Leishmania transmission by sand flies (Diptera: Phlebotominae)

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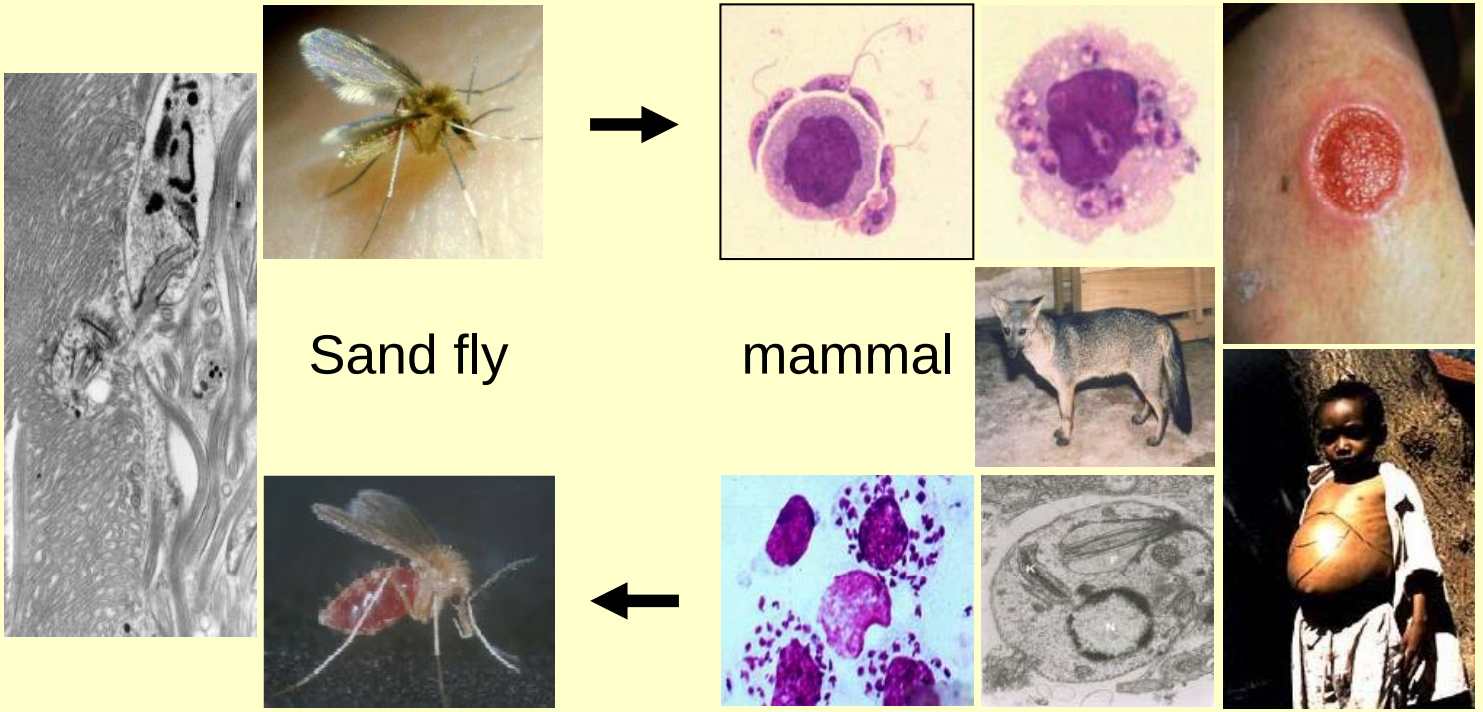



EDENext
Biology and control of vector-borne infections in Europe

EDENext country
EDENext team
2011-2015

Publication activity of sand fly group within EDENext (October 2015):

92 publications in peer-reviewed journals published, **5** accepted, **8** under revision

EDENext: Phlebotome-borne diseases group (PhBD)

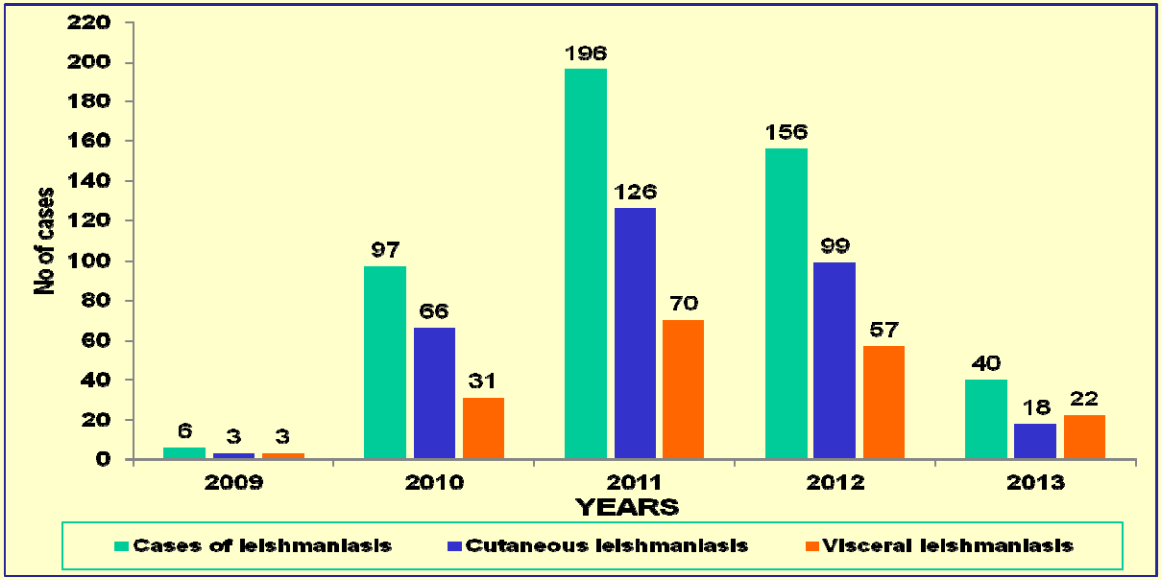
CUNI is coordinator of the PhBD group „Biology and control of sand fly-borne diseases“.

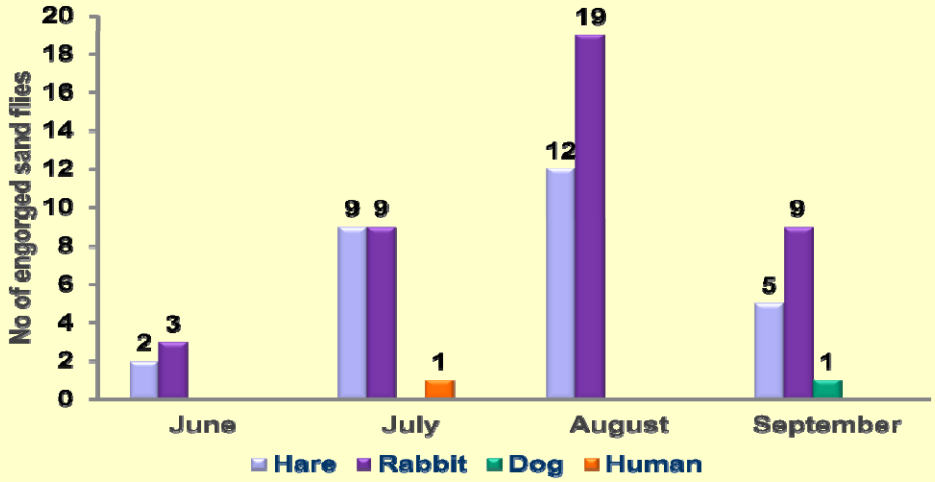
The team is dedicated to following research topics:

- Immunomodulatory effect of sand fly saliva and studies of anti-sand fly saliva antibodies as a tool for monitoring sand fly-host contact.
- Experimental studies on the susceptibility of various sand flies to *Leishmania*.
- Spread of strains and hybrids of the *L. donovani* complex in the Mediterranean.
- The distribution of sand fly species in Mediterranean area and their molecular identification.

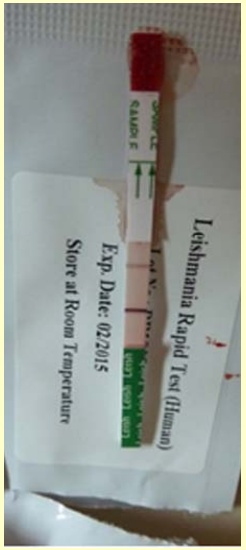
New focus of human leishmaniasis in Madrid

Local epidemy of human cases in urban area of Madrid due to *Leishmania infantum* transmitted by *Phlebotomus perniciosus,* reservoir: Iberian hares (*Lepus granatensis*) and rabbits (*Oryctolagus cuniculus*) abundant due to environmental management of the area



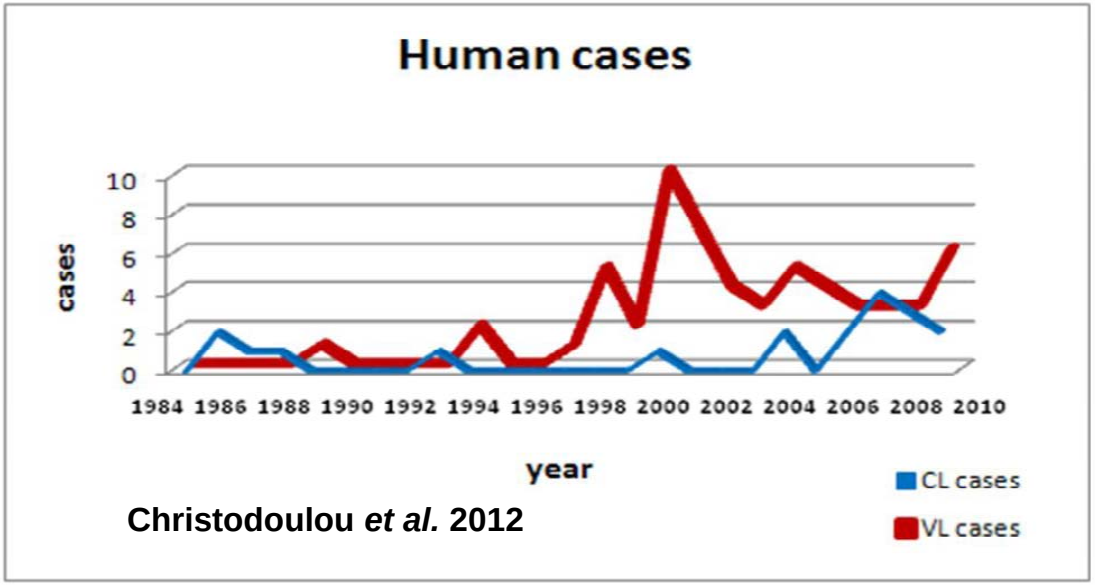


Molina *et al.* 2012, Jimenez *et al.* 2013, 2014

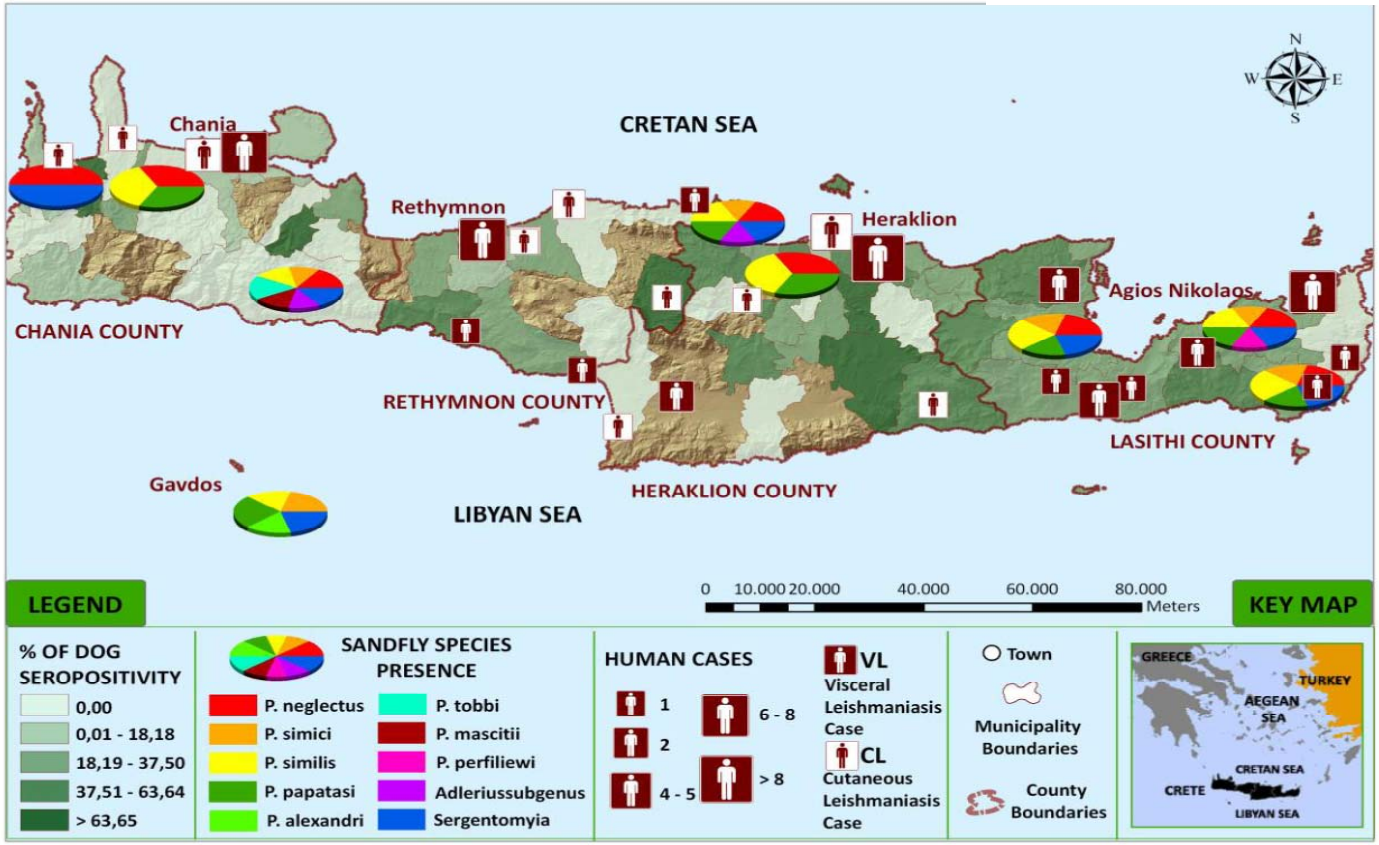


Reactivation of *L. tropica* focus in Crete

L. tropica common in Crete after WW2, erradicated due to anti-malaria campagne.
New cases since 2000, most patients above age of 60, relapses.
3 zymodemes: MON 58 new for Crete, isolated from both Afghan refugee and a local dog, autochtonous transmission!



Christodoulou *et al.* 2012



Ntais *et al.* 2014

Conjunctival swab as a new diagnostic tool for leishmaniasis

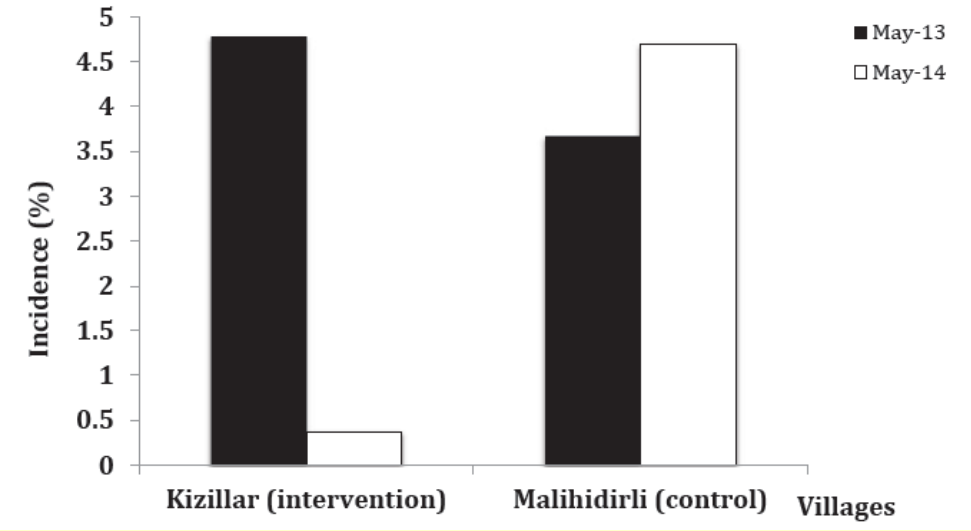
Test performed		IFAT (cut-off 1:160) Positive	IFAT (cut-off 1:160) Negative	% LR+ (95% CI)	% LR- (95%CI)	K value
CS n-PCR	Positive	39	16	71.6% (61.1-80)	92.2% (89.2-94.6)	0.75
	Negative	15	183			
BC n-PCR	Positive	19	3	50% (34.1-65.9)	91,2% (87.4-95)	0.70
	Negative	35	196			
LN-CE	Positive	36	0	80% (68.3-91.7)	95.7% (92.9-98.4)	0.76
	Negative	18	199			

Comparison of IFAT and CS n-PCR, BC n-PCR and LN-CE in the CanL diagnosis

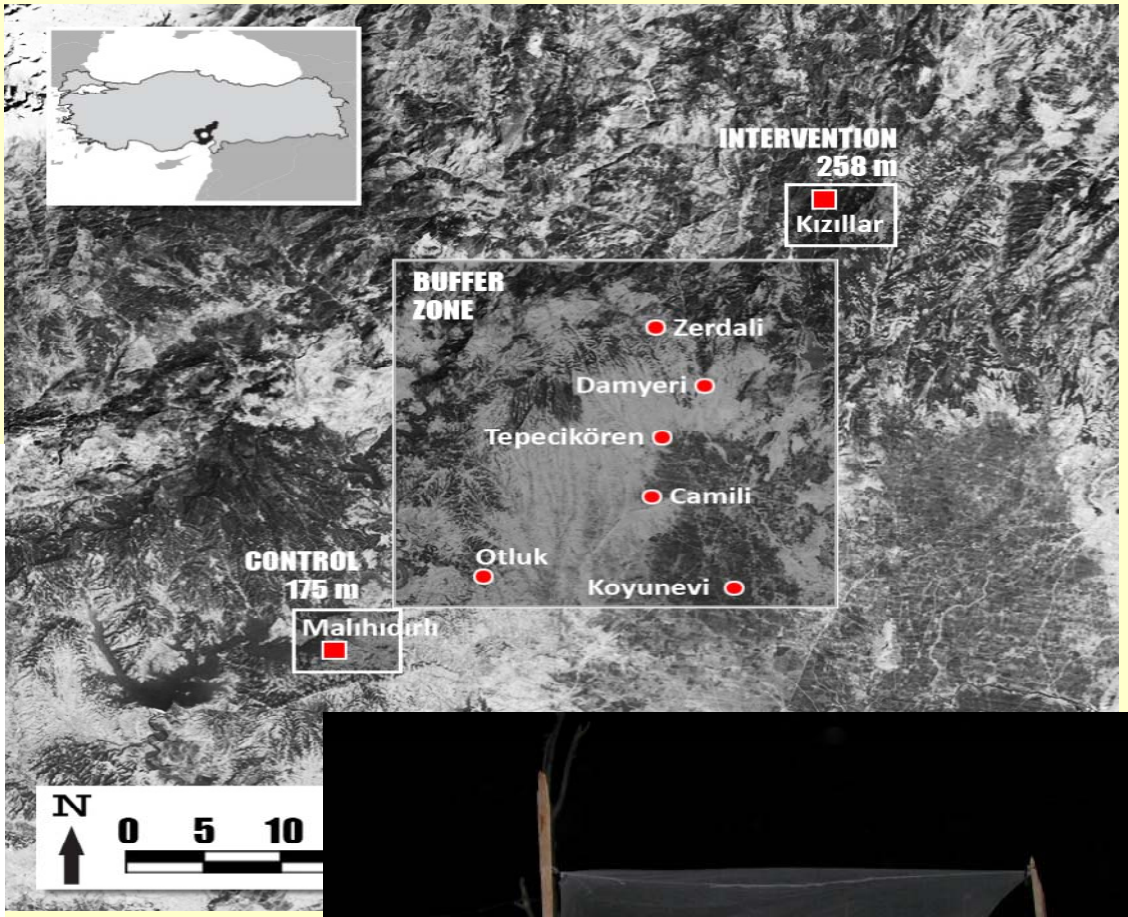
- a non-invasive assay for detecting *Leishmania* infections in dogs and cats
- the best relative diagnostic performance in a large number of randomly surveyed dogs
- validated in several European endemic countries (Italy, Portugal, Turkey, Georgia)
- enables detection in early stage of infection or at the onset of clinical signs
- proposed as a first-line approach for the detection of *Leishmania* infections in Europe.

Evaluation of the protective efficacy of ITNs in a focus of cutaneous leishmaniasis in Turkey

A village-scale trial conducted in Cukurova Plain, Turkey, a focus of CL caused by *L. infantum*/*L. donovan*i hybrid.
Vector species *Phlebotomus tobbi.* Significant decrease of CL cases in village with Olyset® Plus ITNs.

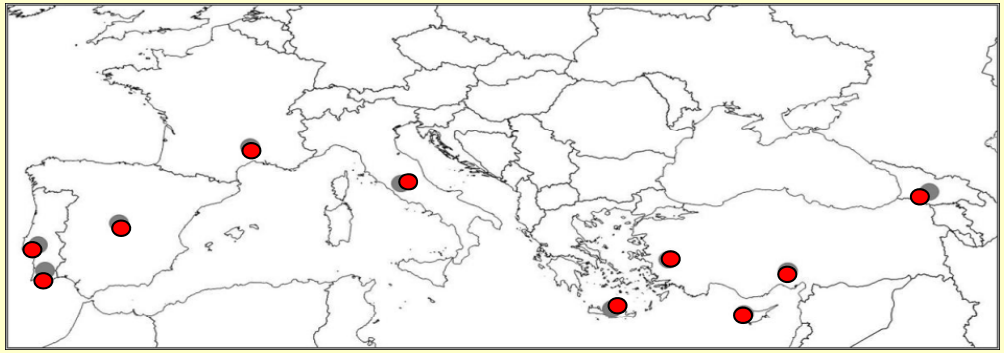


The annual changes of cutaneous leishmaniasis incidence (%) in intervention and control villages between May, 2013 and May, 2014. **Gunay et al. 2014**

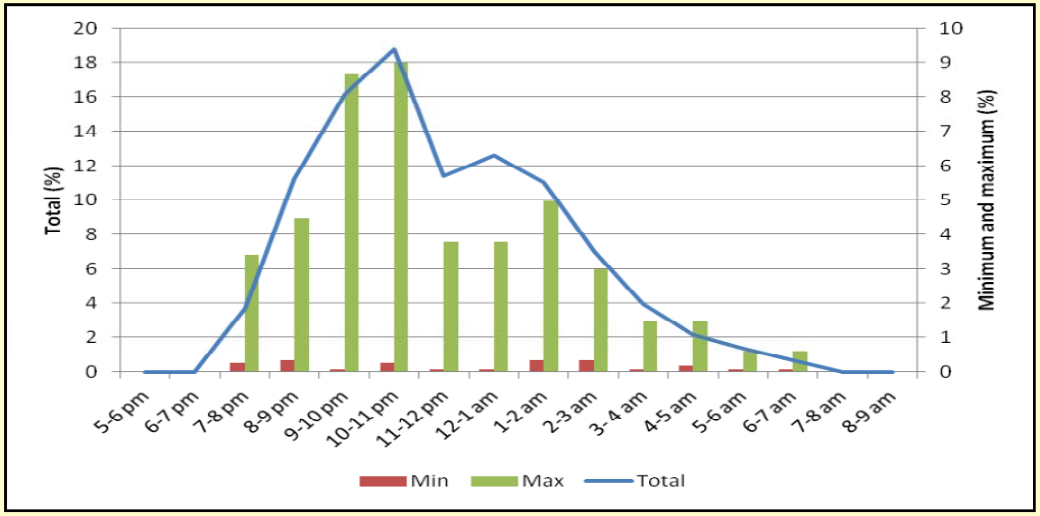


Seasonal dynamics of sand fly vectors of Mediterranean leishmaniasis caused by *Leishmania infantum*

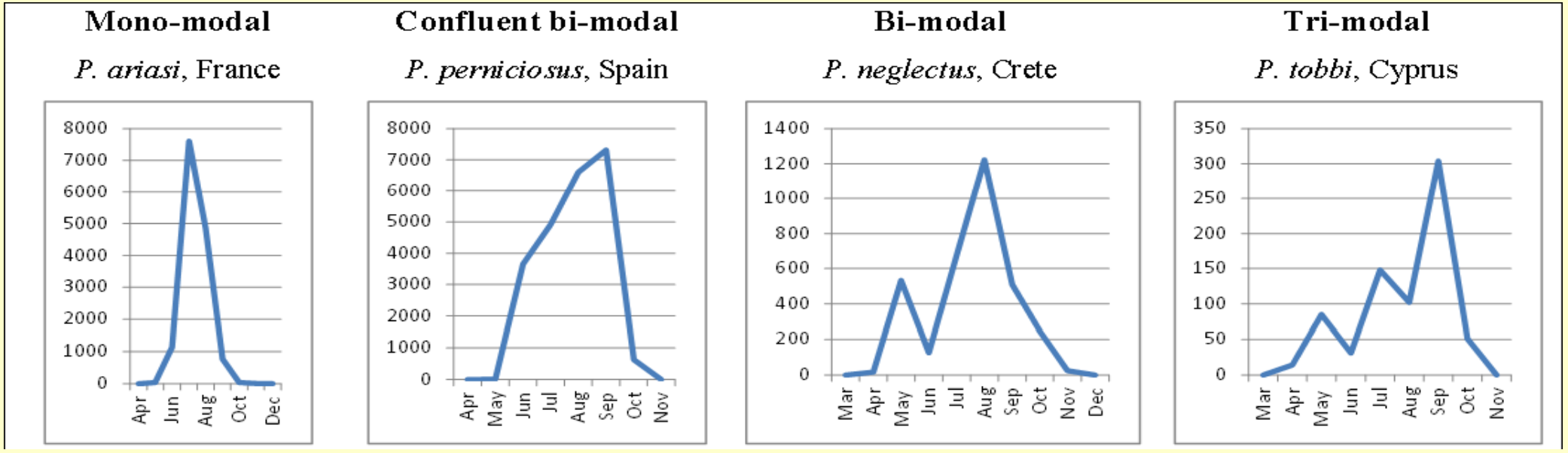
Sand fly seasonal dynamics and nocturnal acitvity were studies at 10 sites in 8 countries.
Significant correlation of seasonal dynymics with the latitude.



Hourly abundance of *L. infantum* vectors. Cumulative data from 6 sites.



Types of abundance trends recorded in 2011-2013 for Mediterranean sand flies.

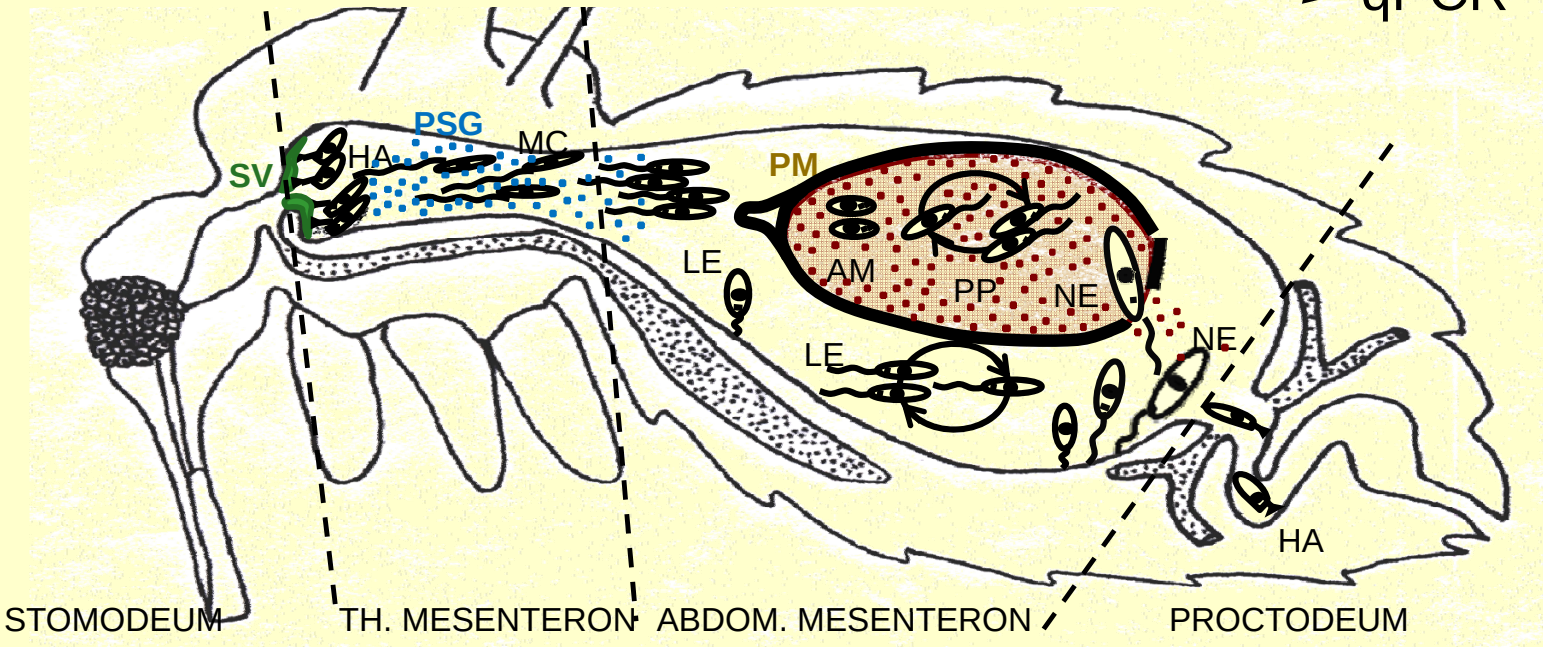


Leishmania development in sand flies and transmission to the host

Experimental infections through membranes with known number of promastigotes or amastigotes.
Dissection in various intervals (days) postinfection:

Light microscopy

qPCR



1. Development inside bloodmeal surrounded by the peritrophic matrix
2. Migration from endoperitrophic space and attachment to midgut epithelium
3. Anterior migration to thoracic midgut and attachment to stomodeal valve
4. Transmission to the host

Sand fly distribution and molecular methods for sand fly identification

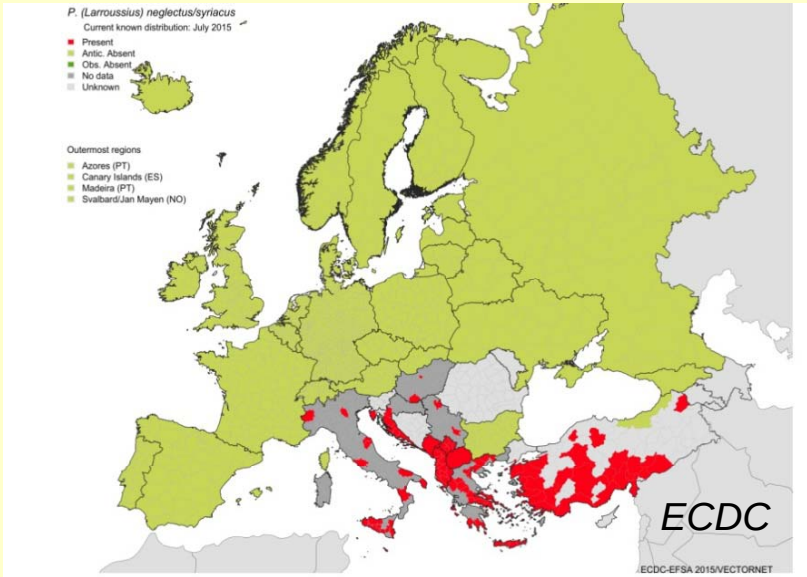
About 20 species of the genus *Phlebotomus* in Europe, proven or suspected vectors of *Leishmania*

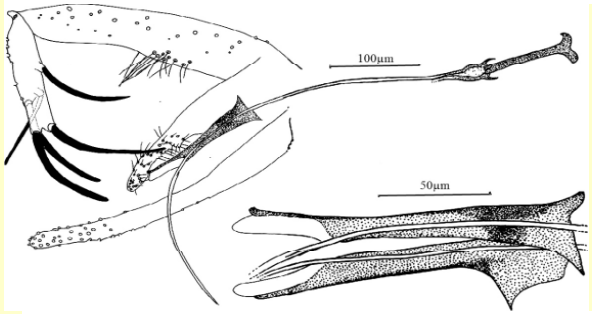
subgenus *Phlebotomus*: *P. papatasi*
subgenus *Paraphlebotomus*: *P. sergenti, P. similis*
subgenus *Larroussius*: *P. ariasi*, *P. perniciosus*, *P. tobbi*, *P. major* s.l., *P. perfiliewi* s.l., *P. kandelakii*, *P. longicuspis*
subgenus *Adlerius*: *P. balcanicus*, *P. simici*
subgenus *Transphlebotomus*: *P. mascittii*, *P. economidesi, P. anatolicus, P. killicki*

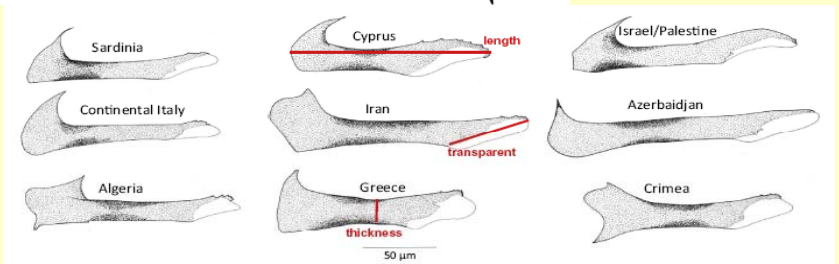
Current distribution maps based on previously published data. Detailed knowledge of recent distribution is missing.

Many species challenging for classical identification, constituting species complexes

A need for molecular approaches in sand fly species identification and distribution mapping





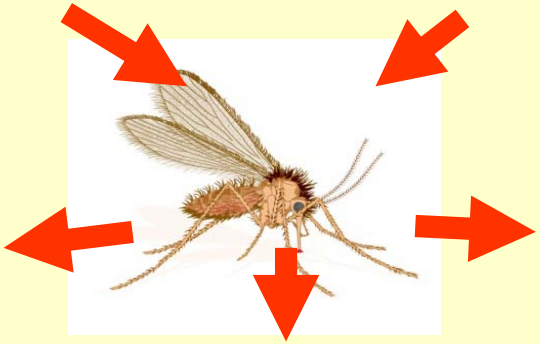


Ph. perfiliewi complex (Depaquit et al. 2013)

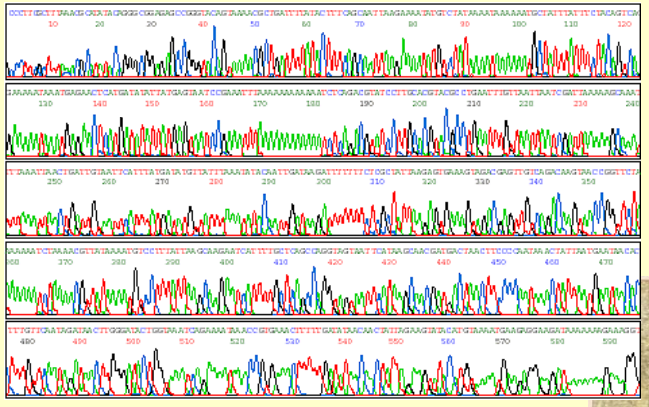
Species identification of sand flies by molecular methods: establishment of multi-approach protocol

Sand fly laboratory colonies

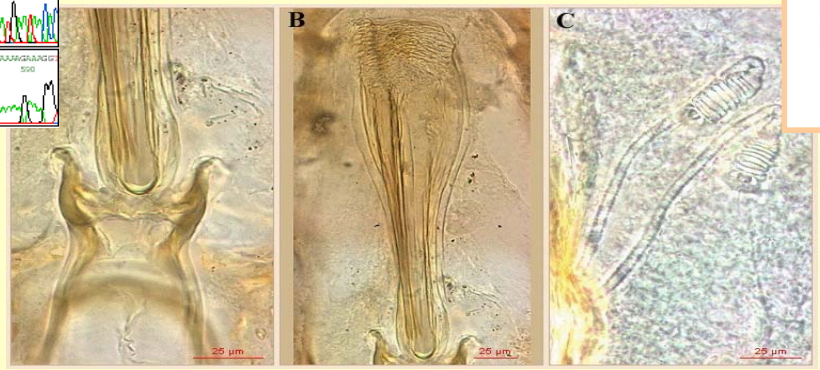
Field surveys in endemic areas

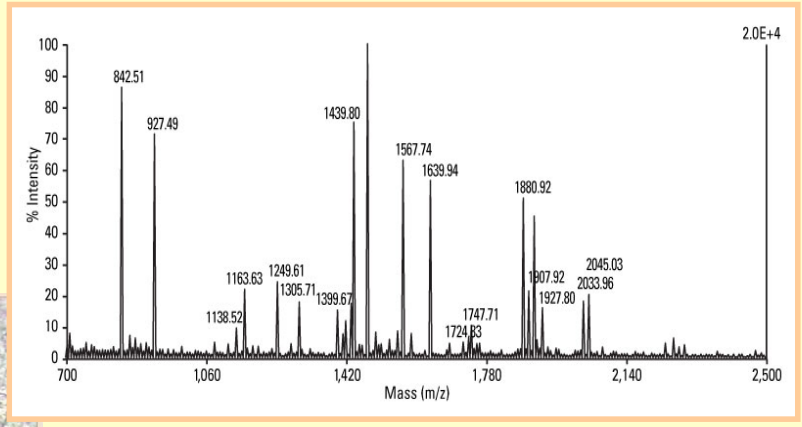


Morphological analysis



DNA barcoding





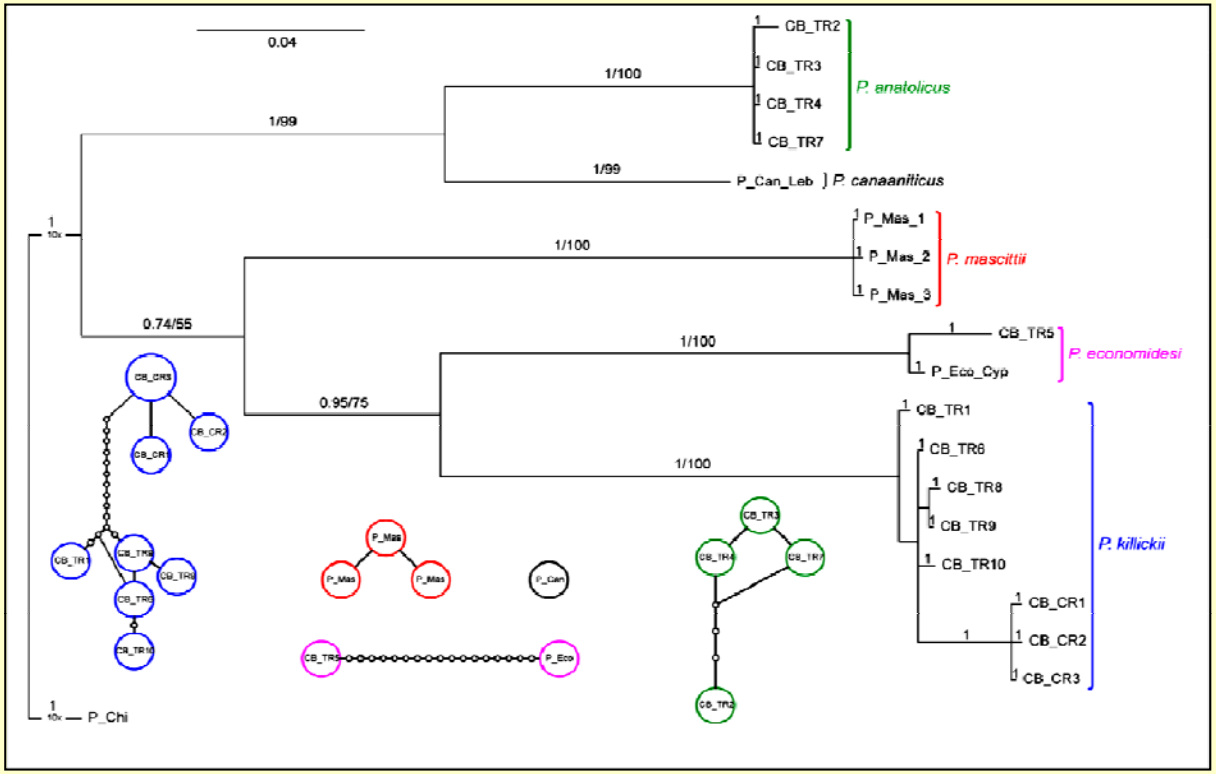
MALDI-TOF protein profiling

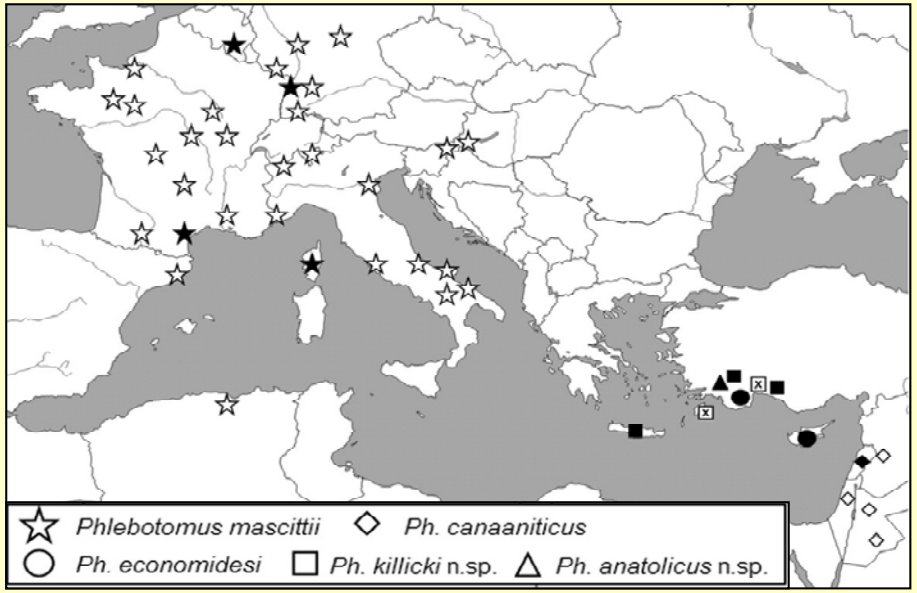
DNA barcoding for species identification and molecular taxonomy of sand flies

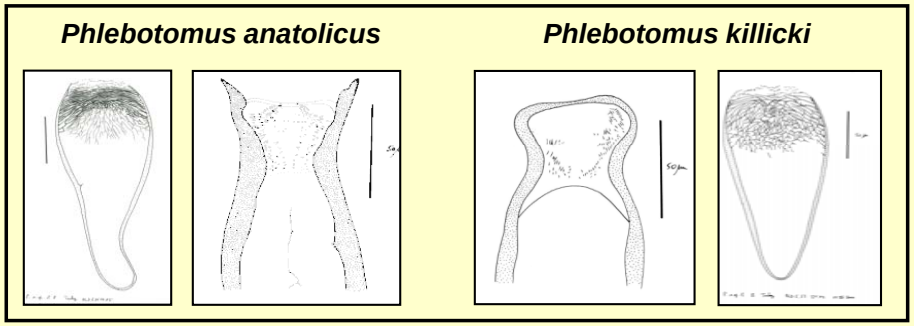
DNA barcodes can provide an insight into morphologically challenging species complexes

Barcode analysis can facilitate further taxonomical studies („reverse taxonomy“) and
recently revealed 2 undescribed species within the subgenus *Transphlebotomus*

Phylogram and haplotype network of 5 species of the subgenus *Transphlebotomus*







Contents lists available at ScienceDirect
Infection, Genetics and Evolution
journal homepage: www.elsevier.com/locate/meegid

Phylogeography of the subgenus *Transphlebotomus* Artemiev with description of two new species, *Phlebotomus anatolicus* n. sp. and *Phlebotomus killicki* n. sp.

Özge Erisoz Kasap [a,*], Vit Dvorak [b], Jérôme Depaquit [c], Bulent Alten [a], Jan Votypka [b], Petr Volf [b]

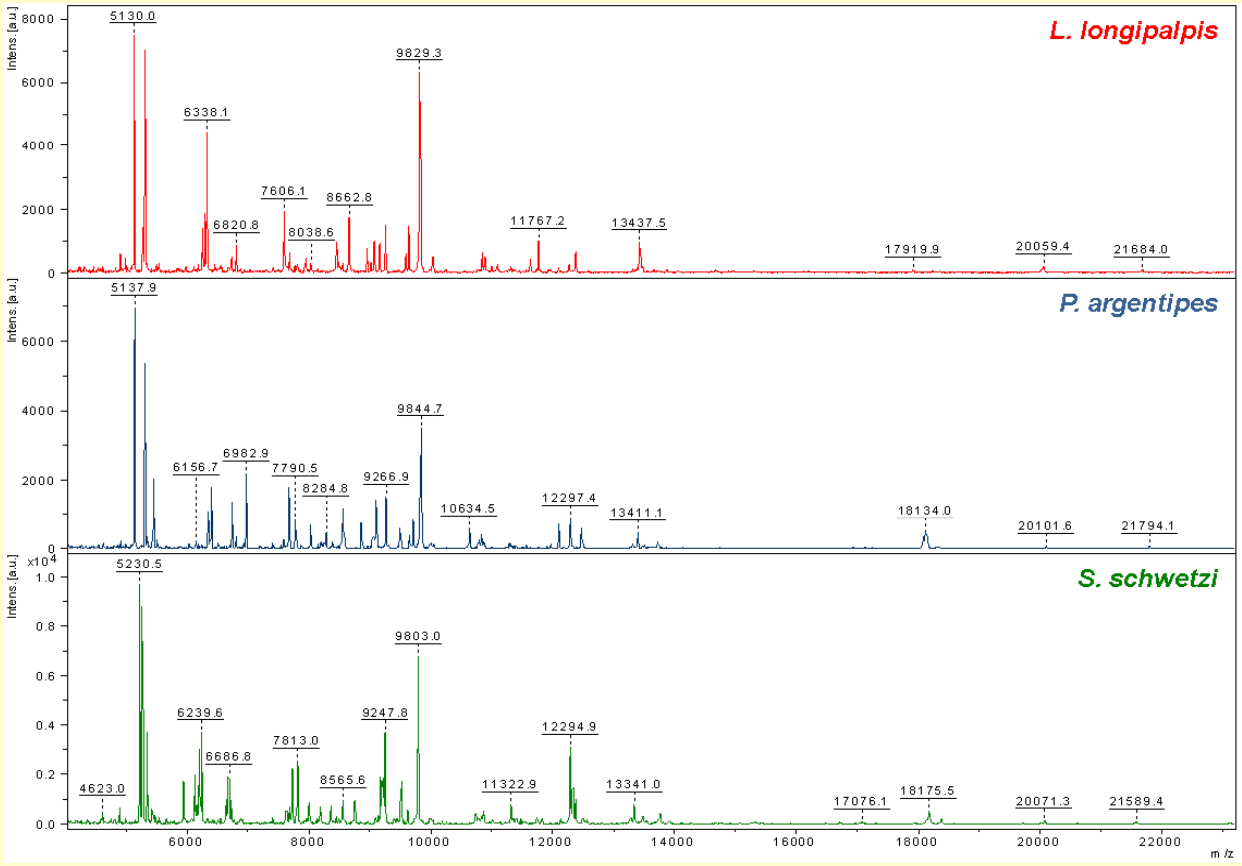
MALDI-TOF protein profiling for species identification

An emerging approach in species identification of medically important arthropods.

Recently successfully applied on sand flies.

Enables conslusive species identification in a rapid and cost-effective manner.

Suitable for large-scale field surveys.



Dvorak *et al. Parasites & Vectors* 2014, **7**:21
http://www.parasitesandvectors.com/content/7/1/21

Parasites & Vectors

RESEARCH Open Access

Identification of phlebotomine sand flies (Diptera: Psychodidae) by matrix-assisted laser desorption/ionization time of flight mass spectrometry

Vit Dvorak[1*], Petr Halada[2], Kristyna Hlavackova[1], Emmanouil Dokianakis[3], Maria Antoniou[3] and Petr Volf[1]

Mathis *et al. Parasites & Vectors* (2015) 8:266
DOI 10.1186/s13071-015-0878-2

Parasites & Vectors

RESEARCH Open Access

Identification of phlebotomine sand flies using one MALDI-TOF MS reference database and two mass spectrometer systems

Alexander Mathis[1*], Jérôme Depaquit[2], Vit Dvořák[3], Holly Tuten[1,9], Anne-Laure Bañuls[4], Petr Halada[5], Sonia Zapata[6], Véronique Lehrter[2], Kristýna Hlavačková[3], Jorian Prudhomme[4], Petr Volf[3], Denis Sereno[4], Christian Kaufmann[1], Valentin Pflüger[7] and Francis Schaffner[1,8]

97% specifity (139/144) of species identification was obtained in a study comprising 19 species.

Protein spectra obtained with different instruments can be analyzed in a centralized database.

EuroLeish.net Training Network

H2020: Marie Sklodowska–Curie – Innovative Training Network

Control of leishmaniasis, from bench to bedside and community

Barcelona Institute for Global Health (ISGlobal)
Barcelona, Spain

BMD Software (BMD)
Aveiro, Portugal

Charles University in Prague (CUNI)

Coris BioConcept (CORIS)
Gembloux, Belgium

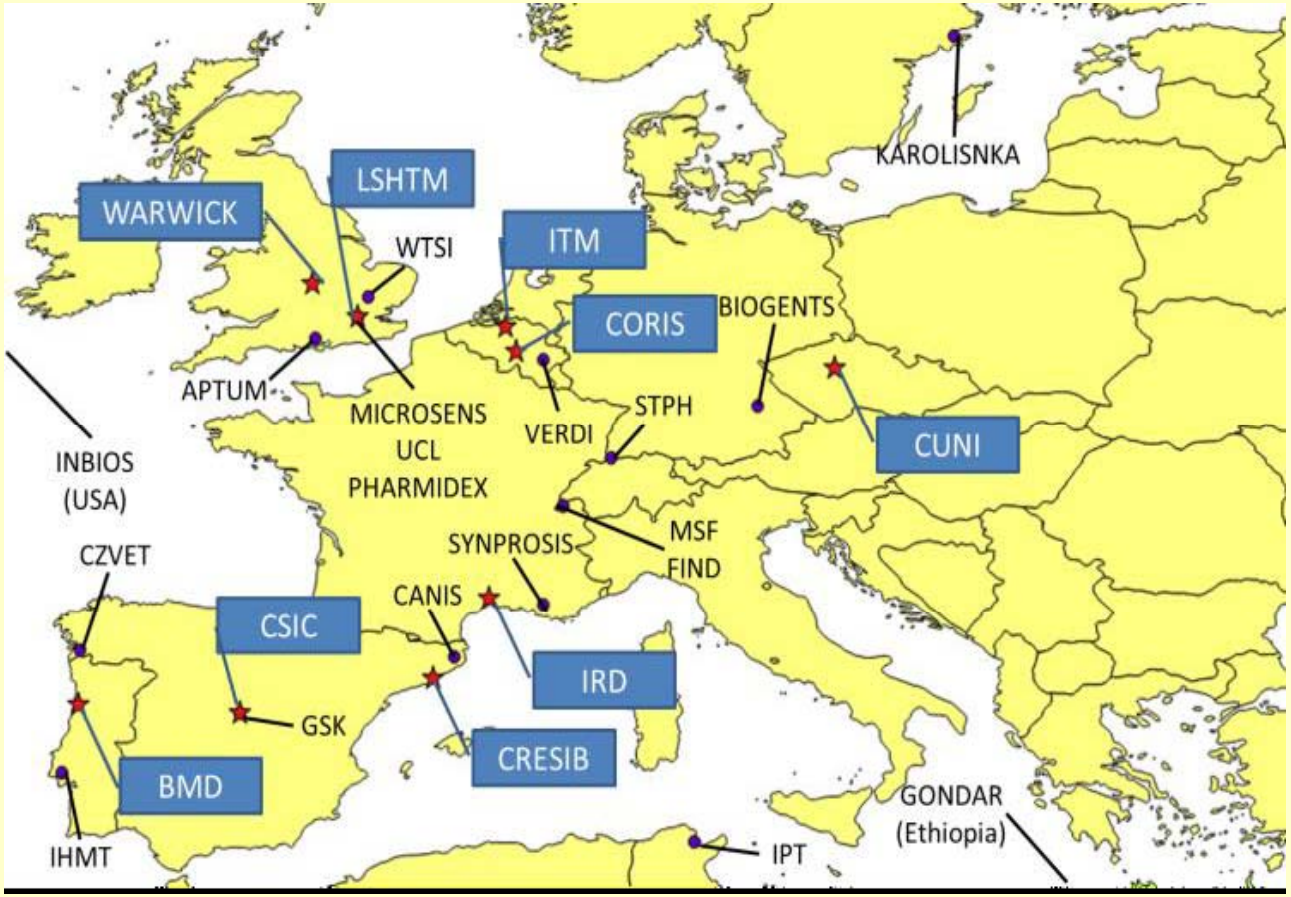
Institut de recherche pour le développement (IRD)
Montpellier, France

Institute of Tropical Medicine (ITM)
Antwerp, Belgium

Consejo Superior de Investigaciones Científicas (CSIC)
Madrid, Spain

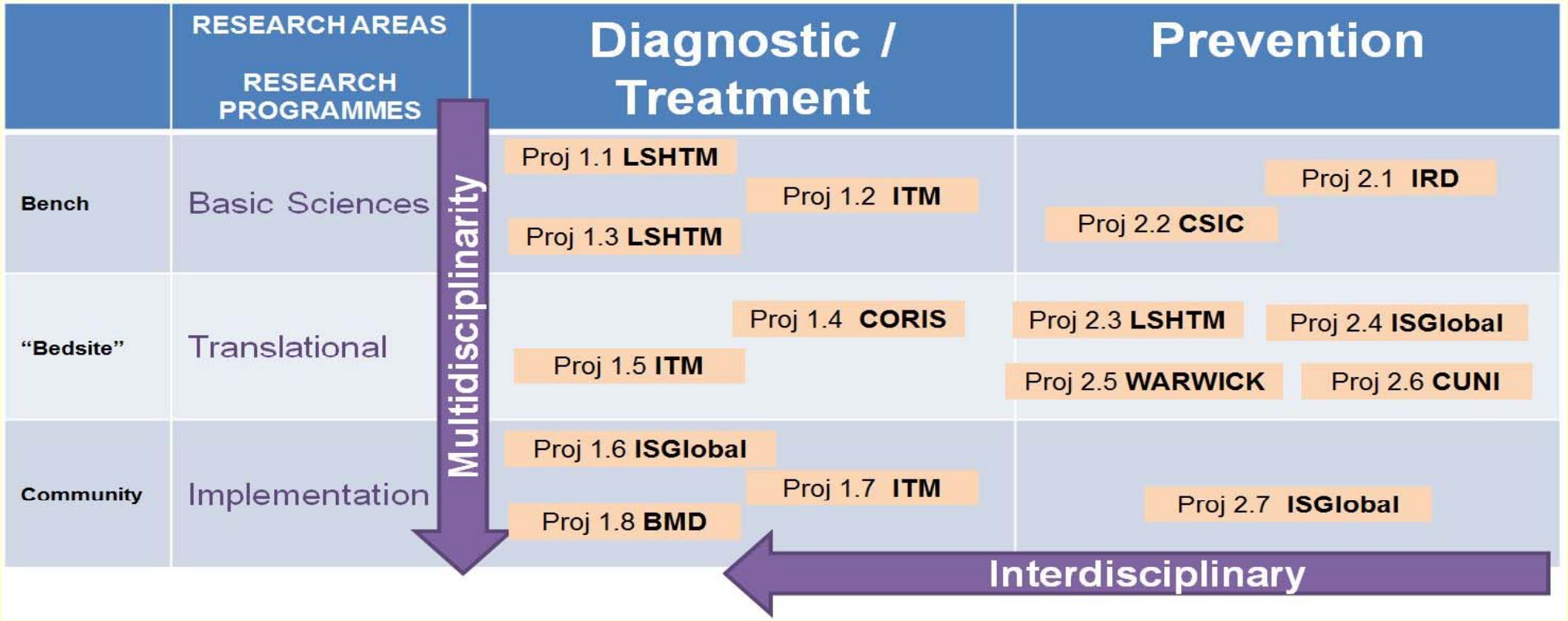
The University of Warwick (WARWICK)
Coventry, United Kingdom

London School of Hygiene and Tropical Medicine (LSHTM)
London, United Kingdom



Euroleish.net - Project overview

- Academic and non-academic institutions in Europe and abroad
- 15 research projects, 15 PhD students
- Multidisciplinary research on leishmaniases
- Molecular biology and epidemiology, drug discovery, vector control and immunology



Euroleish.net: PhD candidate selection process

February 2015	• Background requirements • Position descriptions
March/ Mid-April 2015	• Advertising positions (EURAXESS) • Revision candidatures & shortlisting candidates
April 20th-21st (London meeting)	• Candidates interview for final decision by panels
May/ June 2015	• Eligibility check of documents and records • Formal recruitment process
July 2015	• 15 PhD fellows hired • Start projects

Euroleish.net: PhD Candidate selection process

- 15 PhD students selected – 2 supervisors per student
- 6 months in a non-academic institution (e.g. SME)
- 2-3 months in partner institution

David Santos-Mateus
London, United Kingdom

Temmy Sunyoto
Antwerp, Belgium

Assel Syzdykova
Aveiro, Portugal

Rita Velez
Barcelona, Spain

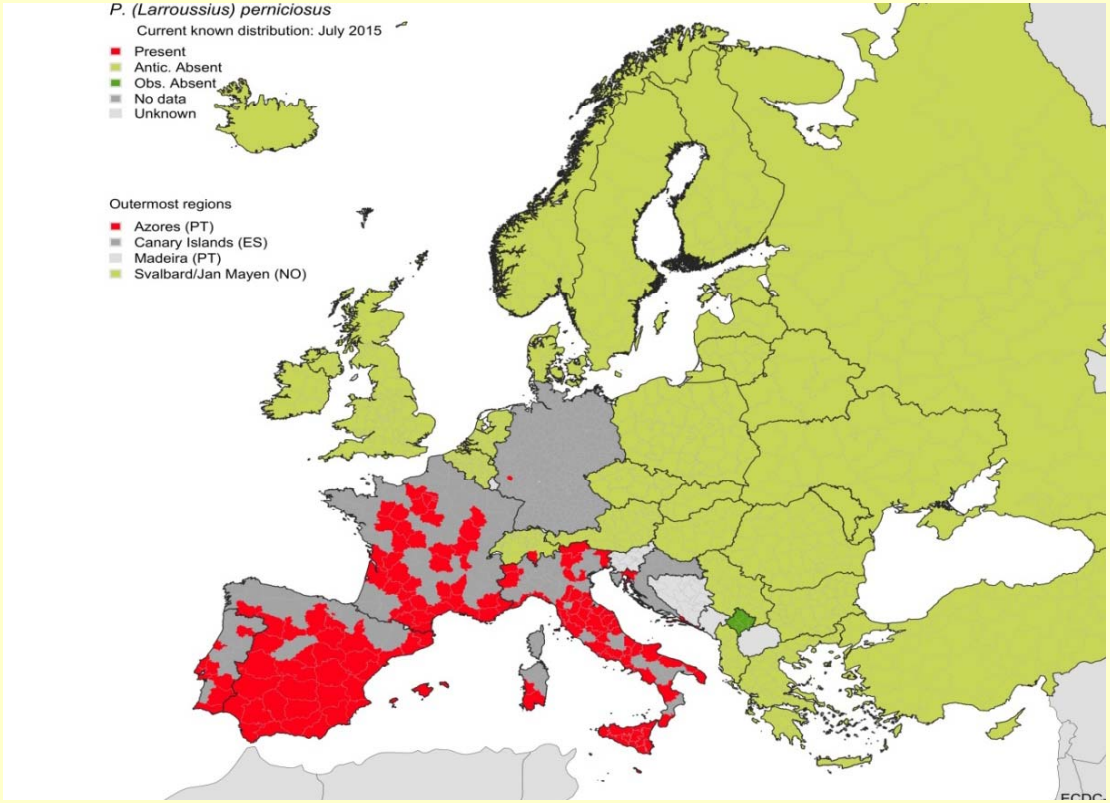
Gert-Jan Wijnant
London, United Kingdom

Laura Willen
Prague, Czech Republic

Laboratory for Vector Biology,
Dept. Parasitol., Fac. Sci., CUNI

2.6. Development of new sand fly exposure markers to evaluate vector control tools

Laura Willen, MSc.
Prof. **Petr Volf**, Charles University in Prague (CUNI), Czech Republic
Dr. **Pascal Mertens**, Coris BioConcept (CORIS), Gembloux, Belgium
Dr. **Montserrat Gallego**, Institute for Global Health (ISGlobal), Barcelona, Spain



Phlebotomus perniciosus

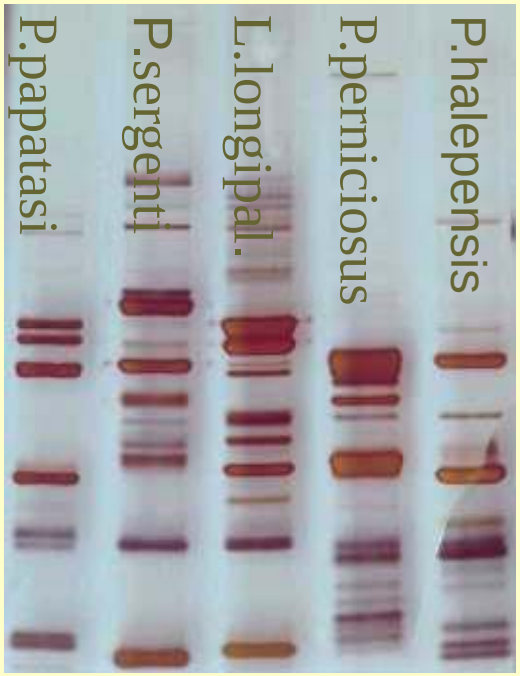
Leishmania infantum

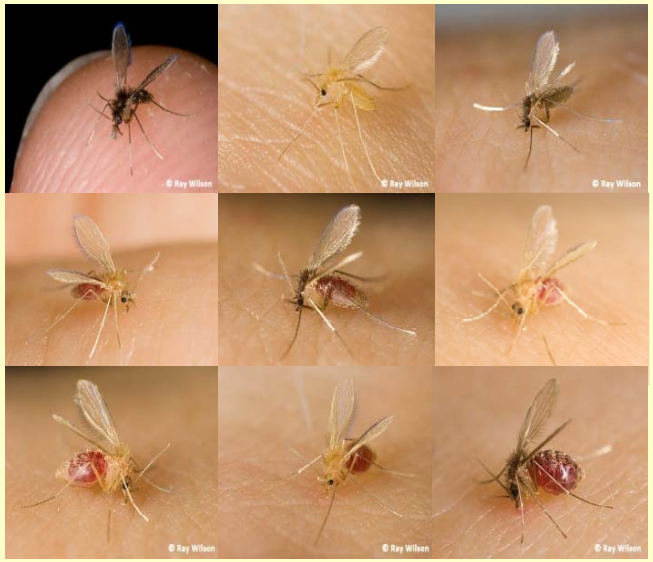
Human visceral leishmaniasis

Canine leishmaniasis

Hosts bitten by sand flies develop antibodies against sand fly saliva. Antibodies are sand fly species-specific

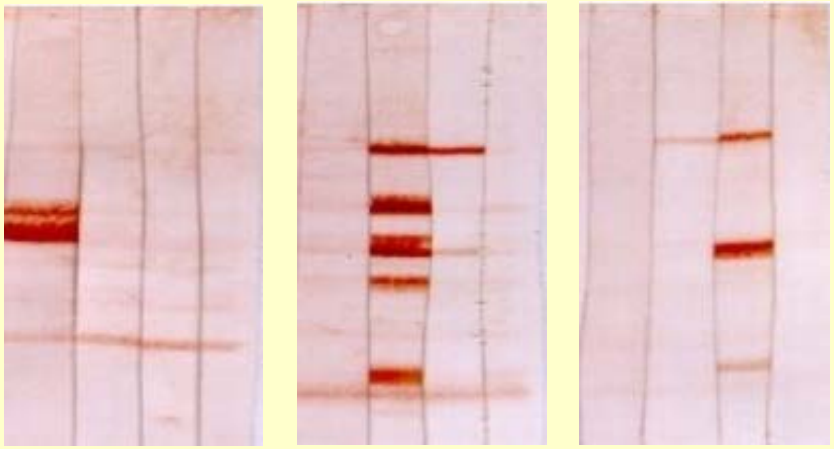
SDS PAGE of saliva





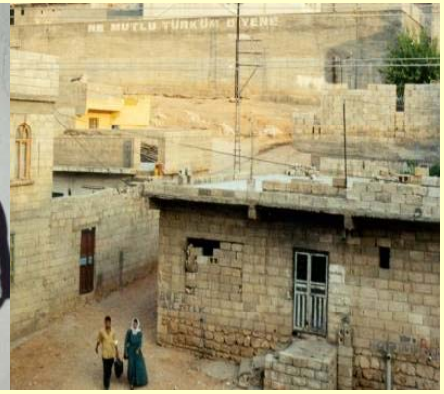
Immunoblotting of salivary lysates

An. stephensi *P. sergenti* *P. papatasi*



ST SE PA CO ST SE PA CO ST SE PA CO

sera of mouse bitten by



Hosts:
Laboratory animals
Humans
Wild animals

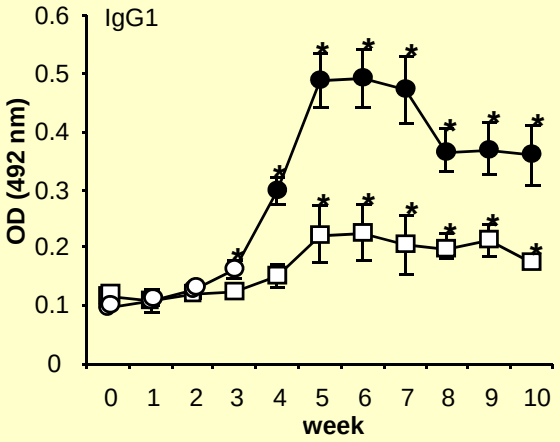
Volf and Rohousova, 2001, Rohousova et al., 2005., Martin-Martin et al. 2014

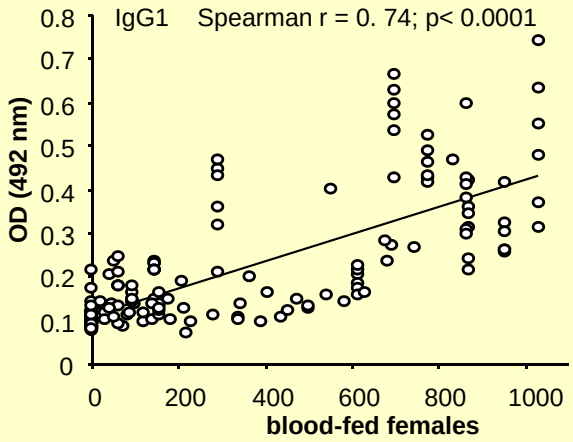
IgG antibodies in sera of dogs bitten by *P. perniciosus* recognize salivary antigens and are good markers of exposure

- ● high-exposed
- □ low-exposed

200 or 50 sand flies/dog
1× week

Vlkova et al. 2012: PLoS NTD

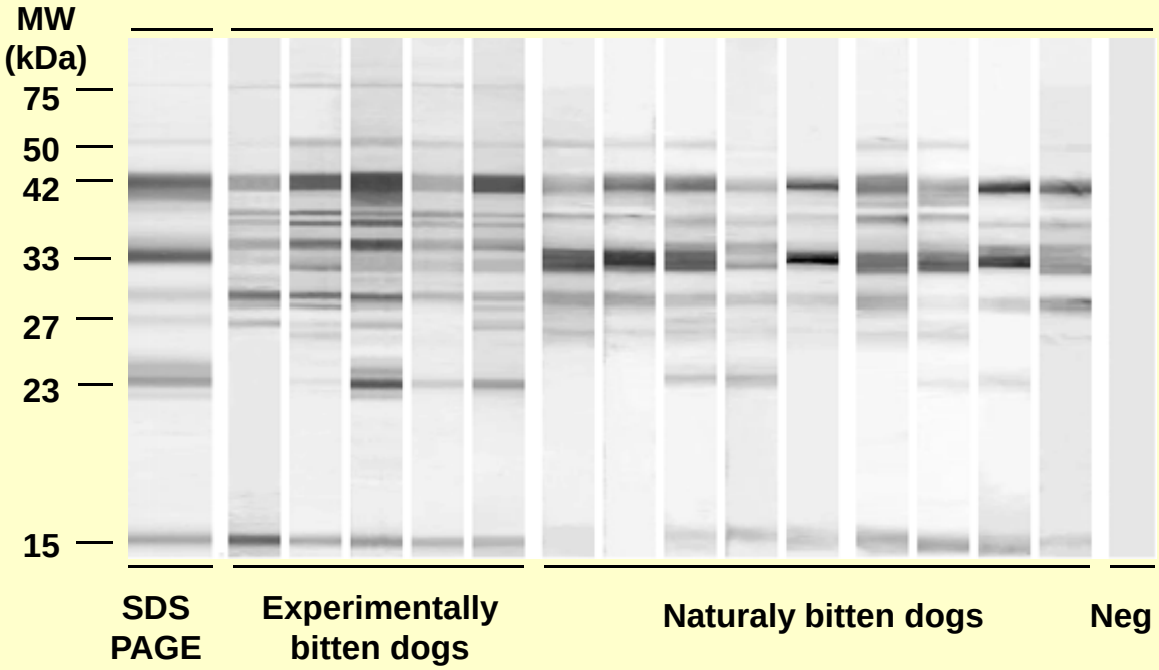




Antibody levels positively correlated with the number of bloodfed *Phlebotomus perniciosus females.*

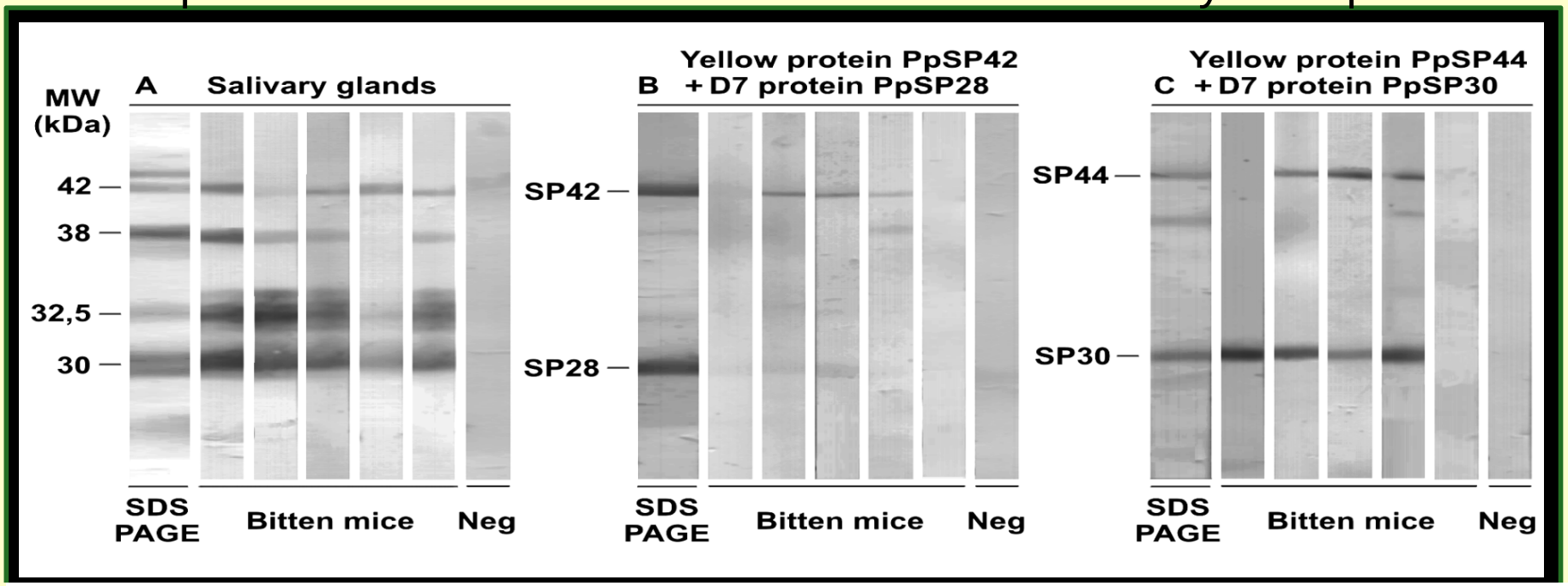
Major salivary antigens were identified as yellow proteins and apyrases.

Next steps: recombinant proteins and synthetic peptides for diagnostic test Phase II study in Spain - **CRESIB**



Recombinant proteins replace salivary gland lysates in western blots and ELISA tests

Phlebotomus papatasi recombinant proteins expressed in *E. coli* react with sera of mice bitten by this species



OPEN ACCESS Freely available online

PLoS NEGLECTED TROPICAL DISEASES

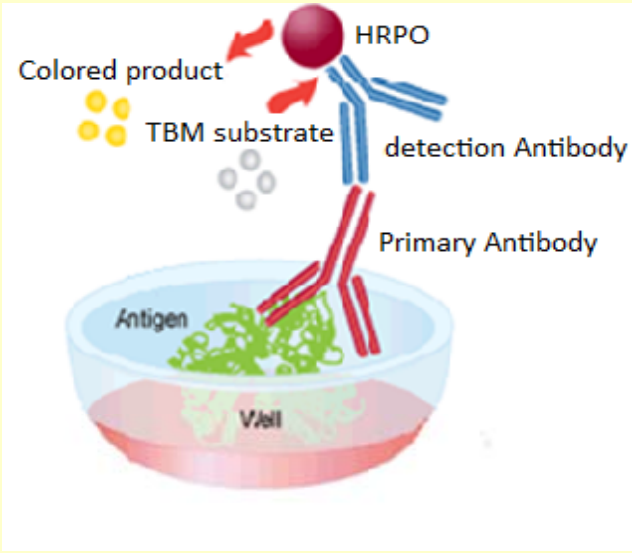
Kinetics of Antibody Response in BALB/c and C57BL/6 Mice Bitten by *Phlebotomus papatasi*

Michaela Vlkova[1]*, Iva Rohousova[1], Jitka Hostomska[1], Lucia Pohankova[1], Lenka Zidkova[1], Jan Drahota[1], Jesus G. Valenzuela[2], Petr Volf[1]

Work plan

To develop a diagnostic test for determination of host exposure to sand fly bites and estimation of the risk of *Leishmania* transmission.

1. Select best recombinant proteins: done, yellow-related protein
2. Select best peptides: in progress
3. Develop semiquantitative rapid test in CORIS (2016-2017)
4. Evaluation of the test in field conditions in Spain
   together with CRESIB (2017)



3D structure of salivary yellow-related protein