

Emergence of sandflies (Phlebotominae) in Austria, a Central European country

Wolfgang Poepl · Adelheid G. Obwaller · Martin Weiler · Heinz Burgmann ·
Gerhard Mooseder · Susanne Lorentz · Friedrich Rauchenwald · Horst Aspöck ·
Julia Walochnik · Torsten J. Naucke

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Abstract The possible existence of autochthonous sandfly populations in Central Europe north of the Alps has long been excluded. However, in the past years, sandflies have been documented in Germany, Belgium, and recently, also in Austria, close to the Slovenian border. Moreover, autochthonous human *Leishmania* and *Phlebovirus* infections have been reported in Central Europe, particularly in Germany. From

2010 to 2012, sandfly trapping (740 trap nights) was performed at 53 different capture sites in Austria using battery-operated CDC miniature light traps. Sites were chosen on the basis of their climate profile in the federal states Styria, Burgenland, and Lower Austria. Sandfly specimens found were transferred to 70 % ethanol for conservation. Identification was based on morphological characters of the male genitalia and the female spermathecae, respectively. Altogether, 24 specimens, 22 females and 2 males, all identified as *Phlebotomus (Transphlebotomus) mascittii* Grassi, 1908, were found at six different sampling sites in all three federal states investigated. The highest number of catches was made on a farm in Lower Austria. Altogether, the period of sandfly activity in Austria was shown to be much longer than presumed, the earliest capture was made on July 3rd and the latest on August 28th. Sandflies have been autochthonous in Austria in small foci probably for long, but in the course of global warming, further spreading may be expected. Although *P. mascittii* is only an assumed vector of *Leishmania* spp.—data on its experimental transmission capacity are still lacking—the wide distribution of sandflies in Austria, a country thought to be free of sandflies, further supports a potential emergence of sandflies in Central Europe. This is of medical relevance, not only with respect to the transmission of *Leishmania* spp. for which a reservoir is given in dogs, but also with respect to the phleboviruses.

W. Poepl · H. Burgmann
Department of Infectious Diseases and Tropical Medicine,
Medical University of Vienna, Vienna, Austria

W. Poepl · G. Mooseder
Department of Dermatology and Tropical Medicine,
Military Hospital Vienna, Vienna, Austria

A. G. Obwaller
Division of Science, Research and Development,
Federal Ministry of Defence and Sports, Vienna, Austria

M. Weiler
Federal Ministry of Defence and Sports, Austrian
NBC-Defence-School, Korneuburg, Austria

S. Lorentz · T. J. Naucke
Parasitus Ex e.V., Niederkassel, Germany

F. Rauchenwald
Bayer Austria Ges.m.b.H., Vienna, Austria

H. Aspöck · J. Walochnik (✉)
Institute of Specific Prophylaxis and Tropical Medicine,
Center for Pathophysiology, Infectiology and Immunology, Medical
University of Vienna, Kinderspitalgasse 15, 1090 Vienna, Austria
e-mail: julia.walochnik@meduniwien.ac.at

T. J. Naucke
Department of Zoology, Division of Parasitology,
University of Hohenheim, Stuttgart, Germany

T. J. Naucke
Laboklin GmbH & Co. KG, Bad Kissingen, Germany

Introduction

Sandflies (Diptera: Psychodidae: Phlebotominae) serve as vectors of various pathogens, including *Leishmania* spp., *Bartonella* spp. and phleboviruses. In Europe, sandflies are typical Mediterranean faunal elements which are widely distributed mainly in southern parts of Europe, but also in extra-Mediterranean regions in Eastern and Western Europe

(Aspöck et al. 2008). The occurrence of sandflies in Central Europe north of the Alps has long been excluded. Then, in 1999, sandflies were recorded for the first time in the south-west of Germany (Naucke and Pesson 2000). In subsequent years, the autochthonous occurrence of sandflies in Central Europe was confirmed by further catches in Germany and Belgium. Recently, the occurrence of *Phlebotomus (Transphlebotomus) mascittii* was also documented in Austria, in Carinthia close to the border to Slovenia (Naucke et al. 2011). These recent sandfly catches together with autochthonous cases of leishmaniasis and *Phlebovirus* infections in Central Europe, particularly in Germany (e.g. Bogdan et al. 2001, Meyer-König et al. 2010), indicate spreading tendencies of the vectors within, as well as of sandfly-borne pathogens by anthropogenic influence towards Central Europe (Aspöck et al. 2008).

Aiming to investigate the possible occurrence of sandflies in isolated areas with favourable climatic conditions within regions previously considered free of sandflies, entomological field studies were performed in Southern and Eastern Austria.

Materials and methods

Austria is a landlocked, largely mountainous Central European country of 83,860 km² located within the alpine arch. The lowlands and plains of the east and southeast, however, are part of the Vienna basin and the Pannoni low country with continental climate characterised by hot summers and cold winters. Austria is bordered by the Czech Republic and Germany to the north, Hungary and Slovakia to the east, Slovenia and Italy to the south, and Switzerland and Liechtenstein to the west, and is divided into nine federal states.

In July 2010, July and August 2011 and 2012, entomologic field studies were carried out in Southern and Eastern Austria to investigate the possible occurrence of sandflies. Battery-operated CDC miniature light traps using fine gossamer collection bags (model #512, John W. Hock Company, Gainseville, Florida) were run from dusk to dawn. Bags were collected daily in the morning and the contents were stored at -20 °C until inspection. Sandfly specimens found were transferred to 70 % ethanol for conservation. For species identification, sandflies were cleared in potassium hydroxide solution, and finally, slide-mounted in Hoyer's fluid (Naucke 1998). The sandflies were measured with a binocular Olympus CH-2 with the help of a camera lucida. The identification was based on morphological and morphometric parameters of males and females, particularly, the morphological characters of the male genitalia and of the female spermathecae, using the identification keys by Theodor (Theodor 1958) and Seccombe (Seccombe 1993).

Altogether, sandfly trapping was performed at 53 different capture sites with a total number of 740 trap nights. The sample sites were chosen on the basis of a risk profile on

climatically most suited areas for the occurrence of sandflies in Austria (Aspöck et al. 2006). Oral informed consent for entomological sampling was obtained from all landowners and residents, respectively. All traps were installed at locations in the vicinity of human dwellings. The number of light traps and trap nights set at each location varied due to logistics and environmental factors and weather conditions.

In Styria, trapping was performed from July 12th to July 16th 2010 at 13 different trapping sites with a total number of 31 trap nights. Each site was examined for 1–2 nights, the number of traps used varied between 1–3 traps at each site.

In Burgenland, trapping was performed from July 18th to July 20th 2011 and for 17 nights between August 1st and August 26th 2011 at 32 different trapping sites with a total number of 203 trap nights. Each site was examined for 1–10 nights, the number of traps used varied between 2–6 traps at each site. The following year, sampling was repeated at one location with 6 traps run for 34 nights between July 2nd July and August 31st 2012 (204 trap nights).

In Lower Austria, trapping was performed at one trapping site with three traps for seven nights from August 16th to 19th and August 22nd to 26th 2011. The following year, sampling was repeated at this location with 8 traps run for 34 nights between July 3rd and August 31st 2012 (272 trap nights). An additional two traps were run on a second trapping site for 16 nights between July 3rd and July 23rd 2012.

Geographical data from trap sites were recorded with a global-positioning-system unit (Garmin, etrex VISTA HCx, USA). Temperature data on trapping regions were retrospectively obtained from the Central Institute for Meteorology and Geodynamics (ZAMG).

Results

Altogether, 24 sandfly specimens, all identified as *Phlebotomus (Transphlebotomus) mascittii* Grassi, 1908, were found at six different sampling sites in all three federal states investigated (Fig. 1). During the entire study period, the earliest capture was made on July 3rd and the latest on August 28th (Table 1).

In Styria, five females were found in Halbenrain (46°43'21.18" N/15°56'30.96" E at 216 m.a.SL) during trapping for two nights (July 14th to 16th 2010) with three traps. The traps had been placed under a concrete stair of a mill and a wooden shed used for storage within a windless group of buildings. A further female was found in Pfarrsdorf (46°42'27.84" N/15°57'51.12" E at 216 m.a.SL) during a trapping for two nights (July 14th to 16th 2010) with two traps. The traps had been installed in an open garage with concrete pavement near a human dwelling. Three females were captured on a third location in Ratzenau (46°43'35.04" N/15°49'4.26" E at 233 m.a.SL) during a trapping for two

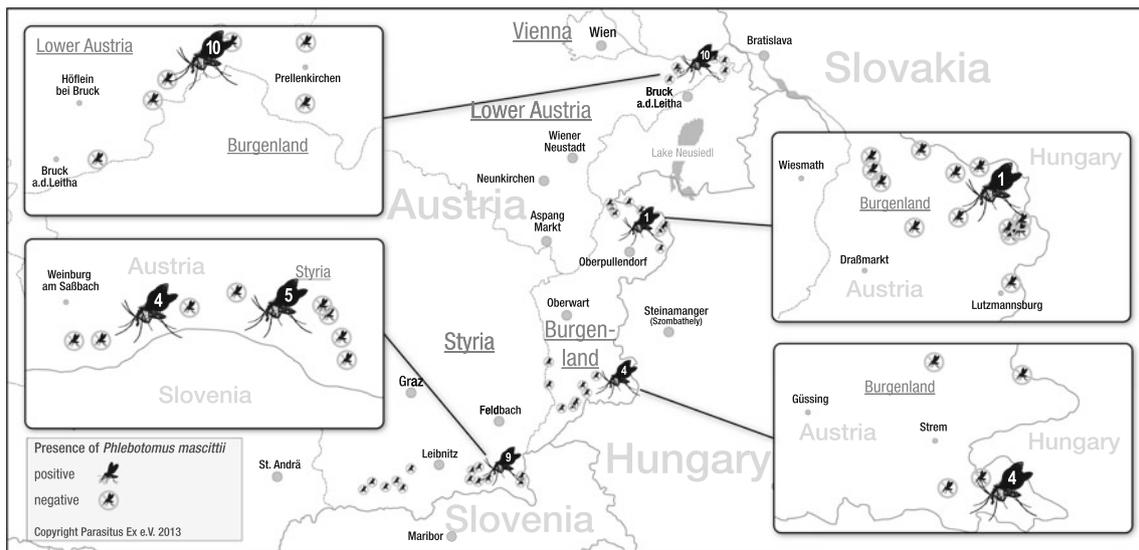


Fig. 1 Map showing capture sites in Styria, Burgenland and Lower Austria. Crosses indicate localities where no sandflies were found, the sandflies symbolise localities where *Phlebotomus* (*Transphlebotomus*)

mascittii Grassi 1908, was detected. The numbers within the sandfly symbols represent the total number of catches made in this area, overlapping capture sites are not shown

nights (July 14th to 16th 2010) with two traps. The traps were placed in a shed with loamy ground which was used as a lumber storage. In the area where those three villages with positive capture results are located, the mean monthly temperature in July 2010 was 18.9 °C at 7 a.m., 26.9 °C at 2 p.m. and 23.4 °C at 7 p.m.

In Burgenland, one female was found in Deutschkreutz (47°35'45" N/16°37'43.2" E at 196 m.a.SL) during trapping for a single night in August (August 2nd 2011) using three traps. The traps had been set up near human dwellings where no animals were kept. In the area of Deutschkreutz the mean monthly temperature in August 2011 was 18.5 °C at 7 a.m., 25.9 °C at 2 p.m. and 21.8 °C at 7 p.m. Further sandflies, namely one female and one male were captured in Luisling (47°0'40.0" N/16°28'46.6" E; 47°0'45.9" N/16°28'50.2" E at 233 m.a.SL), where four traps had been in use from July 18th to 20th and August 2nd to 6th. One female and one male were captured on the 3rd and 19th of August 2011, respectively. The trap sites were located near a human dwelling in a wooden shed with little organic matter on the floor, in a barn with concreted floor used as a storage for equipment and straw bales and in an open garage with concrete pavement used for storage of agricultural equipment and wood. Close to the garage, chicken were kept. In the area of Luisling, the mean monthly temperature in August 2011 was 17.5 °C at 7 a.m., 26.8 °C at 2 p.m. and 21.7 °C at 7 p.m. In Luisling, sandfly trapping was repeated 1 year later for 34 days between July 3rd and August 31st 2012 using six traps. Two females were captured, one on July 24th, the other one on August 20th. The mean monthly temperature in this area was 19.4 °C at 7 a.m., 27.7 °C at 2 p.m. and 23.0 °C at 7 p.m. in July 2012, and 17.7 °C at 7 a.m., 27.8 °C at 2 p.m. and 23.2 °C at 7 p.m. in August 2012.

In Lower Austria, three females were found in Rohrau (48°3'56.664"N/16°51'33.084"E at 148 m.a.SL) during a trapping period of six nights in August 2011 (from July 18th to 20th and from August 2nd to 6th) using three traps. The traps had been located in a nineteenth century open wooden barn used for storage of bales and agricultural equipment, and in an open, concreted garage used for storage. A dog and several cats were held at this farm. In this area, the mean monthly temperature in August 2011 was 18.5 °C at 7 a.m., 26.3 °C at 2 p.m. and 22.2 °C at 7 p.m. In Rohrau, sandfly trapping was repeated in the year 2012 for 34 nights between the 3rd of July to the 31st of August using six traps. Six females and one male were captured between July 3rd and August 7th. A female and a male captured in Rohrau are shown in Figs. 2 and 3, respectively. The mean monthly temperature in this area was 19.5 °C at 7 a.m., 26.6 °C at 2 p.m. and 22.7 °C at 7 p.m. in July 2012 and 19.2 °C at 7 a.m., 26.9 °C at 2 p.m. and 23.6 °C at 7 p.m. in August 2012.

Discussion

Until the first and hitherto unique finding of sandflies in Austria near the Carinthian/Slovenian border in the year 2009 (Naucke et al. 2011), Austria was believed to be free of sandflies. However, it had already been shown that only a moderate increase in temperature would lead to conditions favourable for the occurrence of sandflies in large parts of the country (Aspöck et al. 2006). The expected species were *P. mascittii* in western parts of Austria and *Phlebotomus neglectus* in the east (Aspöck et al. 2006). A more recent study investigating the responses of sandfly vector species to climate change in Europe

Table 1 Capture sites, time periods and results of entomologic field studies on the occurrence of sandflies in Eastern and North-Eastern Austria performed 2010–2012

Locality name (latitude, longitude, altitude*)	Captures	Capture date	Capture period	Mean monthly evening temperature (7 p.m.)	Trap site description
Burgenland					
Deutschkreutz (47°35'45"N, 16°37'43.2"E, 196*)	1 female	2 August 2011	2 August 2011	21.8 °C	Near human dwelling, No livestock, No companion animals
Luisling (47°0'40.0"N, 16°28'46.6"E, 233) (47°0'45.9"N, 16°28'50.2"E, 233)	1 male, 1 female 2 females	3 August 2011, 19 August 2011 24 July 2012, 20 August 2012	18–20 July 2011 1–26 August 2011 2 July–31 August 2012	21.3 °C 21.7 °C 23.0 °C/23.2 °C	Near human dwelling with chickens; (a) Wooden shed with organic matter on the floor; (b) Barn with concrete floor, (c) Open garage with concrete pavement used for storage
Lower Austria					
Rohrau (48°3'56.664"N, 16°51' 33.084"E, 148)	3 females 1 male, 6 females	24 August 2011, 28 August 2011 2 July 2012, 6 July 2012, 11 July 2012, 25 July 2012, 7 August 2012	16–19 August 2011 22–28 August 2011 3 July–31 August 2012	22.2 °C 22.7 °C/23.6 °C	Near human dwelling; dog and cats; (a) Wooden barn, (b) Open, concreted garage used for storage
Styria					
Halbenrain (46°43'21.18"N, 15°56'30.96"E, 216)	5 females	14 July 2010, 15 July 2010	12–16 July 2010	26.9 °C	Wooden shed near human dwelling
Pfarrsdorf (46°42'27.84"N, 15°57'51.12"E, 216)	1 female	14 July 2010	12–16 July 2010	26.9 °C	Open garage, concrete pavement near human dwelling
Ratzenau (46°43'35.04"N, 15°49'4.26"E, 233)	3 females	14 July 2010, 15 July 2010	12–16 July 2010	26.9 °C	Shed with soil bottom near human dwelling

*Altitude in metres above sea level



Fig. 2 A female of *Phlebotomus (Transphlebotomus) mascittii* captured in Rohrau, Lower Austria (Orig.)

by combining their dispersal abilities with climatic prospects, projected sandfly establishment of species with current south-eastern focus (*P. neglectus*, *P. perfiliewi*) in eastern Austrian regions neighbouring Slovenia and Hungary in the near future (Fischer et al. 2011). In that study it was further projected that *P. ariasi* and *P. mascittii* would disperse to south-eastern parts of Austria and would be able to occur in eastern and north-eastern parts at the end of the twenty-first century (Fischer et al. 2011). Thus, it appears surprising that in the present study (1) *P. mascittii* and not *P. neglectus* was found in Eastern Austria and that (2) *P. mascittii* has obviously already established also in the north-eastern parts of Austria. However, the cited projections were based on the assumption that Austria was free of sandflies, and sandflies would invade the country from neighbouring countries. Today, all records of sandflies in Central Europe, including Austria, are considered to represent remnants of warmer periods in Central Europe and not to be associated with a recent immigration due to climate change and



Fig. 3 A male of *Phlebotomus (Transphlebotomus) mascittii* captured in Rohrau, Lower Austria (Orig.)

global warming (Aspöck 2010). Nevertheless, these findings might reflect a range expansion from small refugial areas and studies on climate change scenarios and dispersal abilities of sandfly species suggest that “the development of Central European climate will increasingly support suitable habitats for phlebotomine sandflies” (Fischer et al. 2011).

P. mascittii is widely distributed in the Mediterranean region from Spain in the West to Turkey in the East. North of the Alps, it has been found in France, Switzerland, Belgium, Germany (Aspöck et al. 2008), and recently in the South of Austria (Naucke et al. 2011). Considering the wide distribution, the presence of such remnants of the Holocenian optimum about 6,500 years ago in very localised spots in Austria is not surprising (Aspöck 2008; Aspöck 2010).

Based on the results of the present study, it can be concluded that sandflies are more widely distributed (and most probably have been for long) in Central Europe than previously realised. Sandflies were captured at 6 out of 44 trapping sites and in most areas trapping was performed only for a short period of time (in some areas even for only a single night). Thus, the present study provides clear evidence for the permanent occurrence of sandflies in those areas, but care should be taken when considering areas with negative capture results as sandfly-free. Nonetheless, the total number of sandfly catches made was rather low. Even at catching sites where sandfly trapping was performed over a period of several weeks, only a handful catches were made. In line with this, in several entomological studies, *P. mascittii* was found at low densities (Berdjane-Brouk et al. 2011; Maroli and Bettini 1977; Veronesi et al. 2007). Since the first description of *P. mascittii* in 1908 (Grassi 1908), not more than 20 *P. mascittii* were trapped each season (Raynal 1954). There is only one place known in Europe, where *P. mascittii* can be found in high numbers, and this is a 450-m long and blocked railway tunnel on the island of Corsica (Toumanoff and Chassignet 1954). Adult *P. mascittii* can be found all through the year inside this tunnel (Naucke et al. 2008b). In Germany, a 200-year-old barn could be identified as a breeding place (Naucke 2002). Thus, to evaluate realistic population densities of *P. mascittii*, optimised catching methods are required. In a recent study from Northern Italy comparing sticky and sucking traps with or without light bait over a 4-month sampling period, only one out of 2,253 sandflies was identified as *P. mascittii* (Veronesi et al. 2007). Likewise, in an entomological study performed with CDC miniature light traps in Northern Algeria, only one individual of *P. mascittii* was found among 883 sandflies (Berdjane-Brouk et al. 2011). However, because a sandfly species is probably not survivable in such a low-population density, the number of catches made in the present study apparently does not reflect the real population density of *P. mascittii* in Austria. Our personal observation based on sandfly breeding experiences is that *P. mascittii* is less attracted by light in contrast to other sandfly species. Escaped

sandflies in the laboratory rarely can be trapped in a CDC light trap, but often can be found on apple or peach pieces.

P. mascittii is an assumed vector of *Leishmania* spp., but data on its natural or experimental transmission capacity are still lacking. Nevertheless, in Germany, *P. mascittii* was found as the sole sandfly species in areas with at least most probable autochthonous *Leishmania* infections in humans and animals (Naucke 2002; Naucke et al. 2008a). And, as it is known, that the active dispersal capability of sandflies is very low (e.g. Rioux et al. 1979), it has to be assumed that in these cases, the causative *Leishmania* strains had been transmitted by *P. mascittii*. Also in Austria, two cases with assumed autochthonous *Leishmania* infections have been reported (Beyreder 1965; Kollaritsch et al. 1989). Interestingly, these were both from the eastern low parts of Austria, which is the part of Austria, where now sandflies were found and also where in a recent epidemiological study investigating asymptomatic Austrian adults, the highest seropositivity for *Leishmania* spp. was found (Poepl et al. 2012).

When speculating on sandfly borne pathogens establishing in Austria, a prerequisite for autochthonous infections is not only the presence of a competent vector, but also of a pathogen reservoir. For *Leishmania*, dogs are certainly the most important reservoir hosts. In Mediterranean countries, *Leishmania* infections in dogs, and particularly in stray dogs, are very common (e.g. Brandonisio et al. 1992). Infected dogs usually develop conspicuous clinical symptoms leading to a miserable appearance, so that many infected stray dogs have been brought home by compassionate German and Austrian tourists. This has meanwhile even led to such dogs being offered for sale via the internet. According to conservative estimates about 20,000 dogs infected with *Leishmania* presently live in Germany (Naucke et al. 2008a). In dogs, therapy is usually more complicated than in humans, moreover, dogs are assumed to remain reservoirs even after successful therapy, and also asymptomatic dogs can pass on the infection to sandflies (Alvar et al. 2004, Ashford et al. 1998). Also in Austria, the percentage of infected dogs seems to be high, although until now, the number of dogs that have been investigated for *Leishmania* infection is still rather low (Edelhofer et al. 1995; Leschnik et al. 2008). But also horses, cats and other domestic animals are transported within and imported into Europe, and also these animals may play a role as reservoir hosts (Englund and Pringle 2003). Moreover, a *Leishmania* focus can be maintained by circulation of the parasites between sandflies and rodents.

The period of sandfly activity in Austria was shown to be rather long, long enough to allow *Leishmania* parasites to complete their life cycle in the vectors and following transmission to vertebrate hosts. Although at most sampling sites, traps were run for only a few days, sandflies were caught from early July to late August. Rising temperatures, particularly in summer, may lead to growing populations of autochthonous

sandfly species and also favour the introduction of other sandfly species.

Sandflies can also transmit other pathogens, particularly phleboviruses, which have to be kept in mind, because they are wide-spread in Southern Europe and a sandfly that might lack vector capability for *Leishmania* spp. may still be a potent vector for various phleboviruses. Autochthonous human infections with phleboviruses have recently occurred in Southern Germany (Meyer-König et al. 2010).

In conclusion, it was shown that sandflies are much more widely distributed in Austria than previously assumed, currently in small foci, but in the course of global warming, further spreading may be expected. This is of potential medical relevance, not only with respect to transmission of *Leishmania* spp., for which a reservoir is known to exist in Austria, but also of other pathogens, in particular phleboviruses.

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