CHARLES UNIVERSITY IN PRAGUE
Faculty of Science
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Intraspecific variability of *Phlebotomus sergenti*, a major vector of *Leishmania tropica*

Vít Dvořák

Ph.D. thesis

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Prague 2008

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2003-present	postgraduate study: Department of Parasitology, Faculty of Science, Charles University, Prague Thesis: „Intraspecific variability of *Phlebotomus sergenti*“

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Grant projects and financial support:

Grant Agency of The Charles University:

„Intraspecific variability of *Phlebotomus sergenti*“, GAUK 182/2005, Author (2005-2007)

„Intraspecific variability and molecular taxonomy of phlebotomine sand flies of subgenus *Larroussiu*s, important vectors of human leishmaniosis“, GAUK 268195/2008, Author (2008-2010)

Ministry of Education, Youth and Sports:

„Centre of molecular ecology of vectors and pathogens, LC06009, coordinator J. Kopecký

Mobility Fund of Charles University in Prague:

a partial funding of stay in laboratory of Dr. Gabriele Schönian, Institute of Microbiology and Hygiene, Humboldt University, Berlin, Germany

Socrates/Erasmus Programme:

a funding of stay in laboratory of Dr. Gabriele Schönian, Institute of Microbiology and Hygiene, Humboldt University, Berlin, Germany

Introduction

Phlebotomus sergenti is the main vector of *Leishmania tropica*, a causative agent of human cutaneous leishmaniasis. This disease is endemic in 82 countries and some 10 million people suffer from it with about 1 million of new cases occuring each year in an increasing trend. Therefore, *Phlebotomus sergenti* is a sand fly species of great medical importance. Originally described according to material from Algeria in 1917, it has a very broad range of distribution which covers areas of Southern Meditteran (Morroco, Algeria, Tunisia), Northern Mediterran (Portugal, Spain, Sicilly), Middle East, Arabia, Afghanistan, Pakistan and northern parts of India. In case of such a broad distribution we can expect a notable intraspecific variability.

An intraspecific study using the internal transcribed spacer 2 (ITS2) individualised two branches: one related to the north-eastern Mediterranean area (Cyprus, Pakistan, Syria and Turkey), the other being South and West of the first one (Egypt, Morroco, Israel). These groups may differ in ecology, host preferences and possibly also in vectorial capacity

It seems judicious to consider the potential existence of sibling species, as was proven by means of molecular biology in case of *Lutzomyia longipalpis* , where different cryptic species were established. If sibling species within *P. sergenti* were proven, it would have important implications in diagnostics and epidemiology as well as human medicine.

Objectives

- to hybridise males and females of *P. sergenti* from two colonies of different geographical origin (Turkey and Israel) to examine a possible reproductive barrier among hypothetized sibling species
- to compare these two laboratory colonies by RAPD analysis and compare the results with those obtained by geometric morphometry analysis performed by a collaborating laboratory
- to test RAPD as a method of choice for the comparison of sand flies from different foci of leishmaniasis
- to test cross-applicability of microsatellite markers developed so far for phlebotomine sand flies and if not applicable, design a unique panel of species-specific microsatellite markers for *P. sergenti*
- to compare wild specimens of *P. sergenti* from different populations within the broad range of distribution by several molecular markers: RAPD, sequencing of ITS2 (nuclear marker) and sequencing of cytochrome b (mitochondrial marker). Compare obtained results with the results of geometric morphometric analysis performed by a collaborating laboratory

Results

1. We demonstrated that crossing is possible between *P. sergenti* laboratory-reared specimens from colonies originating from Turkey and Israel. Succesful mating and insemination was observed and viable hybrid F1 and F2 offsprings were obtained from both parental combinations. No statistically significant difference was found in the egg production of the hybrides when compared to the parents.

2. RAPD analysis was able to distinguish clearly between members of Turkish and Israeli colony. When F1 progeny obtained from the cross-mating study were included in the RAPD analysis, these samples formed a distinct group with position intermediate between Turkish and Israeli subgroup, not only sharing a portion of bands with each of the parental colonies but also exhibiting several unique loci. The results of RAPD analysis were in accord with geometric morphometric analysis of wing shape of *P. sergenti* from Turkey and Israel, demonstrating that both molecular biology and morphological approaches are complementary.

3. RAPD proved to be a useful method for identification and comparison of *P. sergenti* specimens from two foci of cutaneous leishmaniasis in northern Israel which differ in the vector.

4. No microsatellite markers previously isolated for other phlebotomine species were found applicable on *P. sergenti*. A development of species-specific microsatellite markers panel was started and two suitable markers were identified so far. More markers are still under development.

5. Wild populations of *P. sergenti* from Turkey, Israel, Syria, and Usbekistan were analysed by three different molecular methods: RAPD, sequencing of ITS2 (nuclear marker), sequencing of cytochrome b (mitochondrial marker).
RAPD analysis of samples from several localities in Turkey and Israel revealed a same grouping pattern; sand flies from each country formed their own clade, one containing all field samples originating from Turkey as well as a specimen colony of Turkish origin, second containing all field samples from Israel plus the Israeli colony specimen. Similar pattern of clades was also obtained with samples from Syria and Usbekistan; specimens from each country formed a unique clade. There was no distinct grouping within the Turkish clade,

although the localities are separated by geographical distance and also by Amanos mountain range of a considerable heigth. Obtained results suggest that these mountains do not represent a sufficient barrier for sand fly dispersion and the mountain passes play a role of transitional gaps which allow a gene flow between the populations.

Results of ITS2 rDNA sequencing corroborated the previously published intraspecific division of *P. sergenti* into two branches, north-eastern and south-western. The specimens from Usbekistan fall within the north-eastern clade, close to the samples from Pakistan, Cyprus and Lebanon. Syrian samples and Turkish samples also cluster in this clade, while Israeli samples fall within the second, south-western clade.

Sequencing analysis of cyt b mtDNA revealed that haplotypes from Turkey, Israel, Syria, and Usbekistan formed three lineages, one containig specimens from Turkey and Israel together. This finding questions the idea of *P. sergenti* species complex.

List of publications

Papers:

Vít Dvořák, A. Murat Aytekin, Bulent Alten, Soňa Škařupová, Jan Votýpka and Petr Volf. 2005. **Intraspecific variability of *Phlebotomus sergenti* Parrot, 1917 (Diptera: Psychodidae)**. Journal of Vector Ecology 31 (2), 229-238.

Milena Svobodová, Jan Votýpka, **Vít Dvořák**, Jitka Pecková, Tap Meier, Julia Sztern, Abdelmageed Nasseredin, Lionel Schnur, Petr Volf and Alon Warburg. 2006. **Distinct Transmission Cycles of Leishmania tropica in 2 Adjacent Foci, Northern Israel**, Emerging Infectious Disease 12 (12), 1860-1868.

Milena Svobodová, Bulent Alten, Lenka Zídková, **Vít Dvořák**, Jitka Hlavačková, Jitka Myšková, Veronika Šeblová, Ozge Erisoz Kasap, Asli Belen, Jan Votýpka and Petr Volf. 2008. **Cutaneous leishmaniasis caused by *Leishmania infantum* transmitted by *Phlebotomus tobbi***, International Journal for Parasitology, in press.

Vít Dvořák, Jan Votýpka, A. Murat Aytekin, Bülent Alten and Petr Volf**.** 2008. **Intraspecific variability of natural populations of *Phlebotomus sergenti*, the main vector of *Leishmania tropica***, Acta Tropica, submitted.

Abstracts:

V. DVOŘÁK, A. M. AYTEKIN, B. ALTEN, S. ŠKAŘUPOVÁ, P. VOLF, J. VOTÝPKA: Discrimination between P*hlebotomus sergenti* from Turkey and Israel. 2005. ISOPS V (5th International Symposium on Phlebotomine Sandflies), Gammarth, Tunisia.

V. DVOŘÁK, A. M. AYTEKIN, B. ALTEN, S. ŠKAŘUPOVÁ, P. VOLF, J. VOTÝPKA: Intraspecific variability of *Phlebotomus sergenti*: comparison of two laboratory colonies. 2006. European SOVE (Society of Vector Ecology) meeting, Serres, Greece.

V. DVOŘÁK, A. M. AYTEKIN, O. ERISOZ, B. ALTEN, P. VOLF, J. VOTÝPKA: Intraspecific variability of *Phlebotomus sergenti*: comparison of Turkish and Israeli laboratory colonies and wild populations. 2008. European SOVE (Society of Vector Ecology) meeting, Cambridge, Great Britain.

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