site in Remich, Luxembourg (cliff base and garden; SFLU-002; 11/07).

Figure S8. Sand fly sampling site in Trier-Saarburg (Nittel), Germany (cliff base and garden; SFDE-001; 12/07).

Table S1. Detailed field data of our survey of Phlebotomine sand flies across their northern distribution range limit in Western Europe, summer 2023.

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Conflict of interest

Francis Schaffner is editor-in-chief of the Journal of the European Mosquito Control Association; he had no influence in the review process and decision making on this manuscript. The other co-authors declare no conflict of interest.

Data availability statement

The detailed data that supports the findings of this study are available in the supplementary material of this article.

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