

Stable populations of sandflies (*Phlebotominae*) in Eastern Austria: a comparison of the trapping seasons 2012 and 2013

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ABSTRACT

Till 2009, when the first sandfly (Diptera: Psychodidae: Phlebotominae) was found in Carinthia, Austria was believed to be free of sandflies. Then, in 2010, the occurrence of sandflies was reported for the first time, also in Eastern Austria. The present study was designed to investigate the density and the period of sandfly activity in this region. During July and August 2012 and 2013, sandfly trapping (476 and 284 trap nights, respectively) was performed at two capture sites in Eastern Austria where sandflies had been detected in 2010. Sandflies trapped with battery-operated CDC (Centers for Disease Control and Prevention) miniature light traps were transferred into 70% ethanol for identification based on morphological characters. In addition, temperature and precipitation data of the trapping regions were retrospectively obtained for the trapping season as well as for the respective previous months. In 2012, a total of 9 sandflies,

1 male and 8 females, were captured. In 2013, 20 specimens, 18 females and 2 males, were captured. All specimens were identified as *Phlebotomus (Transphlebotomus) mascittii* Grassi, 1908. The sandflies were found during the periods July 2nd to August 20th in 2012 and July 10th to August 13th in 2013. No significant correlation between numbers of catches and mean daily temperature was found. However, the mean monthly day temperature was only 24.2 °C in July 2012 compared to 25.9 °C in July 2013 when most specimens were captured. Of course, total numbers are very low. However, although the trapping period was shorter in 2013, more sandflies were caught than in 2012. The present data confirm the existence of stable populations of sandflies in Eastern Austria and suggest a tendency towards optimized conditions for sandfly occurrence in Eastern Austria. These results might become of considerable medical relevance with respect to a possible circulation of *Leishmania* spp. and phleboviruses.

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INTRODUCTION

Sandflies (Diptera: Psychodidae: Phlebotominae) are the vectors of several human pathogens, including *Leishmania* spp., *Bartonella* spp. and the phleboviruses. In Central Europe, the first records of sandflies were reported from Germany and Belgium, in 1999 and 2005, respectively. Austria was long believed to be free of sandflies. Then, in 2009, the first sandflies (*Phlebotomus mascittii*) were found in Southern Carinthia, close to the Slovenian border [1]. Recently, sandflies were also detected in Eastern Austria, however, only a few individuals were found [2]. The aim of the present study was to ascertain whether these sandflies captured in small numbers reflect stable populations and to investigate the density and period of sandfly activity at two capture sites in Eastern Austria.

MATERIALS AND METHODS

Two sampling sites located in Eastern Austria, in the federal states of Burgenland and Lower Austria, were chosen. These regions show a Pannonian climate with a continental influence, with hot summers and cold winters. The land area is mainly in agricultural use.

In July and August 2012 and 2013, entomologic field studies were carried out in Rohrau, Lower Austria (48°3'56.7" N, 16°51'33.1" E, 148 m altitude) and in Luising, Burgenland (47°0'40.0" N, 16°28'46.6" E and 47°0'45.9" N, 16°28'50.2" E, 233 m altitude). At both trapping sites several sandflies had already been found in 2011 [2]. Battery-operated CDC miniature light traps were run from dusk to dawn and emptied every morning. Trapped sandflies were transferred into 70% ethanol for conservation. For species identification, each specimen was cleared with potassium hydroxide solution and slide-mounted in Hoyer's fluid. The identification was based on morphological and morphometrical characteristics, mainly of the male genitalia and female spermathecae using the identification keys of Theodor and Seccombe [3, 4, 5].

In Lower Austria, trapping was performed with 8 traps for 34 nights (272 trap nights) from July 2nd to August 31st 2012. In 2013, sampling was repeated with 6 traps for 28 nights (168 trap nights) from July 9th to August 30th.

In Burgenland, the field study was performed with 6 traps for 34 nights (204 trap nights) from July 2nd to August 31st 2012. In 2013, trapping was repeated with 4 traps for 29 nights (116 trap nights) from July 19th to August 30th.

Temperature and precipitation data on the trapping regions were retrospectively obtained from the Central Institute for Meteorology and Geodynamics (ZAMG), for both trapping seasons as well as for the respective previous months.

RESULTS

Altogether, 29 sandflies were found at the two sampling sites, 9 in 2012 and 20 in 2013. All individuals found were identified as *Phlebotomus (Transphlebotomus) mascittii* Grassi, 1908 (Table 1).

In 2012, two females were captured in Burgenland, between July 24th and August 20th. The traps were located near human dwellings, in wooden barns, partly with concrete floor used for storing wood and hay. Further traps were placed in open garages with concreted floors used for placing harvesters and agricultural equipment. Close to the garages chicken were kept. In this area (Luising), the mean monthly day temperature from 7 a.m. to 6 p.m. in June was 23.6 °C and the mean night temperature from 6 p.m. to 7 a.m. was 17.2 °C. During the trapping season, the mean day temperature was 25.1 °C and the night temperature 18.2 °C in July, similar to August, with 25.2 °C and 17.5 °C, respectively. The amount of precipitation during the trapping season 2012, July and August, was 148.2 mm.

In Lower Austria, six females and one male were captured between July 2nd and August 7th 2012. The trapping location was a typical farmhouse of this region within the village of Rohrau, which is approximately 25 km from the Slovakian boarder. The residential house, stable and barn are connected around an inner yard, whereas the fourth side is closed by a gate wall. The traps were placed in an open garage with concrete pavement and in the nineteenth century-partially open wooden barn with loamy ground, used for storage of agricultural equipment and hay rolls. Horses, several cats and a dog were held on the farm. In this area, the mean monthly day temperature in June was 22.6 °C, while the night temperature was 17.8 °C.

Table 1. Capture sites, trap nights, time periods and climate conditions comparing trapping seasons 2012 and 2013 in Eastern Austria.

Capture site	Burgenland (Luising)		Lower Austria (Rohrau)	
	2012	2013	2012	2013
Captures	2 females	5 females	6 females 1 male	13 females 2 males
Trap nights	204	116	272	168
Trapping period	July 2-August 31	July 19-August 30	July 2-August 31	July 9-August 30
Capture period	July 24-August 20	July 19-August 13	July 2-August 7	July 10-August 8
Catches (July/August)	1/1	1/4	5/2	11/4
Mean monthly temperature in June (°C, day/night)	23.6/17.2	22.1/15.9	22.6/17.8	20.7/16.1
Mean monthly temperature in July (°C, day/night)	25.1/18.2	26.7/18.7	24.2/19.1	25.9/19.2
Mean monthly temperature in August (°C, day/night)	25.2/17.5	25.1/17.7	24.7/18.9	24.3/18.6
Precipitation (mm; June/July/ August)	64.8/116.2/32.0	48.7/10.2/70.9	47.3/129.7/92.2	64.3/0/78.8

During the trapping season, the mean day temperature was 24.2 °C and the night temperature 19.1 °C in July, similar to August, with 24.7 °C and 18.9 °C, respectively. The total precipitation during the trapping season in this area was 158.9 mm.

In 2013, five females were captured in Burgenland, between July 19th and August 13th. In June, the mean monthly day temperature was 22.1 °C and the night temperature was 15.9 °C. The respective temperatures in July were 26.7 °C and 18.7 °C, and in August 25.1 °C and 17.7 °C. The total amount of precipitation during the trapping season 2013 was 81.1 mm. In Lower Austria, 13 females and two males were captured between July 10th and August 8th 2013. Here, the mean monthly day temperature in June was 20.7 °C and the night temperature 16.1 °C. In July and August, the day temperatures were 25.9 °C and 24.3 °C, respectively, and the respective night

temperatures were similar to those in 2012 (Table 1). The amount of precipitation during the trapping season 2013 was 78.8 mm.

DISCUSSION

Of course, with 29 sandflies caught altogether, the numbers are very low. However, although the trapping period was shorter in 2013, more sandflies were caught than in 2012. Thus, the present data together with the first 14 sandflies caught in this region in 2011 [2] confirm the existence of stable populations of sandflies in Eastern Austria.

The first findings of sandflies in Central Europe were primarily attributed to climate change, however, today it is assumed that sandflies have been endemic in Central Europe since the Holocene climate optimum (about 6,500 years ago) and expanded from small climatically advantaged refugial areas [6, 7]. *P. mascittii* is a good example for this and the Viennese thermal

line in Eastern Austria, which forms a geological breakline to the Viennese Basin, might have acted as an advantaged refugium after the postglacial invasion of sandflies to Central Europe from Mediterranean glacial refugial areas. This geological fault line with thermal springs and relatively high tectonic activity has a mean annual temperature between 9 °C to 10 °C.

A recent study on dispersal abilities of sandflies with climatic prospects projected an establishment of sandfly species with a current south-eastern focus for the future, particularly of *P. neglectus* and *P. perfiliewi*, in eastern Austrian regions [8]. However, these two species were not found in the current study. For the current study, trapping sites close to the borders of Slovakia and Hungary were chosen. To our knowledge, no reports on sandfly occurrences exist for Slovakia, but for Hungary the occurrence of four different sandfly species has been confirmed, namely *P. neglectus*, *P. perfiliewi*, *P. mascittii* and *P. papatasi* [9]. *P. perfiliewi* and *P. papatasi* were only found in the southernmost regions of Hungary, but *P. neglectus* was found as far north as Budapest and *P. mascittii* was found also in Northern Hungary (approximately 100 km east of Lusing, the area studied here). *P. mascittii* in association with *P. perfiliewi* and *P. neglectus* was found in Southern Hungary close to the Croatian border. In that study, traps were set close to human dwellings where domestic animals were kept, similarly to the present study. However, in the current study, only *P. mascittii* was found, and always in low numbers, which has also been reported by other authors [1, 9]. In general, little is known on the biology of *P. mascittii*. In southern regions, *P. mascittii* usually occurs syntopically with proven vectors of visceral leishmaniosis, e.g. with *P. ariasi* and *P. perniciosus*, and it has been described as an anthropophilic and aggressive species [10, 11, 12].

Besides the optimal habitat, the presence of sandflies is mainly regulated by their requirements of temperature and humidity [13]. In 2013, most sandflies were caught in Rohrau (Lower Austria). Comparing the two seasons, in July 2013, 11 sandflies were trapped with a mean daily temperature of 25.9 °C, which was 1.7 °C higher

than in July 2012, when only 5 sandflies were found. The temperatures during the nights were similar in both years. Considering the precipitation, there was none in July 2013 and no rain on the capture dates in 2012. Altogether, no unequivocal pattern seems to emerge from the temperature and precipitation data; however, while day temperatures partly differed significantly, the mean night temperatures were very similar over the trapping seasons. In both years, sandflies were already caught on the first day of the trapping period, and the present data revealed an increase of the mean daily temperature by 0.5 °C-1.4 °C already in the month before the respective trapping periods, which was particularly evident for Burgenland. Thus, it might even be conceivable that sandfly activity in this area already starts in June. In Rohrau, the last sandflies were trapped in both years almost at the same date, on the 7th and 8th of August, respectively. These dates mark a temperature drop of the 24 h mean day temperature of 2.7 °C in 2012 and of 7.8 °C in 2013. According to Lindgren *et al.* [14] *P. mascittii* is tolerant to different climates, particularly cold climates with mean annual temperatures of < 20 °C. However, sandfly activity is not only driven by temperature, but also by humidity, particularly soil humidity at the breeding sites, which plays a significant role. In the regions investigated, precipitation is extremely low. Nevertheless, it is remarkable that in both years studied the mean temperatures in July were between 21.6 °C and 22.7 °C, which is very high for this region. A previous study had evaluated a mean July temperature of at least 18 °C for the occurrence of *P. mascittii* and of at least 20.7 °C for the occurrence of *P. neglectus* and *P. perfiliewi* [15].

CONCLUSIONS

The presented data confirm the existence of stable populations of sandflies in Eastern Austria and suggest a tendency towards optimized conditions for sandfly occurrence in this region. These results might become of considerable medical relevance with respect to a possible circulation of *Leishmania* spp. and phleboviruses. Therefore, monitoring of existing and possibly expanding sandfly populations should be taken into consideration, at least for specific areas.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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