First record of *Phlebotomus (Transphlebotomus) mascittii* in Slovakia

Vit Dvorak¹*, Kristyna Hlavackova¹, Alica Kocisova², and Petr Volf¹

¹ Charles University Prague, Faculty of Science, Department of Parasitology, Vinicna 7, Prague 2, 12844, Czech Republic
² University of Veterinary Medicine and Pharmacy, Institute of Parasitology, Komenskeho 73, Kosice 04181, Slovakia

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Abstract – A large-scale entomological survey was carried out in summer 2016 in the Czech Republic and Slovakia. It revealed, for the first time, the presence of the phlebotomine sand fly *Phlebotomus (Transphlebotomus) mascittii* Grassi, 1908 (Diptera: Phlebotominae) in south-western Slovakia. Species identification of a captured female was confirmed by both morphological and sequencing (COI) analyses.

Key words: Sand fly, *Phlebotomus mascittii*, Slovakia, Transphlebotomus, Northern limit.

Résumé – Première mention de *Phlebotomus (Transphlebotomus) mascittii* en Slovaquie. Une enquête entomologique à grande échelle a été réalisée durant l’été 2016 en République Tchèque et en Slovaquie. Elle a révélé, pour la première fois, la présence de *Phlebotomus (Transphlebotomus) mascittii* Grassi, 1908 (Diptera : Phlebotominae) dans le sud-ouest de la Slovaquie. Une femelle a été capturée et son identification repose sur des caractères morphologiques et moléculaires (COI).

Introduction

Phlebotomine sand flies (Diptera: Psychodidae) are vectors of several infectious pathogens including parasitic protozoans of the genus *Leishmania* and phleboviruses and are therefore of great importance in human and veterinary medicine [5, 11]. Although in Europe they occur typically in the Mediterranean countries, some species extend their range of distribution into regions north of their core areas [12]. As the presence of a vector species is one of the risk factors for *Leishmania* transmission [19], it is very important to study the limits of sand fly occurrence because their presence in areas at the edge of their distribution range may be overlooked. This study was conducted as part of the VectorNet project, which focuses on mapping sand fly presence in Europe, including the northern limits of their distribution. To pursue this objective, we surveyed southern parts of the Czech Republic and Slovakia for sand fly presence.

Materials and methods

A field survey to detect sand flies was conducted from July 6 to July 31, 2016 at 41 localities in south-eastern Slovakia, south-western Slovakia (localities from 9 counties) and southern Moravia, Czech Republic (localities from 2 counties) (Table 1). Moreover, collections of insects from past seasons (2012–15) in the same localities in south-eastern Slovakia, as surveyed in 2016 and stored in ethanol, were inspected under a stereomicroscope. Centers for Disease Control (CDC) light traps (John W. Hock) baited with CO₂ (dry ice) were placed mostly inside or close to animal shelters and/or organic material both on commercial farms and in private houses where no insecticide spraying was applied. New collection nets from the manufacturer were deployed to exclude possible contamination by sand fly specimens from previous field studies. The traps were set about 2 h before sunset and collected the next morning. Captured insects were killed by freezing in a polystyrene box with dry ice and manually inspected on a sheet of filter paper and under a stereomicroscope.

The sand fly specimen was transferred to 70% ethanol, head and genitalia were slide-mounted using CMCP-9 mounting medium (Polysciences) and the rest of the body was stored in ethanol for molecular analysis. Morphological identification was carried out using published keys and descriptions [4, 10]. Identification was confirmed by a sequencing analysis of the cytochrome oxidase I (COI) gene. Genomic DNA was isolated with a High Pure PCR Template Preparation Kit (Roche). PCR amplification of COI was performed in a 25 µL reaction volume, using the LCO1490/HCO2198 primer pairs and

*Corresponding author: vidvorak@natur.cuni.cz
| Locality     | County/Province | Country | Date       | No. of traps | Habitat       | Potential hosts                     | N (%) | E (%) | ASL |
|--------------|-----------------|---------|------------|--------------|---------------|-------------------------------------|-------|-------|-----|
| Trebejov     | Košice – okolie | Slovakia| 7.7.2016   | 4            | Horse farm    | Horses, poultry                      | 48.50 | 13'04.00 | 234 |
| Kosiceky     | Košice – okolie | Slovakia| 7.7.2016   | 5            | Village house | Poultry, pigs, rabbits               | 48.43 | 12'50.57 | 191 |
| Olsany       | Trebišov        | Slovakia| 8.7.2016   | 7            | Agricultural farm | Poultry, sheep, rabbits            | 48.31 | 12'57.75 | 175 |
| Velaty       | Trebišov        | Slovakia| 8.7.2016   | 3            | Village house | Poultry                        | 48.27 | 40'57.27 | 193 |
| Velka Trna   | Trebišov        | Slovakia| 8.7.2016   | 6            | Agricultural farm | Sheep, cattle                  | 48.30 | 35'55.94 | 124 |
| Michal'any   | Trebišov        | Slovakia| 8.7.2016   | 9            | Village houses | Poultry, rabbits, pigs              | 48.38 | 33'46.66 | 247 |
| Lipovany     | Lučenec         | Slovakia| 12.7.2016  | 3            | Agricultural farm | Cattle                     | 48.22 | 11'27.16 | 187 |
| Mulka        | Lučenec         | Slovakia| 12.7.2016  | 3            | Agricultural farm | Cattle                     | 48.16 | 26'37.37 | 182 |
| Trebeľ’ovce  | Lučenec         | Slovakia| 12.7.2016  | 3            | Village houses | Poultry, rabbits, cattle            | 48.17 | 02'50.52 | 182 |
| Laza         | Lučenec         | Slovakia| 12.7.2016  | 3            | Village house | Sheep                      | 48.17 | 30'75.55 | 172 |
| Dubovany     | Hlohovec        | Slovakia| 13.7.2016  | 3            | Village house | Poultry, pigs                   | 48.31 | 48'33.33 | 160 |
| Šalgocka     | Hlohovec        | Slovakia| 13.7.2016  | 8            | Village houses | Poultry, rabbits, pigs            | 48.20 | 39'64.64 | 140 |
| Limbach      | Pezinok         | Slovakia| 14.7.2016  | 3 + 2        | Village house | Sheep                         | 48.17 | 13'12.44 | 203 |
| Sváty Jur    | Pezinok         | Slovakia| 27.7.2016  | 3            | Agricultural farm | Dogs, horses            | 48.14 | 08'19.93 | 130 |
| Pernek       | Malacky         | Slovakia| 14.7.2016  | 5 + 7        | Horse farm   | Dogs, elephant                  | 48.21 | 06'54.59 | 257 |
| Jablonové    | Malacky         | Slovakia| 26.7.2016  | 2            | Dog kennel   | Dogs                         | 48.21 | 04'48.48 | 201 |
| Lozorno      | Malacky         | Slovakia| 26.7.2016  | 3            | Horse farm   | Dog kennel                     | 48.21 | 04'51.48 | 199 |
| Čierna Voda  | Senec           | Slovakia| 27.7.2016  | 3            | Horse farm   | Pigs, horses                   | 48.13 | 16'46.11 | 130 |
| Popice       | Znojmo          | Czech Rep.| 15.7.2016  | 4 + 4        | Village house | Goats, horses                | 48.49 | 05'40.20 | 287 |
| Havraníky    | Znojmo          | Czech Rep.| 15.7.2016  | 2            | Sheep pasture | Sheep                        | 48.49 | 09'36.26 | 231 |
| Naceradice   | Znojmo          | Czech Rep.| 15.7.2016  | 2            | Village house | Poultry                      | 48.49 | 09'51.18 | 231 |
| Jecmeniste   | Znojmo          | Czech Rep.| 15.7.2016  | 2            | Sheep pasture | Deer, wildlife                | 48.44 | 09'49.93 | 237 |
| Oblekovice   | Znojmo          | Czech Rep.| 15.7.2016  | 3            | Sandstone quarry | Sheep                       | 48.50 | 16'57.90 | 246 |
| Sobes,       | Znojmo          | Czech Rep.| 17.7.2016  | 8 + 8        | Organic vineyard | Wildlife                 | 48.48 | 58'36.05 | 283 |
| Podmolini    | Znojmo          | Czech Rep.| 17.7.2016  | 5            | Large cattle | Cattle, dogs                | 48.58 | 54'00.00 | 201 |
| Uherčice     | Znojmo          | Czech Rep.| 18.7.2016  | 5            | Village house | Goats, horses              | 48.48 | 45'81.39 | 182 |

(Continued on next page)
amplification conditions previously described [7]. The amplification products were separated and visualized on 1% agarose gel, purified using a High Pure PCR Product Purification Kit (Roche) and directly sequenced in both directions using the primers used for DNA amplification (ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit). The new COI sequence of the Ph. mascittii specimen from Slovakia (length 620 bp) was deposited in GenBank (Accession Number KX963380). It was blasted against the GenBank database for identification and then aligned and compared with sequences of Ph. mascittii (KX869078, KX981913–KX981916) downloaded from GenBank.

Results

Inspection of insects collected in previous seasons in south-eastern Slovakia did not reveal the presence of sand flies. Out of 41 localities surveyed in summer 2016, a single female sand fly was found in one locality, namely Pernek in Slovakia. This village is situated at the western slope of the Small Carpathians, a low mountain range that forms a part of the Western Carpathians mountain system (Fig. 1). The sand fly was trapped in a partly disused barn on a former cattle farm where only about 25 horses are bred at present (Fig. 2).

The specimen was identified as a female Phlebotomus mascittii by traditional morphological characters of the pharynx (Fig. 3) and genitalia. The obtained part of the COI gene sequence (GenBank KX963380) was blasted against the GenBank database and identified as Ph. mascittii. A constructed alignment of the sequence of the Slovak specimen with the above-mentioned sequences of Ph. mascittii from Slovenia confirmed the GenBank identification and revealed only a single polymorphic site at position 106.

Discussion

This study presents the first finding of phlebotomine sand fly Phlebotomus mascittii in Slovakia that adds to the several northernmost records of this species in Europe. The fact that it was this particular species is not surprising; it has been assumed that Ph. mascittii has a large range of distribution and it is present throughout most European countries of the Mediterranean basin [9] as well as adjacent areas north of this region, including sporadic findings in Belgium [4], Germany [13, 15], Austria [16] and Hungary [6]. A recent single record in Algeria also suggests its occurrence in North Africa [2]. Other species of the subgenus Transphlebotomus seem to have markedly more restricted distribution. However, a recent description of two new species of this subgenus, Phlebotomus killicki and Ph. anatolicus [8], raised the question of whether the widespread presence of Ph. mascittii may be partly due to these two previously unrecognised species and suggests that exact distribution of species within the genus Transphlebotomus has not yet been delineated unambiguously.

Our finding of Ph. mascittii in southern Slovakia confirms the presence of this species at the northern limit of subgenus Transphlebotomus distribution. This species was previously recorded in neighbouring countries Austria and Hungary. In Hungary, specimens of Ph. mascittii were sporadically recorded in Baranya county at the southern border with Croatia, in Veszprém county close to Lake Balaton and in Pest county in the suburbs of the capital Budapest in 2006–2009 [6]. The latter observation was supported theoretically by climate modelling, suggesting that the peri-urban environment at the outskirts of Budapest would be favourable for this species under certain scenarios [1]. Our survey, however, did not record any sand flies in areas close to the Slovak-Hungarian border. In Austria, Ph. mascittii was first recorded during entomological surveys in Carinthia (2009–2010), the southernmost region of the country neighbouring Slovenia [16] and thus very distant from our positive site in Slovakia. However, a more detailed survey in the following seasons (2012–2013) revealed small but stable populations of Ph. mascittii in localities in Styria, Burgenland and Lower Austria with the northernmost record in the village of Rohrau close to the capital Vienna and Austrian-Slovak borders [17]. This area, called Hundsheimer Berge, is in fact the southernmost extension of the Small Carpathians where our specimen of Ph. mascittii was collected. Future genetic comparison of Austrian and Slovak specimens should reveal whether they belong to one or two closely related populations. Interestingly, the specimen from Slovakia showed almost 100% identity with sequences of P. mascittii specimens from Slovenia in sequences
of COI, which is a mitochondrial marker often used in molecular systematics of sand flies [3].

Our knowledge of the biology, ecology and epidemiological significance of Transphlebotomus species in the transmission cycles of leishmaniases is incomplete and sometimes contradictory: while some authors have speculated that Ph. mascittii is autogenous and hence not important for Leishmania transmission [4], others assume that this species readily feeds on dogs and humans and it has been proposed as a potential vector of Leishmania infantum in several small foci of presumably autochthonous canine leishmaniasis in Germany [15]. More importantly, an ITS1 (internal transcribed spacer 1) real-time PCR assay recently revealed one female positive for L. infantum DNA among ten tested ungorged females of Ph. mascittii caught in Austria [18]. However, experimental infections of this species have not yet been studied. It is also unresolved whether Transphlebotomus species share similar habitats with other sand fly species or inhabit special niches. While one of the newly described species, Ph. anatolicus, was collected in typical sand fly habitats near domestic animals [8], other Transphlebotomus species are represented in low numbers in usual sand fly surveys, and Ph. mascittii was recorded mainly from cavernicolous habitats [14]. The disused barn found positive in our study may simulate this type of habitat. Curiously, one Asian elephant (Elephas maximus) belonging to a commercial circus company was also kept close by, although a CDC trap which was placed near to it did not reveal any sand fly specimens.

Our single finding suggests that detailed entomological survey is needed to elucidate the extent of sand fly presence in the region of southern Slovakia, northern Austria and Hungary, as their eventual establishment may have implications concerning possible future transmission of canine or human leishmaniases.

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