

**EXPANSION OF THE SAND FLY FAUNA OF SLOVENIA:
FIRST RECORD OF *PHLEBOTOMUS PERFILIEWI* PARROT, 1930
(DIPTERA: PSYCHODIDAE)**

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Abstract - Phlebotomine sand flies (Diptera: Psychodidae) are important vectors of human and animal pathogens, including *Leishmania* spp. parasites and phleboviruses. In this study, we report the first record of *Phlebotomus perfiliewi* Parrot, 1930 in Slovenia, based on material obtained during systematic vector surveillance conducted in 2023 and 2024 in the Primorska region. Sampling was performed monthly from May to October on a rural tourist farm Medljan in Cetore, using CDC light traps. Morphological identification of specimens was based on key characters of cibarial armature, spermathecae, and male genitalia.

In 2023, three specimens of *P. perfiliewi* (2♂, 1♀) were collected. In 2024, nine additional individuals (8♂, 1♀) were recorded, suggesting the presence of an emerging local population. This species, previously widespread in Italy and parts of the central and southern Balkans, had not been recorded in northern Dalmatia, Istria, or Slovenia until now. Given the proximity of the sampling site to northeastern Italy, the population likely represents a recent expansion across the border.

As *P. perfiliewi* is a proven vector of *L. infantum*, its presence raises concerns about the possible establishment of autochthonous transmission cycles in Slovenia. Further molecular studies are needed to confirm the origin and genetic relatedness of the population. These findings highlight the importance of continuous entomological monitoring in response to environmental change and expanding vector distributions in Europe.

KEY WORDS: sand flies, *Phlebotomus perfiliewi*, first record, Slovenia; vector surveillance

Izvleček - RAZŠIRITEV FAVNE PEŠČENIH MUH V SLOVENIJI: PRVA NAJDBA VRSTE *PHLEBOTOMUS PERFILIEWI* PARROT, 1930 (DIPTERA: PSYCHODIDAE)

Peščene muhe (Diptera: Psychodidae) so pomembni prenašalci povzročiteljev bolezni pri ljudeh in živalih, vključno s paraziti rodu *Leishmania* in flebovirusi. V tej raziskavi poročamo o prvi najdbi vrste *Phlebotomus perfiliewi* Parrot, 1930 v Sloveniji, ki temelji na materialu, zbranim v okviru sistematičnega monitoringa vektorjev v letih 2023 in 2024 v Primorski regiji. Vzorčenje je potekalo mesečno od maja do oktobra na podeželski turistični kmetiji Medljan v Cetorah, z uporabo svetlobnih pasti CDC. Morfološka identifikacija osebkov je temeljila na diagnostičnih znakih cibarialnega oborožja, spermatek in moških genitalij.

Leta 2023 so bili zbrani trije osebki vrste *P. perfiliewi* (2 ♂, 1 ♀), leta 2024 pa dodatnih devet osebkov (8 ♂, 1 ♀), kar kaže na prisotnost nastajajoče lokalne populacije. Vrsta, ki je sicer razširjena v Italiji ter delih osrednjega in južnega Balkana, do sedaj ni bila zabeležena v severni Dalmaciji, Istri ali Sloveniji. Glede na bližino vzorčnega mesta severovzhodni Italiji je verjetno, da gre za nedavno širitev populacije čez državno mejo.

Ker je *P. perfiliewi* potrjen vektor *Leishmania infantum*, njegova prisotnost predstavlja potencialno tveganje za vzpostavitev avtohtonih prenosnih ciklov v Sloveniji. Za potrditev izvora in genetske sorodnosti populacije so potrebne nadaljnje molekularne raziskave. Ugotovitve poudarjajo pomen neprekinjenega entomološkega monitoringa v luči okoljskih sprememb in širjenja vektorjev v Evropi.

KLJUČNE BESEDE: peščene muhe, *Phlebotomus perfiliewi*, prva najdba, Slovenija, spremljanje prenašalcev.

Introduction

Phlebotomine sand flies (Diptera: Psychodidae, subfamily Phlebotominae) are small hematophagous insects of major medical and veterinary importance. About 1000 species have been described worldwide, classified in genera including *Phlebotomus*, *Sergentomyia*, *Lutzomyia*, *Brumptomyia*, *Warileya*, and *Chinius* (Galati et al., 2025). Of these, *Phlebotomus* species in the Old World and *Lutzomyia* species in the New World represent the principal vectors of human disease (Killick-Kendrick, 1990; Lane, 1993; Maroli et al., 2013; Cecilio et al., 2022).

The taxonomic history of phlebotomine sand flies spans more than 250 years. The first known description of a phlebotomine sand fly species was provided by Giovanni Antonio Scopoli in 1763, who described *Bibio papatasi* in *Entomologia Carniolica* from material collected in Carniola (present-day Slovenia) (Scopoli, 1763). At that time, small Diptera were often placed in broad genera such as *Bibio* or *Culex*, and the species was later transferred to genus *Culex* by Fabricius (1787). A major advance came with Latreille (1818), who established the genus *Phlebotomus* and included *P. papatasi*, providing the taxonomic basis for Old World sand flies. The subfamily Phlebotominae within Psychodidae was subsequently erected by Rondani (1840), formally recognising the distinct lineage of sand flies. Throughout the 20th century, taxonomic revisions by Theodor (1958), Abonnenc (1972) and Lewis and Lane (1982) clarified species boundaries and established the modern subgeneric classification.

These contributions laid the foundation for contemporary sand fly taxonomy, from Scopoli's original description to the current classification system.

The life cycle of sand flies is holometabolous. Females lay 15–100 ovoid eggs in humid, organic-rich microhabitats; larvae pass through four instars, are scavengers of organic matter, and may undergo winter diapause (Killick-Kendrick, 1999; Ready, 2013). Pupation lasts 7–14 days, while adults live a few weeks to months. Only females take blood meals, feeding on humans, dogs, rodents, reptiles, and birds. Most species are exophagic, nocturnal, and rest in shaded, humid shelters such as animal burrows, caves, or cracks in walls (Killick-Kendrick, 1990; Lane, 1993).

Besides nuisance from biting, sand flies are vectors of several pathogens of major public health concern. These include protozoan parasites (*Leishmania* spp.), viruses (sandfly fever phleboviruses such as *Toscana*, *Sicilian*, and *Naples* virus), and bacteria *Bartonella bacilliformis*, the agent of Carrion's disease (Maroli et al., 2013; Depaquit et al., 2010; Ivović et al., 2025). Leishmaniasis occurs in over 98 countries, with three main clinical forms: cutaneous, mucocutaneous, and visceral leishmaniasis (also known as kala-azar). An estimated 1.3 million new human cases and 20,000–30,000 deaths occur annually (WHO, 2022). The disease disproportionately affects the poorest populations, with risk factors including malnutrition, poor housing, population displacement, and environmental changes such as deforestation and urbanisation (Ready, 2013).

Sand fly fever, also known as “pappataci fever” or “three-day fever,” is a non-fatal but highly incapacitating arboviral disease transmitted by several *Phlebotomus* species. It has been of considerable military importance, historically affecting troops in the Mediterranean during World War II, and continues to be reported in endemic areas (Depaquit et al., 2010; Ayhan et al., 2017). *Toscana* virus in particular can cause neuroinvasive infections such as meningitis and encephalitis in humans (Ayhan et al., 2017).

Due to their role in transmitting *Leishmania*, phleboviruses, and other pathogens, phlebotomine sand flies have become increasingly important in Europe. Climate change and changes in land use are facilitating their northward spread, raising concerns about the potential emergence of sand fly-borne diseases in areas that were previously unaffected (Morchón et al., 2023). In Slovenia, recent climate trends characterised by increasing mean annual temperatures, milder winters, and prolonged warm seasons are likely to enhance habitat suitability for phlebotomine sand flies, particularly in the sub-Mediterranean Primorska region. Such conditions may facilitate the northward expansion, establishment, and seasonal persistence of sand fly populations, as already observed in neighbouring regions. These environmental changes underscore the growing relevance of sand fly surveillance in Slovenia in the context of climate-driven shifts in vector distribution (Morchón et al., 2023; Ivović et al., 2025).

Research on phlebotomine sand flies in Slovenia began relatively recently compared to other Mediterranean countries. The first records were published by Ivović et al. (2015), who reported *Phlebotomus neglectus*, *P. perniciosus*, *P. papatasi*, *P. mascittii* and *Sergentomyia minuta* from illegal waste sites located in Slovenian Istria (Malija, Rakitovec and Velike Žablje). The species were collected during entomological

surveys conducted between 2011 and 2013, suggesting that such habitats could serve as microfoci for *Leishmania infantum* transmission. This discovery marked the start of systematic entomological investigations in the country. Subsequent study by Praprotnik et al. (2019) provided morphological and molecular confirmation of *P. mascittii* populations, while a broader regional synthesis by Dvořák et al. (2020) placed the Slovenian fauna at the northernmost range of sand flies in the Balkans. In recent years, monitoring and modelling approaches have significantly expanded knowledge of sand fly ecology in Slovenia. Ivović et al. (2025) documented a wider distribution of *Phlebotomus* species and identified environmental conditions favourable for their establishment, while Ivović (2025) highlighted the potential role of domestic dogs in sustaining local *Leishmania* cycles. The most recent and epidemiologically important finding was reported by Bongiorno et al. (2025), who provided the first molecular evidence of *Leishmania* infection in phlebotomine sand flies from Slovenia, with *Leishmania* sp. isolated from *Phlebotomus neglectus* and *P. mascittii* at the Cetore locality, where *P. perfiliewi* was also recorded for the first time. Together, these studies demonstrate rapid development in sand fly research in Slovenia, from the initial findings to integrative monitoring and pathogen detection, positioning the country within the expanding range of Mediterranean sand flies.

During ongoing vector monitoring, we have recorded for the first time in Slovenia the presence of *Phlebotomus perfiliewi* Parrot, 1930, a proven vector of *Leishmania infantum* in southern Europe. This paper aims to document this new record and update the checklist of sand fly species known from Slovenia.

Material and methods

Study area

As part of the national research project “Establishment of a Vector-Borne Disease Vector Monitoring System in Slovenia” (V3-1903), a representative site in the Primorska region of Slovenia, Cetore (tourist farm Medljan), was selected for the study of sand fly species composition (Fig. 1). This locality was chosen because all sand fly species previously recorded in Slovenia have been detected there. Sampling was conducted once per month from May to October during the 2023 and 2024 seasons.

The site is a tourist farm surrounded by typical Mediterranean vegetation and rural structures. Various domestic animals are present, including rabbits, poultry, dogs, cats, horses, and donkeys, providing diverse potential hosts and suitable microhabitats for sand fly breeding and resting.

Trapping and identification

Sand flies were collected monthly from May to October using CDC miniature light traps (John W. Hock Company, USA). Two traps were placed, usually near animal shelters (with chickens, rabbits, or dogs) and dry-stone walls. Additional BG-Sentinel and CDC gravid traps were also used within a broader mosquito surveillance programme and occasionally captured sand flies. After field collection, all specimens

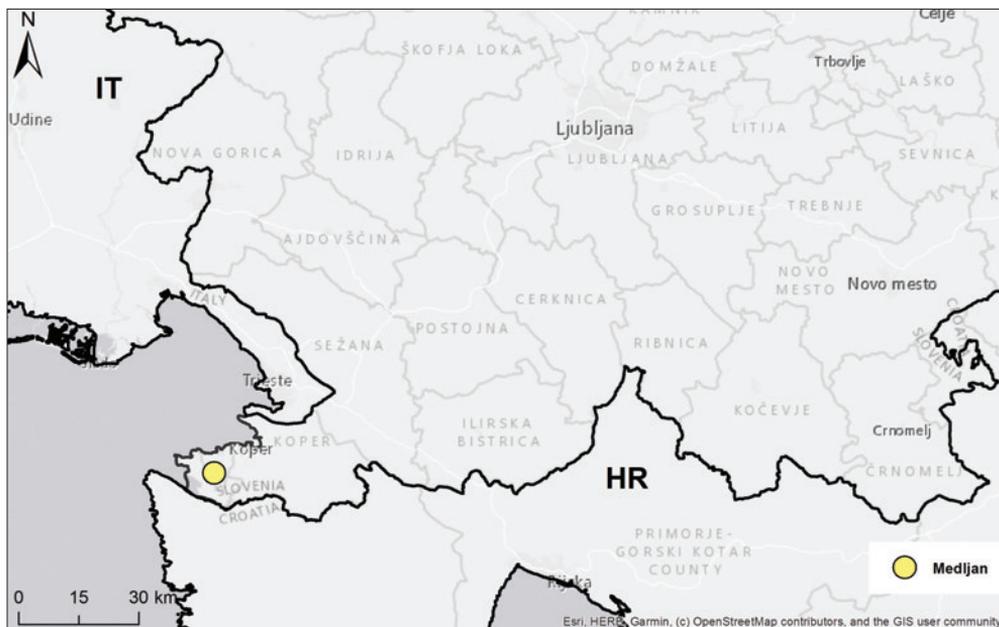


Fig. 1. Geographical location of the study area in the Primorska region, Slovenia.

were frozen at -80°C . Heads and terminal abdominal segments were dissected on ice and mounted on slides for morphological identification, while the remaining body parts were stored for molecular analyses. Identification to the species level was conducted using standard morphological keys (Artemiev and Neronov, 1984; Lewis and Lane, 1976; Perfiliew, 1966), based on the cibarial armature, spermathecae in females, and male terminalia. Slides were prepared in Marc-André solution and mounted in CMP-9 medium (Polyscience, Cat. No. 16299).

Results

A total of 142 sand flies were collected in Medljan in 2023 and 1,394 specimens in 2024. Four species of the genus *Phlebotomus* were identified in both years: *P. neglectus*, *P. perniciosus*, *P. mascittii*, and *P. perfiliewi* (Tab. 1). Additionally, seven specimens collected in 2024 were identified only to the genus level (*Phlebotomus* spp.) due to morphological deterioration. In 2023, *P. neglectus* was the dominant species, representing 76.5% of all captures, followed by *P. perniciosus* (20%), while *P. mascittii* and *P. perfiliewi* were rare, each accounting for 2% of specimens. The presence of *P. perfiliewi* was confirmed by the capture of three specimens (two males and one female) in July and August 2023, representing the first record of this species in Slovenia (Figs. 2–4). In 2024, *P. neglectus* again predominated (88.67%), followed by *P. perniciosus* (8.54%) and *P. mascittii* (1.65%). *P. perfiliewi* was recorded again, with nine specimens (one female and eight males) collected in June and July, sug-

gesting the possible establishment of this species in the area (Tab. 1). Overall, sand fly activity extended from June to October, with peak abundance observed in July and August.

Table 1: Seasonal dynamics and relative abundance of *Phlebotomus* species in Cetore (Medljan), Slovenia during 2023 and 2024.

MEDLJAN (2023)		June		July		Aug		Sep		Oct		Sum	%
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂		
	<i>Ph. neglectus</i>	11	10	23	34	11	10	4	4		2	109	77,00
	<i>Ph. perniciosus</i>		1		9	4	8		6			28	20,00
	<i>Ph. perfiliewi</i>				2	1						3	1,50
	<i>Ph. mascittii</i>	1				1						2	1,50
	Total	12	11	23	45	17	18	4	10	0	2	142	100,00

MEDLJAN (2024)		June		July		Aug		Sep		Oct		Sum	%
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂		
	<i>Ph. neglectus</i>		11	391	824			4	4		2	1236	88,67
	<i>Ph. perniciosus</i>	4	1	46	60	1		0	6	1		119	8,54
	<i>Ph. perfiliewi</i>	1		2	6							9	0,65
	<i>Ph. spp.</i>	1		1	5							7	0,50
	<i>Ph. mascittii</i>	1		8	13	1						23	1,65
	Total	7	12	448	908	2	0	4	10	1	2	1394	100,00

Discussion

Vector-borne diseases are increasingly recognised as a major public health concern in Europe, driven by environmental changes, globalisation, and climate change. These factors facilitate the spread of haematophagous vectors such as mosquitoes, ticks, and phlebotomine sand flies into new ecological niches and geographic regions previously considered unsuitable for their survival and reproduction (Morchón et al., 2023). Notably, the northward expansion of phlebotomine sand flies has been documented in several European countries, including Germany, Austria, and Switzerland, where autochthonous cases of leishmaniasis and phlebovirus infections have raised concerns about the emergence of new transmission foci (Medlock et al., 2014; Ivović et al., 2025). Such distributional shifts are largely attributed to rising average temperatures, changes in land use, and increased movement of animals and people, all of which create favourable conditions for the establishment of competent vector populations.

The detection of *Phlebotomus perfiliewi* in southwestern Slovenia marks a notable expansion of the known distribution of this species in the region. It is a well-established vector of *Leishmania infantum*, playing a dominant role in zoonotic visceral leishmaniasis (ZVL) transmission across the western Mediterranean basin, including Italy,

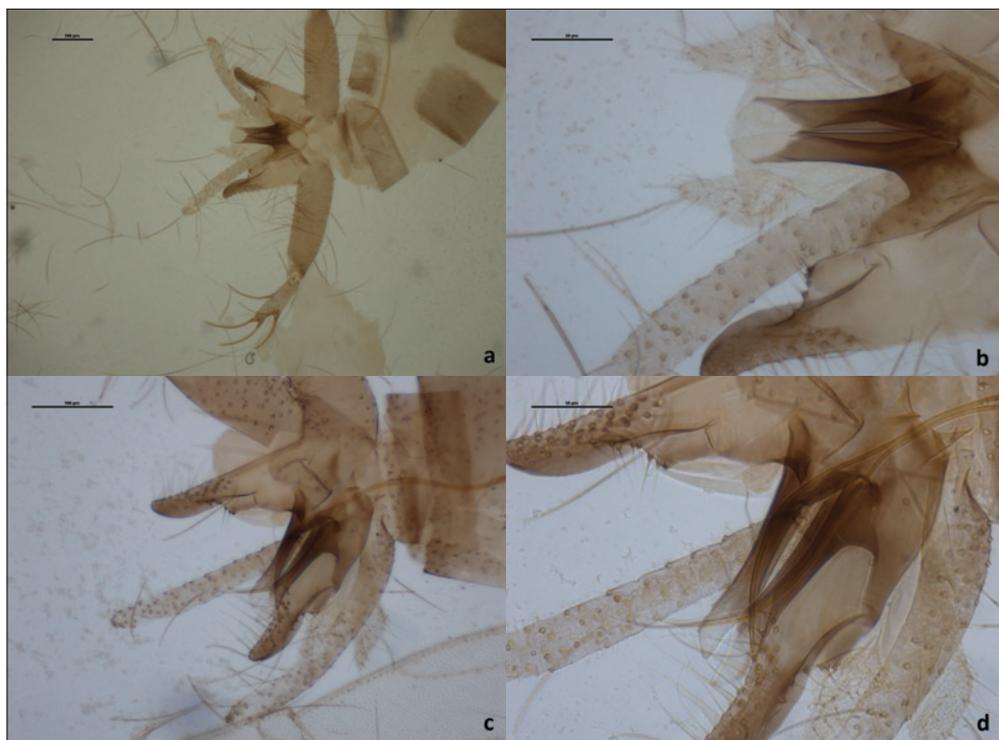


Fig. 2. Male genital structures of *P. perfiliewi*: (a) gonostyle and surstyle of the first male (10×); (b) parameres of the same male (40×); (c) parameres of a second male (20×); (d) parameres of a third male with characteristic serrated distal outer margins (40×).

Tunisia, Algeria, and parts of the Balkans (Maroli et al., 1987; Chelbi et al., 2021; Chelbi et al., 2022; Alten et al., 2016; Bosnić et al., 2006). In Italy, *P. perfiliewi* is among the most abundant species and has long been considered the main vector in the Emilia-Romagna region, where it thrives in both rural and peri-urban environments (Maroli et al., 1987; Baldelli et al., 2011).

Beyond Europe, *P. perfiliewi* has been reported in various ecological contexts, from humid northern Tunisia, where it was traditionally endemic, to highly irrigated arid zones, where its range has expanded in recent decades due to anthropogenic environmental change, particularly agricultural irrigation. Molecular evidence has confirmed local genetic variability of these newly established populations, suggesting long-term adaptation to emerging habitats (Chelbi et al., 2022).

Although *Phlebotomus perfiliewi* is widely distributed in central and southern Balkan countries such as Serbia, North Macedonia, Albania, and Greece (Ivočić et al., 2007; Dvořák et al., 2020), it has not been recorded in northern Dalmatia or Istria, regions geographically adjacent to southwestern Slovenia. This notable discontinuity

in documented distribution suggests that the newly detected Slovenian population most likely originated from northeastern Italy, where *P. perfiliewi* is both abundant and ecologically well-established (Maroli et al., 1987; Baldelli et al., 2011; Calzolari et al., 2022).

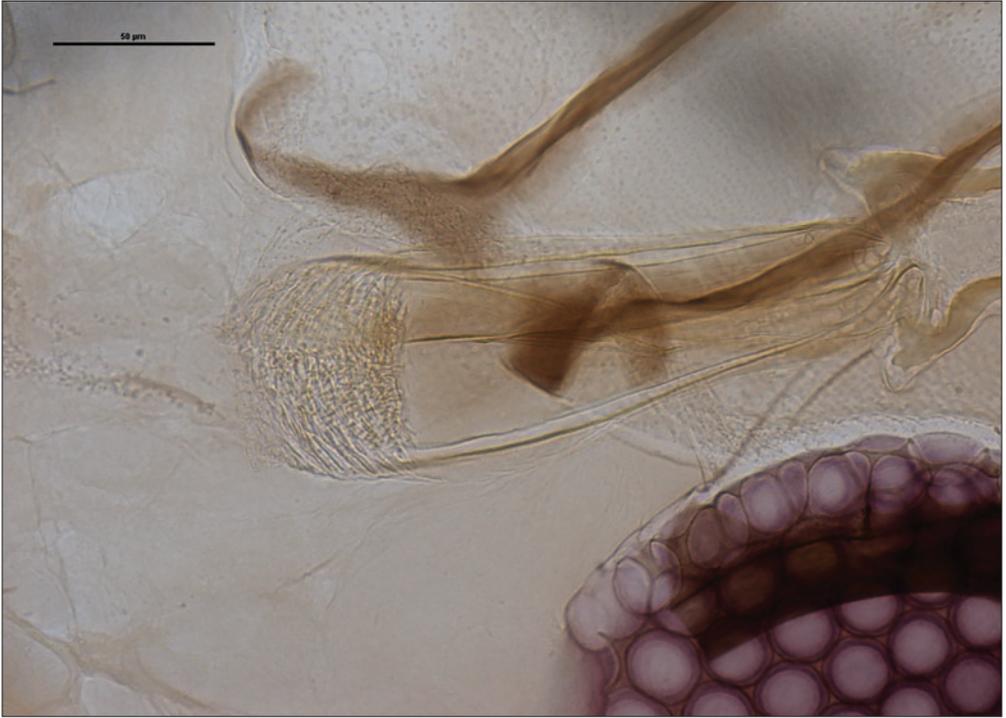


Fig. 3. Female of *P. perfiliewi* (pharynx, pharyngeal teeth).

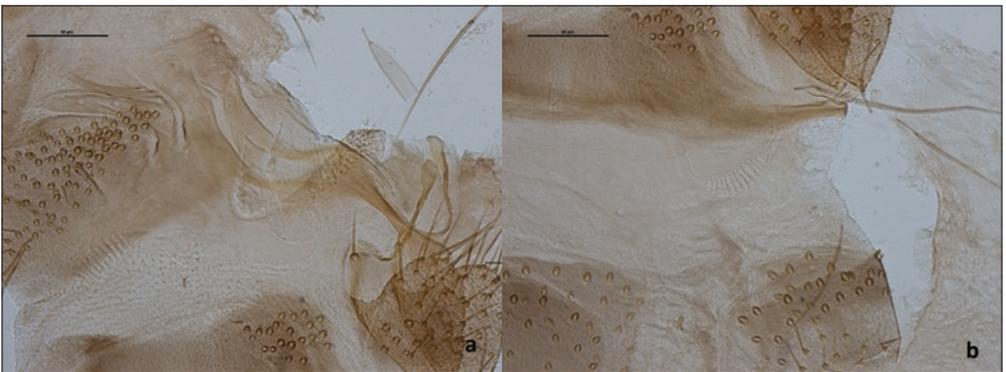


Fig. 4. Female of *P. perfiliewi*: (a) base of the spermatheca (40×) showing a characteristic large triangular wrinkled pocket; (b) spermathecal body (20×) with distinct annulations and individual spermathecal ducts.

Furthermore, recent records have confirmed the presence of *P. perfiliewi* not only in the eastern Mediterranean, but also in western regions, such as southeastern France and the Balearic Islands of Spain (Prudhomme et al., 2024; González et al., 2023). In France, although present at low abundance, the species has been historically recorded in the Alpes-Maritimes region (Prudhomme et al., 2024), and its potential vectorial role is still under investigation. The recent first report from Spain on the island of Mallorca, confirmed both morphologically and genetically, adds further evidence to its broader Mediterranean dispersal and capacity to expand beyond its historically recognised range.

The detection of *P. perfiliewi* near the Slovenian-Italian border supports the hypothesis of west-to-east expansion, likely driven by environmental changes, animal movement, or passive dispersal via prevailing winds - mechanisms that have been implicated in the shifting distribution of other sand fly species in Europe (Medlock et al., 2014). Continued monitoring and molecular characterization of the Slovenian population will be essential to determine its origin and to assess the potential for local *Leishmania* transmission.

Our findings show that *P. perfiliewi* was first recorded in Slovenia in 2023 with three individuals, and again in 2024 with nine individuals, suggesting that the species may be in the early stages of establishment. Although small in number, the presence of both males and females over two consecutive years implies that reproduction may be occurring locally. These findings align with patterns observed in Tunisia, where environmental modification enabled *P. perfiliewi* to become one of the most abundant species within a few years after introduction (Chelbi et al., 2022). Ongoing presence and increasing numbers over time, if confirmed, would further support this interpretation.

Given its vector competence for *L. infantum*, the presence of *P. perfiliewi* in Slovenia carries important epidemiological implications, especially in areas with high densities of domestic dogs, the principal reservoir host for zoonotic visceral leishmaniasis (Chelbi et al., 2021; Calzolari et al., 2022). Experimental and field studies have shown that sand flies are more attracted to infected dogs, likely due to parasite-induced changes in host odor profiles, thus increasing the risk of parasite transmission (Chelbi et al., 2021; Bongiorno et al., 2025; Ivovic, 2025).

Future molecular population genetics studies will be crucial to determine whether Slovenian *P. perfiliewi* specimens are genetically closer to Italian or Balkan populations. Such analyses would clarify whether the observed individuals are part of a founder population or the result of repeated migration events. Meanwhile, continued entomological surveillance and vector-pathogen screening remain essential to detect any potential emergence of leishmaniasis or sand fly-borne viral infections.

Conclusions

The first record of *Phlebotomus perfiliewi* in Slovenia is a significant addition to the country's sand fly fauna. Although previously unrecorded in Istria and northern Dalmatia, this species has long been established in northeastern Italy and the central

parts of the Balkans. Its detection in Slovenia indicates a recent eastward expansion, likely facilitated by suitable ecological conditions, cross-border animal movement, or climatic shifts.

Although the number of captured specimens remains low, the increase observed between 2023 and 2024, together with the presence of both sexes, may indicate an early stage of local population establishment. Given the known vector competence of this species for *Leishmania infantum*, further entomological and molecular studies are warranted to better assess its epidemiological significance and to clarify the origin of the population. Continued surveillance remains essential in the context of the changing ecology of vector-borne diseases in Central Europe.

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