

## Variations in the population structure of Phlebotomine sandflies (Diptera: Psychodidae) in relation to the presence of potential dog hosts

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### ABSTRACT

The seasonal and circadian population dynamics of Phlebotomine sandflies depend on many environmental factors, especially temperature and humidity. However, the factors that influence fluctuations in species composition are more uncertain. During the regular monitoring of arthropod vectors in the Slovenian coastal region, an interesting change in the population dynamics of these insects was observed, depending on the presence or absence of domestic dogs. To investigate the changes in species composition, we set traps for adult flies at two different locations (Medljan and Cetore) in the Coastal-Karst region (Izola, Slovenia). The collections were carried out in June and July 2017 and 2018. In total, 632 specimens of four species (*Phlebotomus papatasi*, *P. neglectus*, *P. perniciosus* and *P. mascittii*) were collected in the study area in both years. The females of all sandfly species were examined for the presence of *Leishmania* parasites in both years, but no *Leishmania* DNA was detected. The relocation of hunting dogs as potential reservoir hosts from Cetore to another location in early 2018 significantly changed the species composition of the sandflies. Two species, *P. neglectus* and *P. perniciosus*, were no longer present at this site. Changes in the species composition and population dynamics of phlebotomine sandflies are probably related to the presence or absence of the *Leishmania* reservoir host.

**Key words:** Phlebotomine sandflies; population dynamics; dogs

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### Introduction

Phlebotomine sandflies (Diptera: Psychodidae), small and delicate nematoceran insects, are proven vectors of human and animal diseases in the Old and New World ([ALTEN et al., 2016](#)). The subfamily Phlebotominae comprises more than 900 species, most of which occur in the tropics and subtropics, but some also in the warmer temperate zones ([DEPAQUIT et al., 2010](#)). Both males and females feed on plant secretions and the honeydew

of aphids. In addition, the females suck blood from mammals, birds or reptiles, which they need for the maturation of their eggs. The larvae are terrestrial and develop in detritus of various kinds, decaying organic material from animal shelters, caves, crevices in rocks, cracks in the walls of old buildings and ruins, and other dark and humid habitats. The adults are mainly active on quiet evenings at dusk, and in the early hours of the night. Some species

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suck blood from humans and can thus transmit pathogens such as various phlebovirus species, bacteria (*Bartonella bacilliformis*) or *Leishmania* protists ([DEPAQUIT et al., 2010](#); [MAROLI et al., 2013](#)).

About 25 species of phlebotomine sandflies from the Old World, belonging to two genera (*Phlebotomus* and *Sergentomyia*), have been recorded in Europe so far ([MAROLI et al., 2013](#), [DVORAK et al., 2018](#)). In general, sandflies are considered typical Mediterranean dipterans, with a small local distribution area. Outside the European Mediterranean region, there are several records of sandflies in climatically favorable regions of Western, Central and Eastern Europe ([OERTHER et al., 2020](#)). However, the occurrence of sandflies in Central Europe has been the subject of extensive research in recent years. In 1999, sandflies were found for the first time in southwestern Germany, and further evidence in the following years confirmed the indigenous occurrence of sandflies in this country ([NAUCKE and PESSON, 2000](#)). These insects have also been found in Hungary, Austria, Slovakia and Slovenia ([FARKAS et al., 2011](#); [IVOVIC et al., 2015](#); [DVORAK et al., 2016](#); [KNIHA et al., 2020](#)).

The Mediterranean region is a recognized endemic focus for both leishmaniasis and phlebovirus infections ([WHO 1990](#)). The coastal region of Slovenia is short (43 km long), but given the Mediterranean climate factors, the presence of vector sandfly species, and the pathogen reservoirs, it is only a matter of time before more local cases of both human and canine leishmaniasis are reported. So far, only one case of indigenous canine leishmaniasis has been documented, in a dog from the border region between Slovenia and Croatia ([KOTNIK, 2020](#)). A 2018 study, involving 465 dogs from 10 different regions, found that nine (1.9%) tested positive with the ELISA test, while both PCR and IFAT tests gave negative results ([KOTNIK et al., 2021](#)).

Previous studies have shown the presence of some of the most important, conclusively incriminated and potential vector species, such as *Phlebotomus papatasi*, *P. neglectus*, *P. perniciosus* and *P. mascittii* ([IVOVIC et al., 2015](#)). *Phlebotomus*

*perniciosus* is the most important *L. infantum* vector in Western Europe ([ECDC, 2020](#)). *Phlebotomus neglectus* belongs to the *P. major* complex, which currently comprises five other species, widely distributed in the Old World ([CAZAN et al., 2021](#)). This species is the most important vector of visceral leishmaniasis (VL) in Eastern Europe ([DVORAK et al., 2018](#)). In the Mediterranean part of Europe, the species *P. papatasi* is mainly anthropophilic and is found in human dwellings ([MAROLI and BETTINI, 1997](#); [IVOVIC et al., 2015](#)). Nevertheless, it has also been caught in the vicinity of chickens or other domestic animals ([SCHLEIN et al., 1989](#)). *Phlebotomus mascittii* is mainly found in chicken coops and barns with the presence of horses, cats and dogs ([IVOVIC et al., 2015](#); [DVORAK et al., 2016](#); [OBWALLER et al., 2016](#)), but has also been collected in abandoned houses and other human dwellings, as well as in cemeteries, not only with CDC miniature light traps, but also with mosquito Bg sentinelTM and gravid traps (Biogents AG, Regensburg, Germany).

Diverse research initiatives have been undertaken to explore innovative approaches for monitoring and controlling various sandfly species, with a specific focus on employing chemical baits. Recent studies have highlighted the potential of volatile organic compounds (VOCs) emitted from dog hair as biomarkers for detecting the presence or absence of *L. infantum* infection ([MAGALHÃES-JUNIOR et al., 2014](#); [STANIEK and HAMILTON, 2021](#)). Moreover, these compounds may serve as indicators of disease intensity, offering insights into variations in parasitism corresponding to different clinical conditions.

Laboratory investigations have demonstrated the allure of exhaled compounds from foxes (*Vulpes vulpes*, Carnivora: Canidae) to *Lutzomyia longipalpis* ([DOUGHERTY et al., 1999](#)). Additionally, research has shed light on the attraction of *Lu. longipalpis* to the alcohols found in plants ([MAGALHÃES-JUNIOR et al., 2014](#)). Notably, studies involving *Lu. longipalpis* have revealed a preference for volatile compounds emitted by *L. infantum*-infected hamsters compared to their uninfected counterparts ([NEVATTE et al., 2017](#)).

In the context of this article, the aim was to delve into the shifts in the population dynamics of sandflies, and explore their potential correlation with the presence or absence of dogs, identified as the primary reservoirs of *Leishmania* parasites in the Mediterranean region.

### Materials and methods

To gain insight into the population dynamics and species composition of phlebotomine sandflies due to the presence/absence of reservoirs, these insects were collected in June and July 2017 and 2018 at two locations near the village of Cetore in the Slovenian coastal region (Fig.1). The first location (Medljan) is a tourist household with several species of domestic animals. Chickens, geese, ducks, donkeys, horses, rabbits and a

dog were present. At the other location (Cetore), in addition to chickens, there were also 4 adult hunting dogs on the farm, which were moved to another location at the beginning of 2018. The hunting dogs were kept in kennels in the backyard of the farm. The locations are about 700 m away from each other.

*Sampling and identification of phlebotomine sandflies.* The sandflies were collected using CDC miniature light traps without CO<sub>2</sub> bait, which were placed in or near animal shelters with dogs, rabbits and poultry. Three traps in each location were set in the evening and operated all night. The caught sandflies were collected early in the morning, sorted, and stored dry at -80°C.

The head and the rear part of the abdomen of all the collected sandflies were mounted on slides in

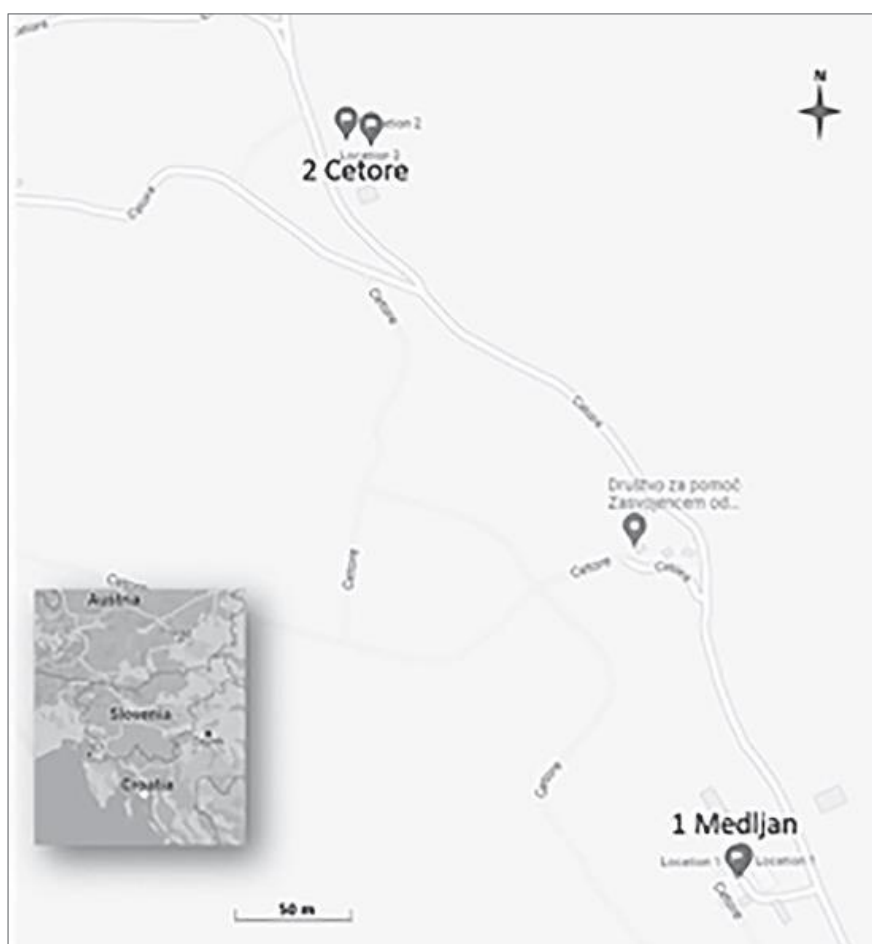


Figure 1. Map showing locations investigated 1. Medljan and 2. Cetore.

CMCP-10 High viscosity mountant (Polysciences, Inc.) and species identification was performed according to morphology-based keys ([PERFILIEV, 1966](#); [LEWIS, 1982](#); [ARTEMIEV and NERONOV, 1984](#); [ERISOZ KASAP et al., 2021](#)). The rest of the body was used for molecular identification of *Leishmania* parasites.

We obtained the data on the average and minimum monthly temperatures (T) as well as data on the average precipitation during the months in which the study was conducted from the archives of the meteorological station at Portorož Airport (<https://meteo.arso.gov.si/met/sl/archive/>), which is only 4 km away from both locations.

*DNA extraction and Leishmania identification.* All unfed females, 217 from both years, were tested

for the presence of *Leishmania* DNA. Fed females were excluded from the analysis, as the possible presence of *Leishmania* DNA in these specimens may be a direct consequence of blood feeding on an infected host. Regrettably, we could not ascertain the source of the blood meals in engorged female specimens. Genomic DNA was extracted from individual insects using the DNeasyBlood & Tissue Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions. A conventional PCR targeting the ITS-1 region and using the LITSR/L5.8S primers ([EL TAI et al., 2000](#)) was performed with a ready-to-use PCR master mix (Helixamp®T500N) to obtain sequence data for the positive sample. The PCR products were visualized in a 2% agarose gel stained with GelRed (VWR

Table 1. Nearest meteorological station (Portorož airport) data for monthly average T, average minimal T and rainfall in mm

Portorož airport	Aver. T (°C)	Aver. min. T (°C)	Rainfall (mm)
2017/May	17.6	12.2	39.9
2017/June	23.3	16.6	52
2017/July	24.2	17.6	30.9
2018/May	19.3	14.1	62.5
2018/June	22.5	16.7	78.3
2018/July	24.1	18.4	35.9

Table 2. Phlebotomine sandfly collection results from 2017; M for Medljan and C for Cetore

Species	<i>P. perniciosus</i>		<i>P. neglectus</i>		<i>P. mascittii</i>		<i>P. papatasi</i>	
	M	C	M	C	M	C	M	C
8.6. 2017	6	2	8	5	1	0	0	0
16.6.2017	4	2	11	2	0	1	0	0
30.6.2017	9	5	21	6	2	2	1	0
14.7.2017	17	3	23	5	2	2	2	0
27.7.2017	6	9	31	14	4	2	0	0
<b>Total</b>	93	40	148	53	21	12	4	1

Table 3. Phlebotomine sandfly collection results from 2018; M for Medljan and C for Cetore

Species	<i>P. perniciosus</i>		<i>P. neglectus</i>		<i>P. mascittii</i>		<i>P. papatasi</i>	
	M	C	M	C	M	C	M	C
4.6.2018	4	0	6	0	5	0	0	0
11.6.2018	4	0	9	0	3	1	0	0
29.6.2018	9	0	19	0	0	1	1	0
13.7.2018	14	0	23	0	3	2	0	2
27.7.2018	9	0	31	0	6	1	0	0
<b>Total</b>	<b>76</b>	<b>0</b>	<b>144</b>	<b>0</b>	<b>24</b>	<b>8</b>	<b>5</b>	<b>3</b>

International PBI, Milan, Italy) using a GelLogic 100 gel documentation system (Kodak, New York, USA).

*Statistical analysis.* The abundance of the species at two locations (Medljan and Cetore) in two consecutive years (2017 and 2018) was monitored, and possible differences in their abundance were tested between the two consecutive years for each of the two locations and for Cetore alone. For this purpose, Chi-square likelihood statistics were used, and significant differences were found at  $P < 0.05$  (Table 2). Likelihood statistics with standardized residuals ( $SR > |2|$ ) were used to determine the specific cells that contributed significantly to the overall Chi-squared test ( $P < 0.05$ ). Likelihood ratio (LR) statistics were used due to the small sample size, which in some cases resulted in expected frequencies of less than 1.

## Results

In 2017, we collected 372 sandfly specimens at the two locations, 266 at the first location and 106 at the second. Four species were identified on the basis of their morphological characteristics (Table 3). In 2018, 260 sandflies were collected at the two locations, 249 at the first location and 11 at the second location, with the difference that only two

species (*P. mascittii* and *P. papatasi*) were present at the second location (Table 4).

Molecular analysis revealed that all unfed female sandflies tested for the presence of *Leishmania* parasites were negative. Unfortunately, we did not obtain the consent of the dog owners to test the dogs for *Leishmania*.

*Statistics.* The statistical analysis of the data for both years showed that there was no significant difference between the 4 species at the first location (Mediljan;  $P = 0.678$ ). Nevertheless, as expected, the absence of *P. neglectus* and *P. perniciosus* at the second location (Cetore) in 2018 resulted in statistically significant differences between the two years analyzed ( $P = 0.001$ ). The analysis also showed that there was no statistical difference between 2017 and 2018 in the abundance of *P. mascittii* and *P. papatasi* at the second location (Cetore;  $P = 0.194$ ) (Table 3).

## Discussion

Scientists specializing in the study of sandflies, especially medical entomologists conducting field collections, are aware of the significant influence of climatic and microclimatic conditions on both the capture and emergence of these insects from their hiding places. In addition to precipitation

Table 4. Statistical analysis of the data for both years, 2017 and 2018, and both locations, Medljan and Cetore

	2017			2018		
	N	%	SR	N	%	SR
<b>Medljan, 4 species: LR = 1.518; df = 3; p = 0.678</b>						
<i>P. mascitii</i>	21	7.9	-0.5	24	9.6	0.5
<i>P. papatasi</i>	4	1.5	-0.3	5	2.0	0.3
<i>P. perniciosus</i>	93	35.0	0.6	76	30.5	-0.6
<i>P. neglectus</i>	148	55.6	-0.2	144	57.8	0.2
<b>Cetore, 4 species: LR = 1.518; df = 3; p = 0.678</b>						
<i>P. mascitii</i>	12	11.3	-1.4	8	72.7	4.5
<i>P. papatasi</i>	1	0.9	-1.4	3	27.3	4.3
<i>P. perniciosus</i>	40	37.7	0.6	0	0.0	-1.9
<i>P. neglectus</i>	53	50.0	0.7	0	0.0	-2.2
<b>Medljan, 2 species: LR = 1.518; df = 2; p = 0.392</b>						
<i>P. mascitii</i>	12	92.3	0.4	8	72.7	-0.4
<i>P. papatasi</i>	1	7.7	-0.8	3	27.3	0.9

and temperature, the daily activity of these insects appears to be determined not only by atmospheric pressure, which indicates impending changes in temperature and precipitation, but also by the presence or absence of suitable *Leishmania* reservoirs ([DVORAK et al., 2018](#)).

In general, it is difficult to determine from previous studies how the presence or absence of a host or reservoir affects the abundance of sandflies in an area. Some older studies suggest that in great gerbil (*Rhombomys opimus*) colonies in the southern parts of the former USSR (now Uzbekistan and Armenia), the abundance of *P. andrejevi* and *P. caucasicus* varies depending on the population density of the reservoir ([DERGACHEVA and ZHERIKHINA, 1974](#); [DERGACHEVA and OGANESIAN, 1987](#)). Studies on the population dynamics and distribution of sandfly species in a rural settlement in the Amazon region in Brazil indicate the importance of changes in human occupancy and vegetation management in rural settlements ([RODRIGUES RAMOS et al., 2014](#)). Regarding the two species that were absent from

the second location in 2018, *P. perniciosus* and *P. neglectus*, a study on the host preferences of sandflies in the hypoendemic focus of canine leishmaniasis in central Italy showed that *P. perniciosus* is an opportunistic feeder. The females fed on all available warm-blooded hosts, humans, dogs, horses, sheep and birds ([BONGIORNO et al., 2003](#)). A similar study in France also showed that the females of *P. perniciosus* had no particular preference for a specific host ([COTTEAUX-LAUTARD et al., 2016](#)).

As far as *P. neglectus* is concerned, the results of blood meal analyses to date have shown that this species is also an opportunistic feeder, and feeds on cattle, dogs, rabbits, chickens and humans ([VELO et al., 2005](#); [IVOVIĆ et al., 2010](#)). In Central Europe, including Slovenia, the females of the species *P. mascitii* often feed on chickens, as this species is most commonly found in chicken pens ([KNIHA et al., 2021](#)). The females of *P. papatasi* are also opportunistic feeders, and the results of blood meal analysis show that the highest percentage of the blood originates from the host species that



dominates at the collection site (SVOBODOVA et al., 2003; AZMI et al., 2020).

On the other hand, the results of this study showed radical changes in the presence and absence of the two main vector species. When the owners moved the hunting dogs to another location, we no longer caught *P. neglectus* and *P. perniciosus* at the Cetore location the following summer.

Since the early twentieth century, several studies have shown changes in the feeding behavior of *Leishmania*-infected sandflies, suggesting increased transmission of the parasite (ROGERS et al., 2002; HURD, 2003). The difficulty in obtaining a blood meal led to increased scrutiny, resulting in documented cases of multiple bite attempts by a single fly (HURD, 2003). Previous studies have shown that some sandfly species are attracted to volatile organic compounds (VOCs) from dog hair. Hence, the non-appearance of two confirmed vector species at Cetore in the second year of the study might also be linked to the absence or relocation of hunting dogs. Given the intriguing results of this study, it would be interesting to see if female sandflies are generally more attracted to dogs rather than other warm-blooded species, such as rabbits or poultry.

The climate data, which focused on temperature and precipitation, did not reveal any deviating values that could significantly influence sandfly species diversity. Despite the higher rainfall in June and July 2018, as shown in Table 1, recurrent rainfall during this period would be expected to affect the occurrence and abundance of the entire phlebotomine sandfly population at the two nearby locations, rather than affecting just two species at one location.

The statistical analysis of data for both years indicated no significant difference among the four species at the initial location (Medljan). However, as anticipated, the absence of *P. neglectus* and *P. perniciosus* at the second location (Cetore) in 2018 led to statistically significant variances between the two analyzed years.

## Conclusions

Sandflies were collected in June and July 2107 and 2018 at both locations, and the results obtained

showed significant changes in the composition of the population of phlebotomine sandflies in relation to the presence/absence of potential dog hosts.

## Declaration of competing interest

No potential conflict of interest was reported by the author.

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**SAŽETAK**

Sezonska i cirkadijalna dinamika populacije flebotomina odnosno papatača ovisi o mnogim čimbenicima okoliša, posebice o temperaturi i vlažnosti. No čimbenici koji utječu na fluktuacije u sastavu vrsta još su uvijek nepoznati. Tijekom redovitog praćenja člankonožnih prenositelja različitih patogena u slovenskom primorju uočena je zanimljiva promjena u populacijskoj dinamici ovih kukaca, ovisno o prisutnosti ili odsutnosti domaćih pasa. Kako bismo istražili promjene u sastavu vrsta, postavili smo klopke za odrasle papatače na dvije različite lokacije (Medljan i Cetore) u obalno-kraškoj regiji (Izola, Slovenija). Sakupljanja su obavljena u lipnju i srpnju 2017. i 2018. U obje godine na području istraživanja sakupljena su 632 primjerka četiriju vrsta (*Phlebotomus papatasi*, *P. neglectus*, *P. perniciosus* i *P. mascittii*). Ženke svih vrsta flebotomina pretražene su na prisutnost parazita lišmanije u obje godine, ali DNA lišmanije nije otkrivena. Preseljenje lovačkih pasa kao potencijalnih rezervoarskih domaćina iz Cetora na drugo mjesto početkom 2018. znakovito je promijenilo sastav vrsta papatača. Dvije vrste, *P. neglectus* i *P. perniciosus*, više nisu bile prisutne na ovom lokalitetu. Promjene u sastavu vrsta i dinamici populacije flebotomina vjerojatno su povezane s prisutnošću ili odsutnošću domaćina rezervoara lišmanija.

**Ključne riječi:** papatači; dinamika populacije; psi

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