Surveillance and Control of Sand flies

Dr. Yusuf ÖZBEL

Ege University Faculty of Medicine

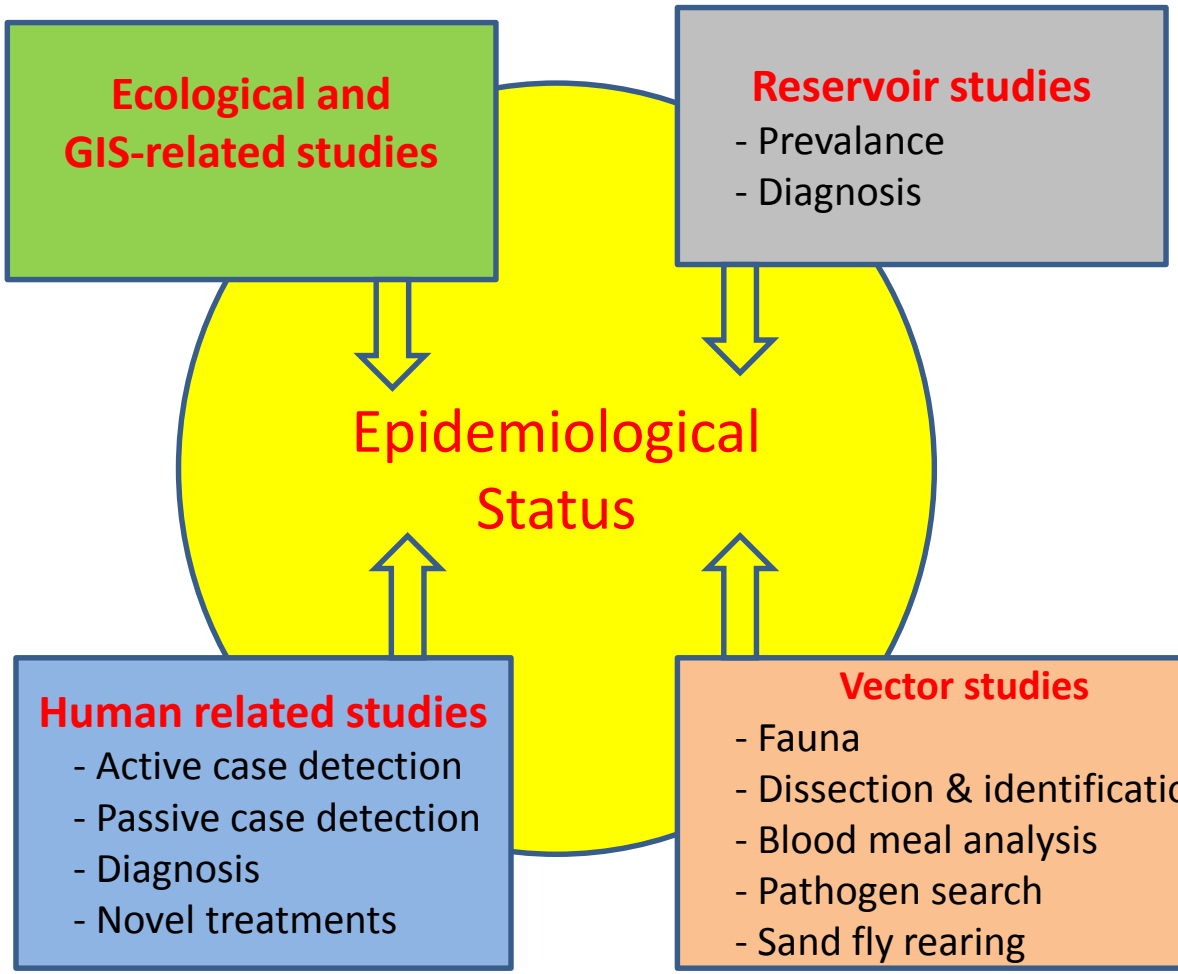
Department of Parasitology

İzmir, TURKEY

yusuf.ozbel@ege.edu.tr

What are we doing in Turkey?

EGE UNIVERSITY
Dept. of Parasitology
Fac. of Medicine
IZMIR



HACETTEPE UNIVERSITY
Dept. of Ecology
Fac. of Science
&
Dept. of Virology
Fac. of Medicine
ANKARA

- International collaborations (European, Asian & Northern African countries)
- There is no control program for sand flies in Turkey
- The situation and endemic regions are known, we need to arrange surveillance system in different levels

Sand fly Species in Turkey

- **Phlebotomus**
  - **P. papatasi**
- **Paraphlebotomus**
  - P. sergenti (CL)
  - P. similis (CL)
  - P. alexandri
  - P. jacusieli
  - P. caucasicus
- **Adlerius**
  - P. halepensis (CL)
  - P. balcanicus
  - P. simici
  - P. kyreniae
  - P. brevis

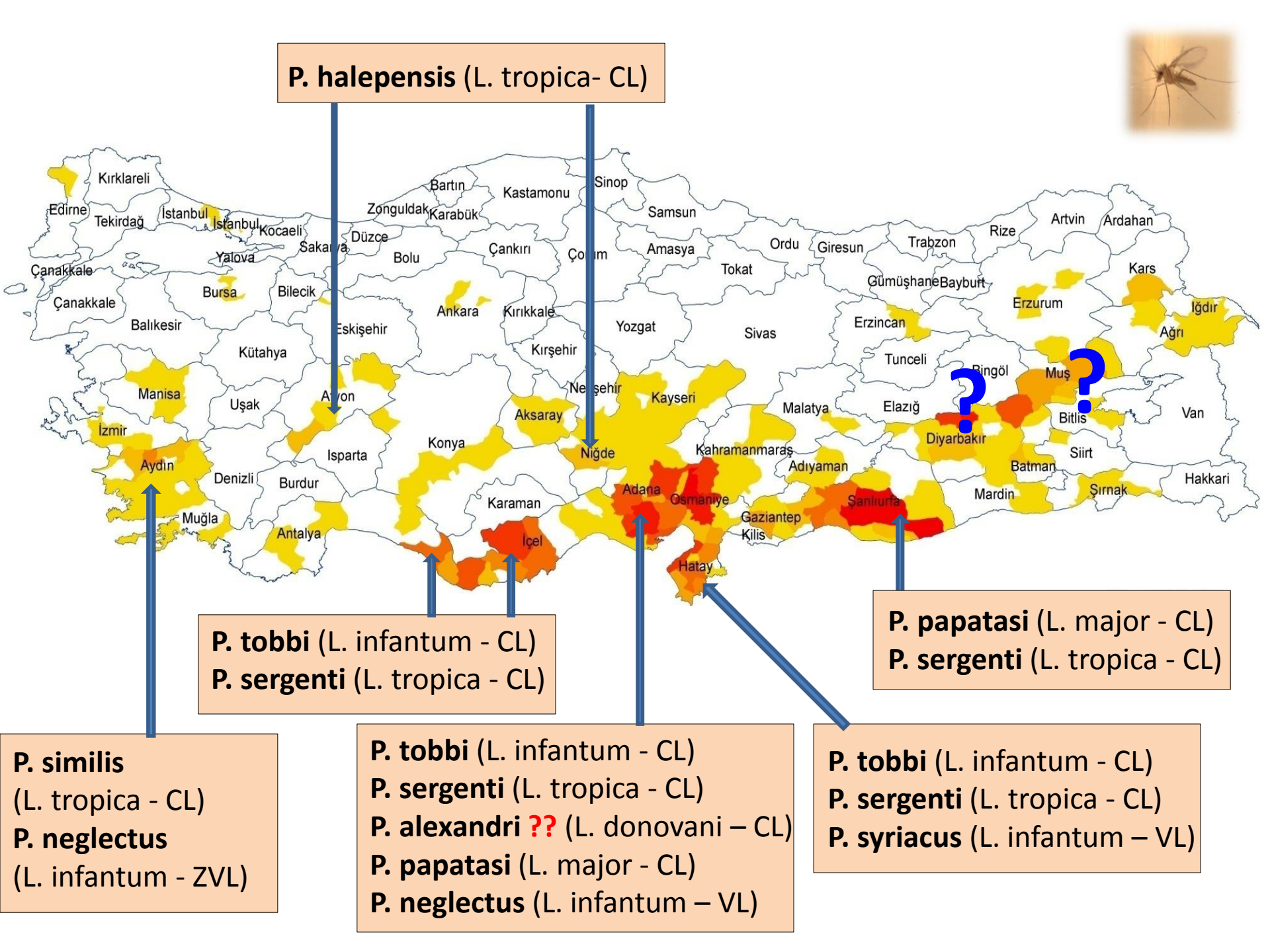
5 subgenus

25 species

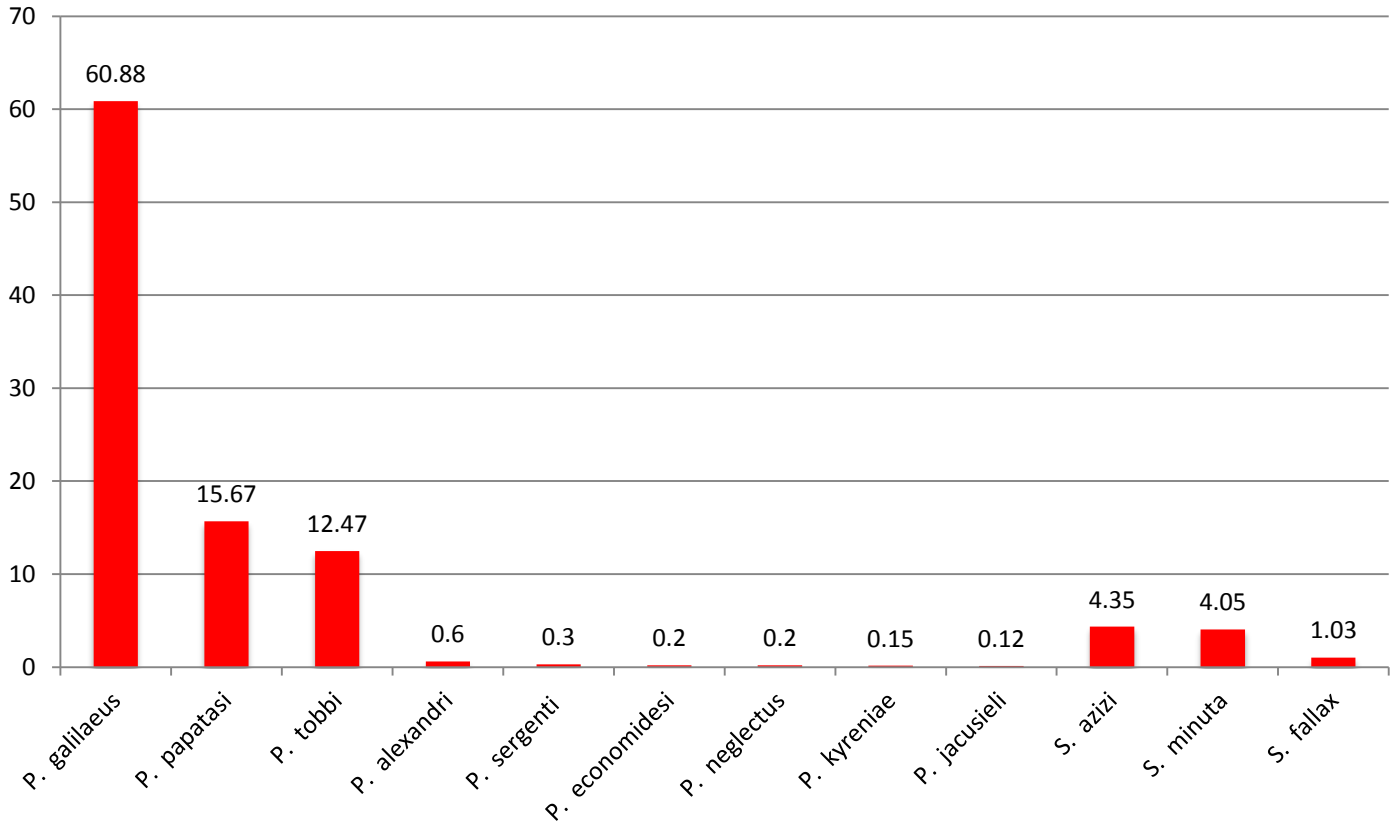
Transphlebotomus

- P. anatolicus
- P. killicki

- **Larroussius**
  - P. tobbi
  - P. major
  - P. neglectus
  - P. syriacus
  - P. transcaucasicus
  - P. perfiliewi
  - P. kandelakii
  - P. mascittii
  - P. galilaeus
  - P. burneyi


P. halepensis (L. tropica- CL)
P. tobbi (L. infantum - CL)
P. sergenti (L. tropica - CL)
P. papatasi (L. major - CL)
P. sergenti (L. tropica - CL)
P. similis
(L. tropica - CL)
P. neglectus
(L. infantum - ZVL)
P. tobbi (L. infantum - CL)
P. sergenti (L. tropica - CL)
P. alexandri ?? (L. donovani – CL)
P. papatasi (L. major - CL)
P. neglectus (L. infantum – VL)
P. tobbi (L. infantum - CL)
P. sergenti (L. tropica - CL)
P. syriacus (L. infantum – VL)
Kırklareli
Edirne
Tekirdağ
İstanbul
İstanbul
Kocaeli
Sakarya
Düzce
Bartın
Kastamonu
Zonguldak
Karabük
Sinop
Samsun
Yalova
Çanakkale
Çanakkale
Bursa
Bilecik
Bolu
Çankırı
Amasya
Ordu
Giresun
Trabzon
Rize
Artvin
Ardahan
Tokat
Gümüşhane
Bayburt
Kars
Erzurum
Iğdır
Ağrı
Balıkesir
Eskişehir
Ankara
Kırıkkale
Yozgat
Sivas
Erzincan
Kütahya
Kırşehir
Tunceli
Bingöl
Muş
Manisa
Uşak
Afyon
Nevşehir
Kayseri
Malatya
Elazığ
Bitlis
Van
İzmir
Aksaray
Konya
Isparta
Niğde
Kahramanmaraş
Adıyaman
Diyarbakır
Batman
Siirt
Aydın
Denizli
Burdur
Karaman
Adana
Osmaniye
Şanlıurfa
Mardin
Sırnak
Hakkari
Muğla
Antalya
İçel
Gaziantep
Kilis
Hatay

Sand flies of Cyprus



- P. perfiliewi (Dokianakis et al., 2018; Ergünay et al., 2014)
- P. killicki (Trans) sympatric with P. economidesi (Dokianakis et al., 2018)
- P. mascittii (?) (Adler, 1944; Leger&Depaquit, 1998)
- P. jacusieli (Adler, 1944; Leger&Depaquit, 1998)

Surveillance of sand flies

(Prepared for Turkish MoH in 2016)

Table 1 Different types of VBD context based on the current presence (√) or absence (-) of disease (endemic human cases), pathogen or vector, exemplified for the Netherlands

Context	Endemic Disease*	Pathogen	Vector	Examples of diseases holding for the Netherlands
1	√	√	√	Lyme borreliosis
2	-	√	√	Dirofilariasis
3	-	-	√	Tick-borne encephalitis
4	-	√	-	Leishmaniasis
5	-	-	-	Crimean-Congo haemorrhagic fever

* Endemic infections with human cases.

Braks et al. Parasites&Vectors 2011, 4:192

- From a public health point of view, 5 different types of VBD situations (contexts) are identified
- All endemic VBDs fall under context 1 and the various non-endemic VBDs fall under one of the remaining four contexts (2-5)
- To identify, assess, communicate and ultimately control VBDs, monitoring and surveillance tools, appropriate to the context, are needed

Braks et al. Parasites&Vectors 2011, 4:192

Surveillance system for sand flies

1	Categorization of endemic areas (from 0 to 5)	According to absence/presence of case/pathogen/vector
2	Clarification of the causative Leishmania species	By using previous data AND new studies (Changes is always possible)
3	Completion of existing information on sand flies	By using previous data AND filling the gaps with new studies
4	Preparation of the maps	Maps showing case AND sand fly distribution Risk maps by including climatic data
5	Establishing monitoring system	The system needs to be established according to the level of endemic area
6	Defining surveillance strategy	For creating early detection system

The contents of surveillance program for sand flies

- Medical importance of sand flies
- Sand fly general information
- Sand fly collection, transportation and identification methods
- Sand fly species recorded in Turkey
- Procedures for sand fly pathogen screening
- Procedures for the collection of environmental parameters
- The aims of the surveillance program
- The responsible institutions for applying the program
- Applications of surveillance program
- The components of control methods

Main aims of the Program

- Detection of sand fly season in the area
- Detection of the changes in the distribution and abundance of sand flies by time
- Ensuring timely decision about preventive measures that can be taken
- Detection of the places for insecticide applications (possible combination with mosquito control applications)
- Evaluation of the integrated control programs

Main Actors

- Ministry of Health (MoH) Headquarters
- Provisional branches of MoH
- Reference centers and labs
- Universities (for advising and research)

Creating basic data

- Identity Card for each endemic area
- Creating an Index
- **HOW?**
  - Historical data on vector, parasite and cases
  - Detection of target vector species
  - Creating a “real time system” for case recording (allowing to follow cases every day)
  - Environmental variables

Procedures for applying surveillance

- Surveillance should serve positively to the control measures
- Determination of responsible institutions and persons
- Determination of the techniques and procedures to be used
- Determination of selected localities
  - Domestic
  - Peridomestic
  - Sylvatic
  - Microhabitats !!!
- Timing and frequency of the collections according to the place and work power
- Determination of cut-off value to help early warning system

Why mapping is important?

The VBORNET project is focused on preparing distribution maps of important arthropods vectors (mosquitoes, ticks, phlebotomines)

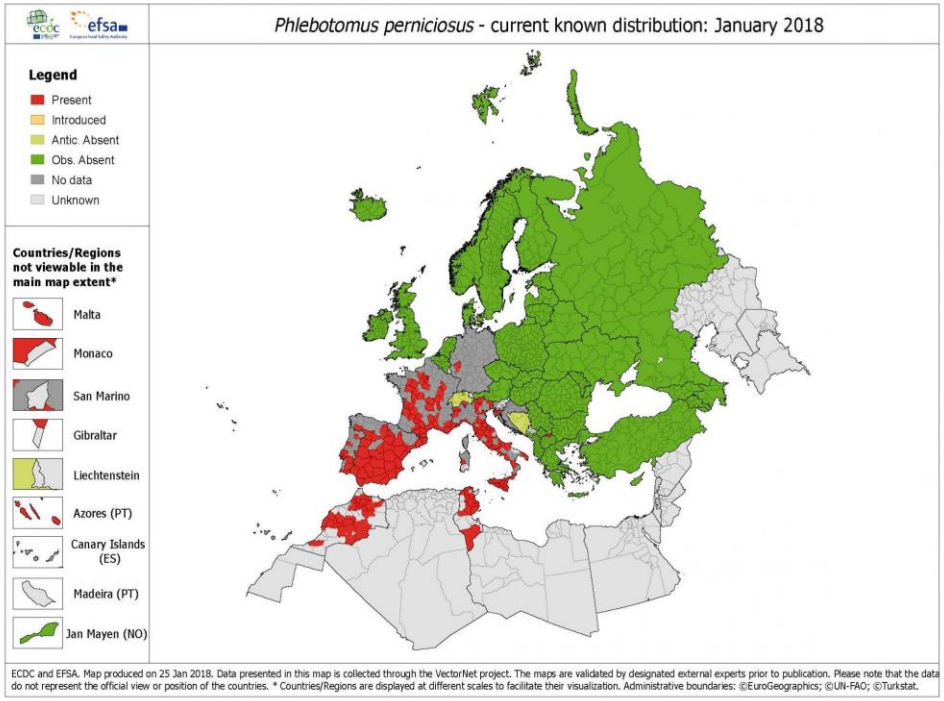
Important sand fly species in Europe

- P. (Larroussius) ariasi
- P. (Larroussius) perniciosus
- P. (Larroussius) perfiliewi
- P. (Larroussius) neglectus/syriacus
- P. (Larroussius) tobbi

- P. (Phlebotomus) papatasi

- P. (Paraphlebotomus) sergenti
- P. (Paraphlebotomus) similis
- P. (Paraphlebotomus) alexandri

- P. (Transphlebotomus) mascittii

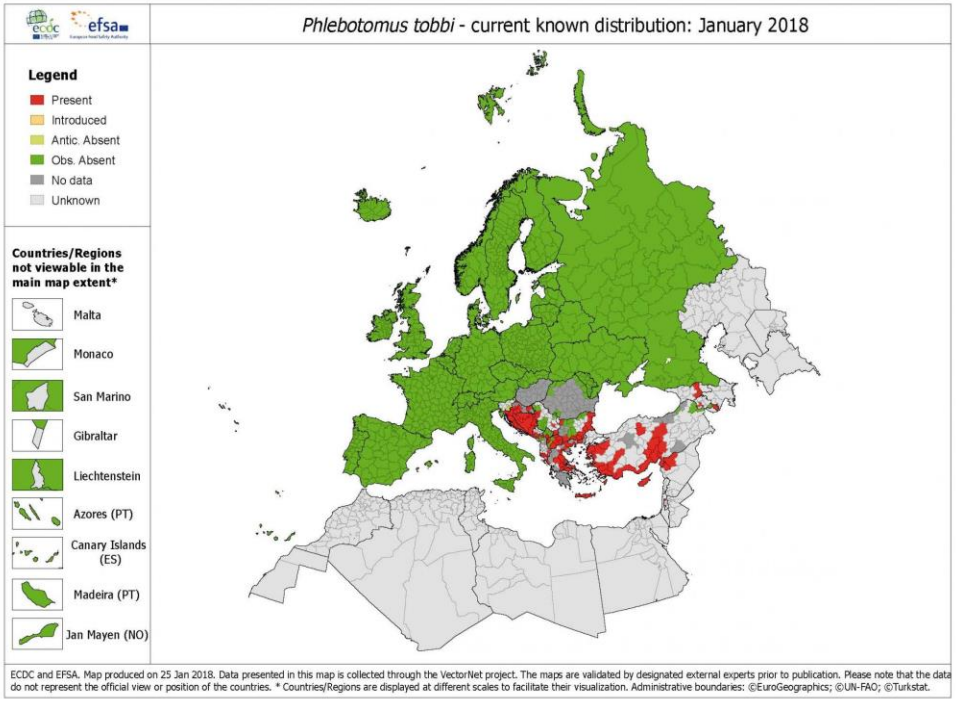


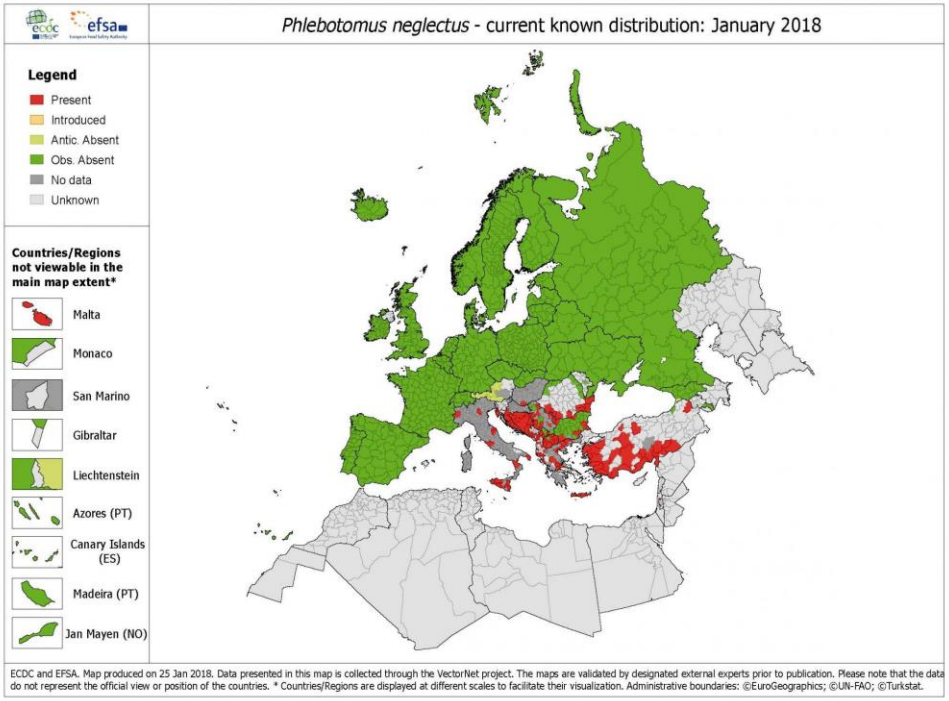
P. perniciosus

Eastern limit of P. perniciosus
and
western limit of P. tobbi
is overlapping

P. tobbi

Why their distribution is limited in the same geographical area in opposite directions?

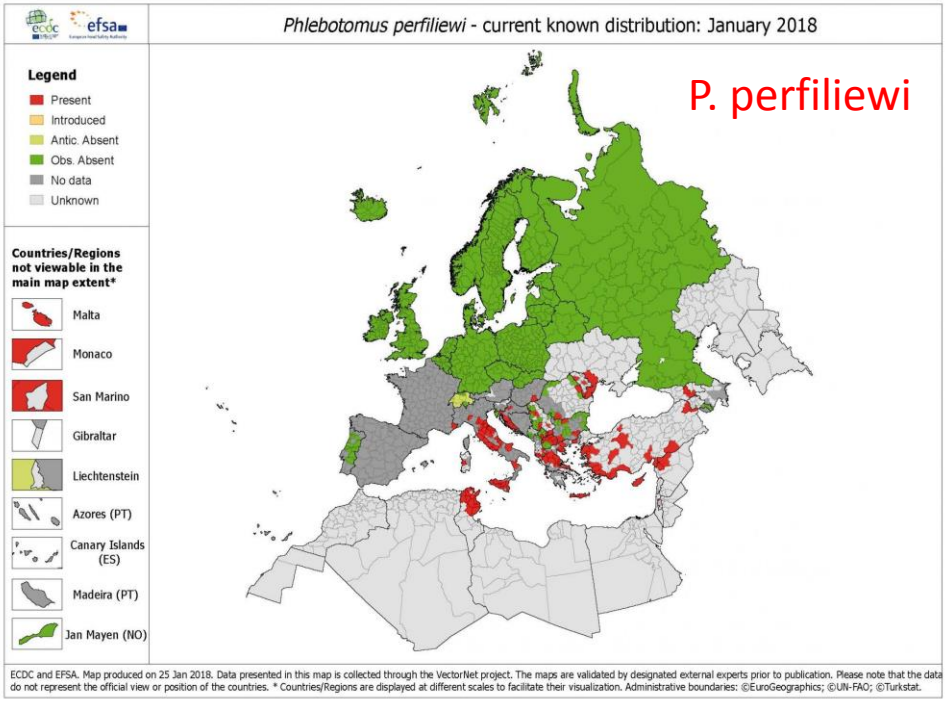


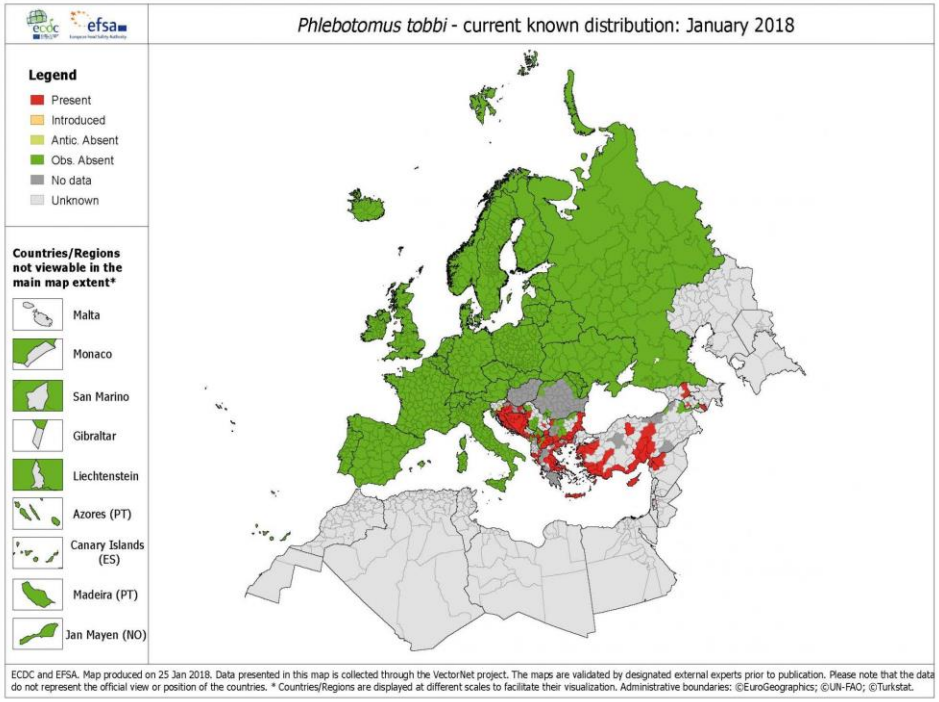


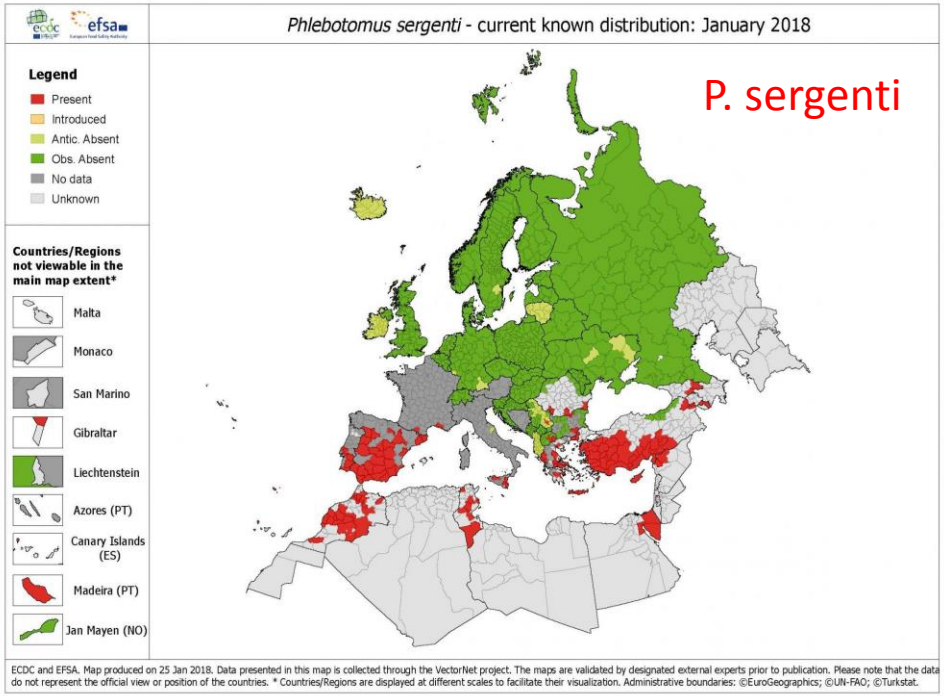
P. neglectus/syriacus

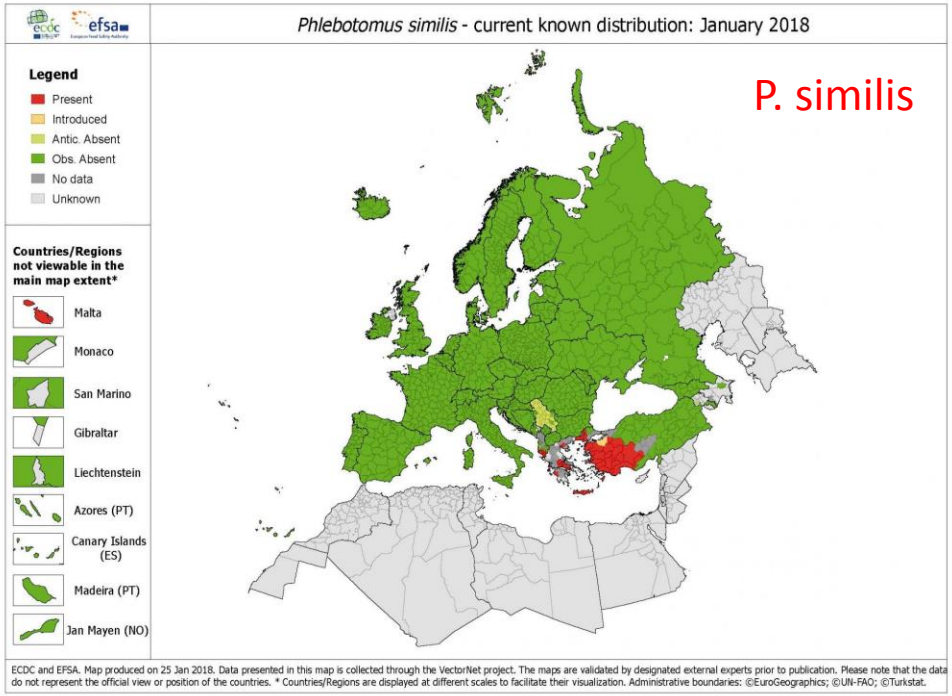
Why P. neglectus/syriacus
and
P. perfiliewi
succeed to spread
to more western limit
not P. tobbi ?

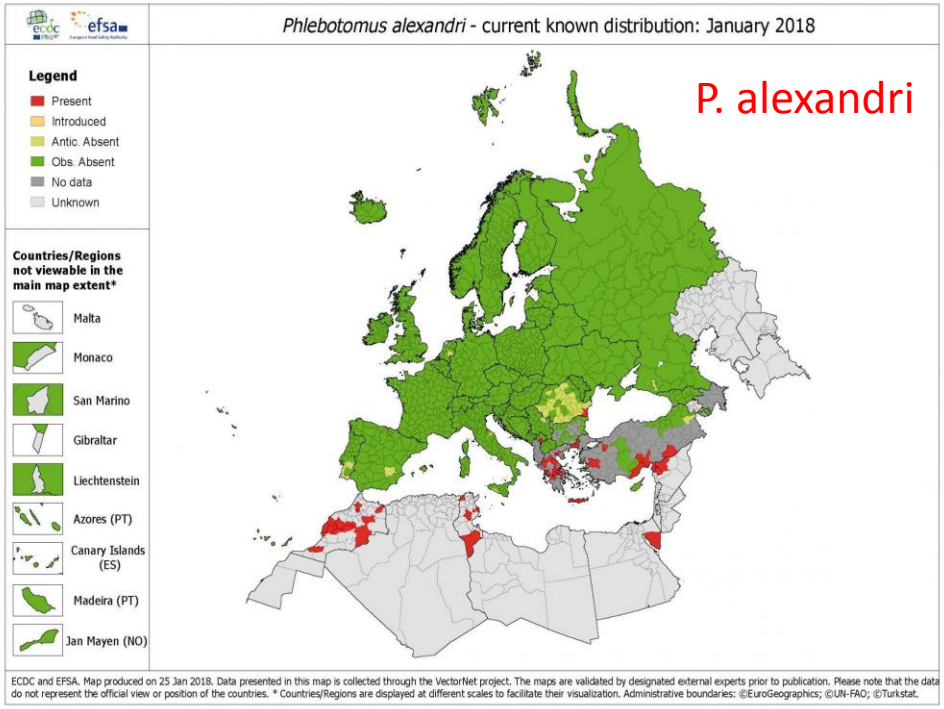
P. tobbi





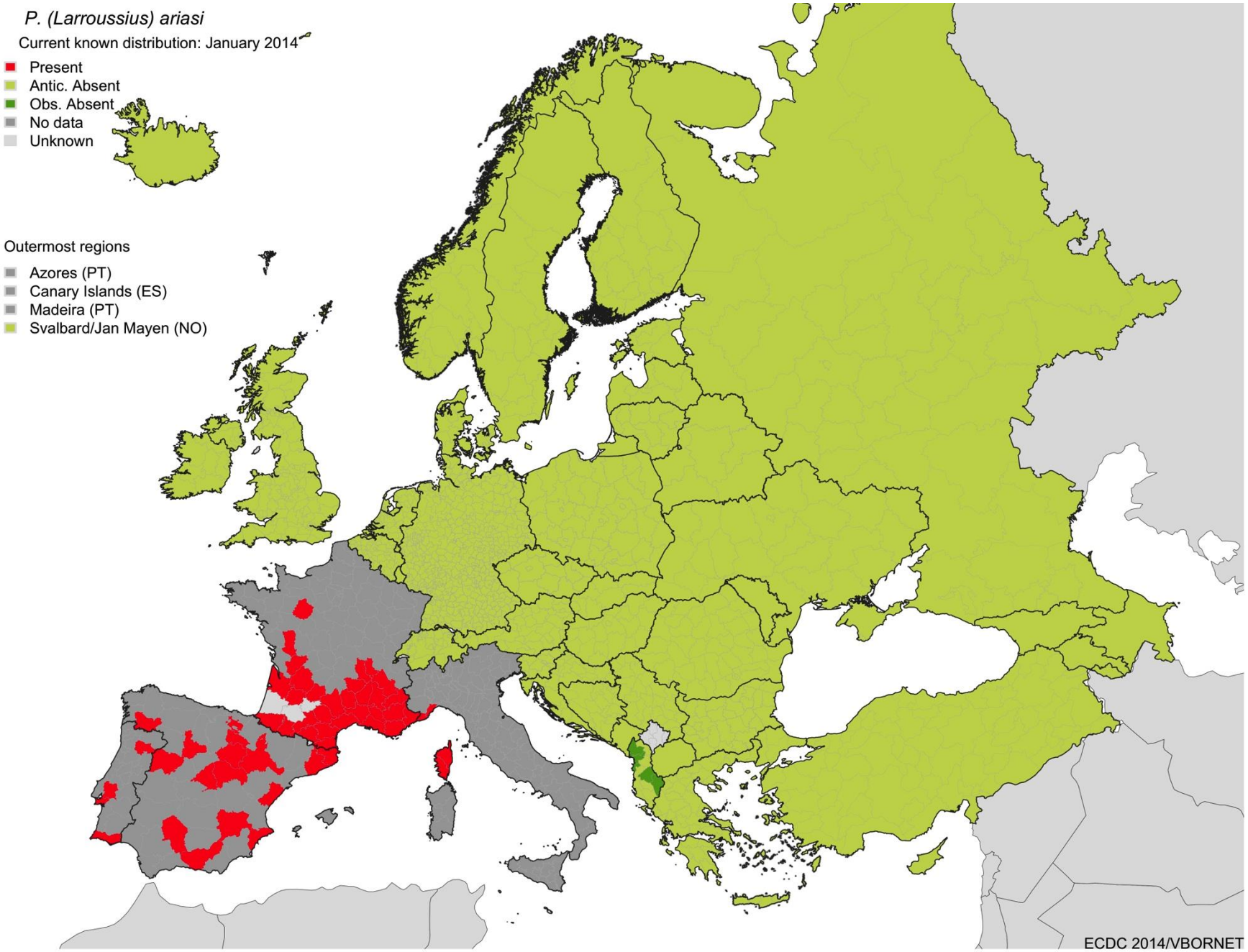


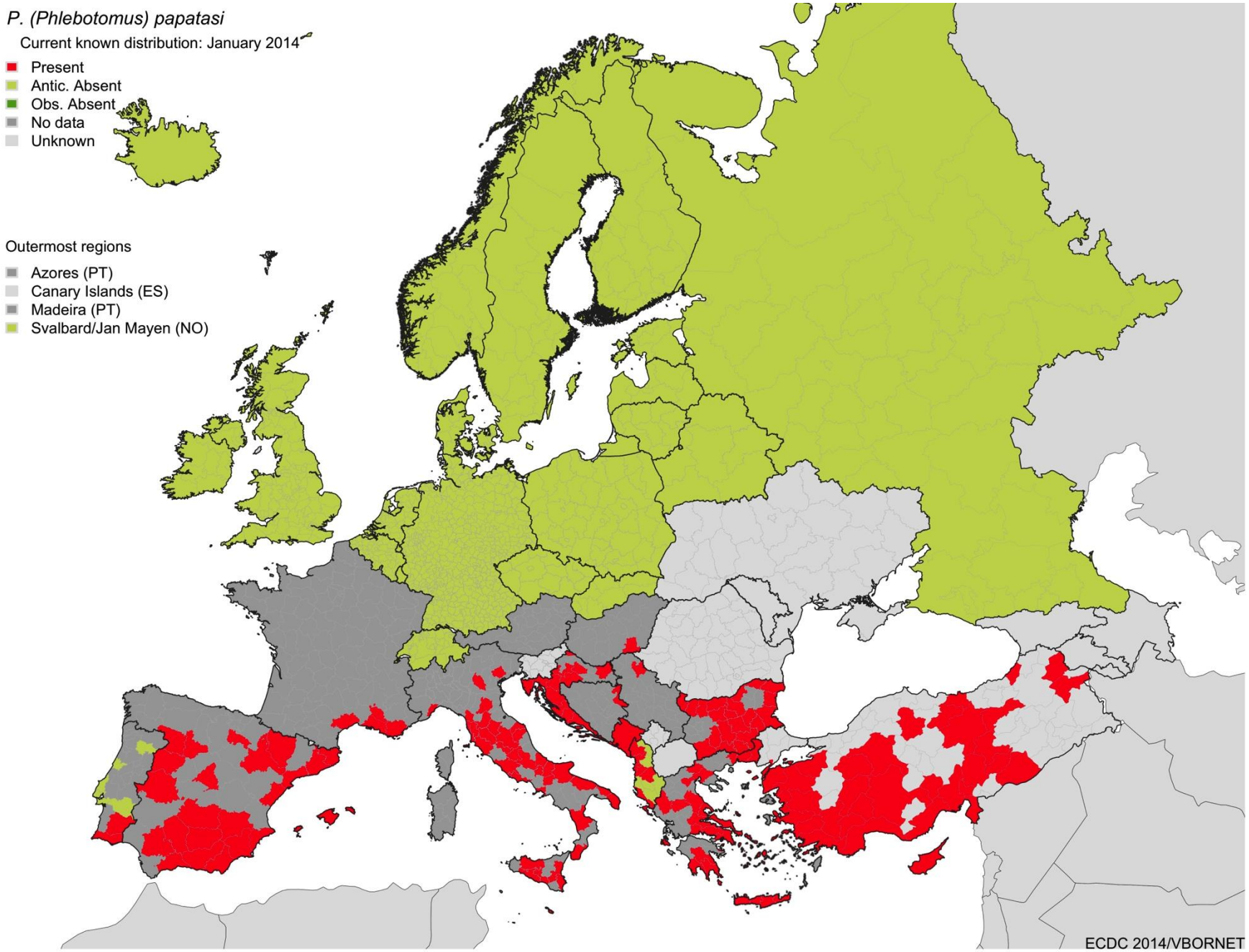


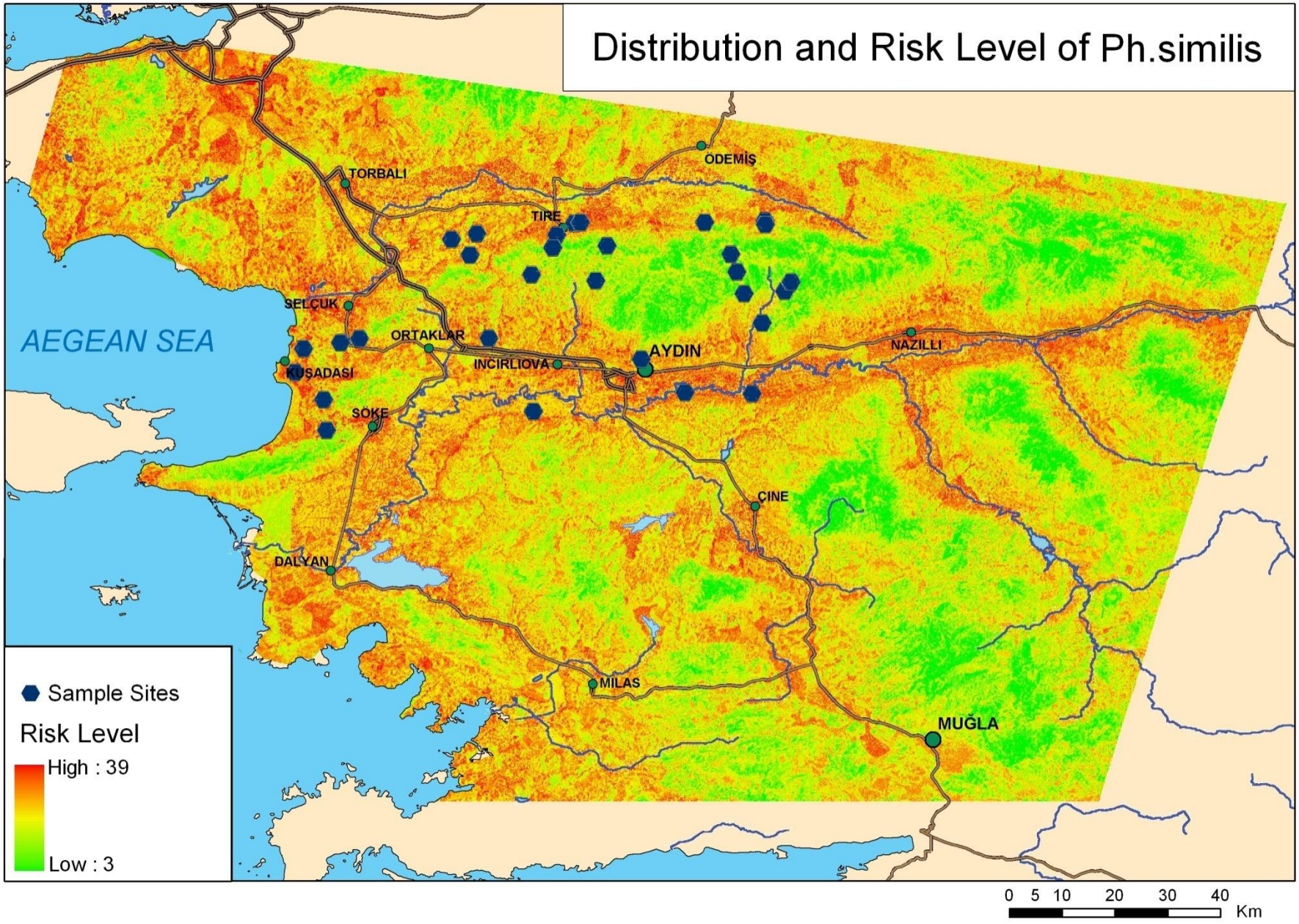


Subgenus Paraphlebotomus species

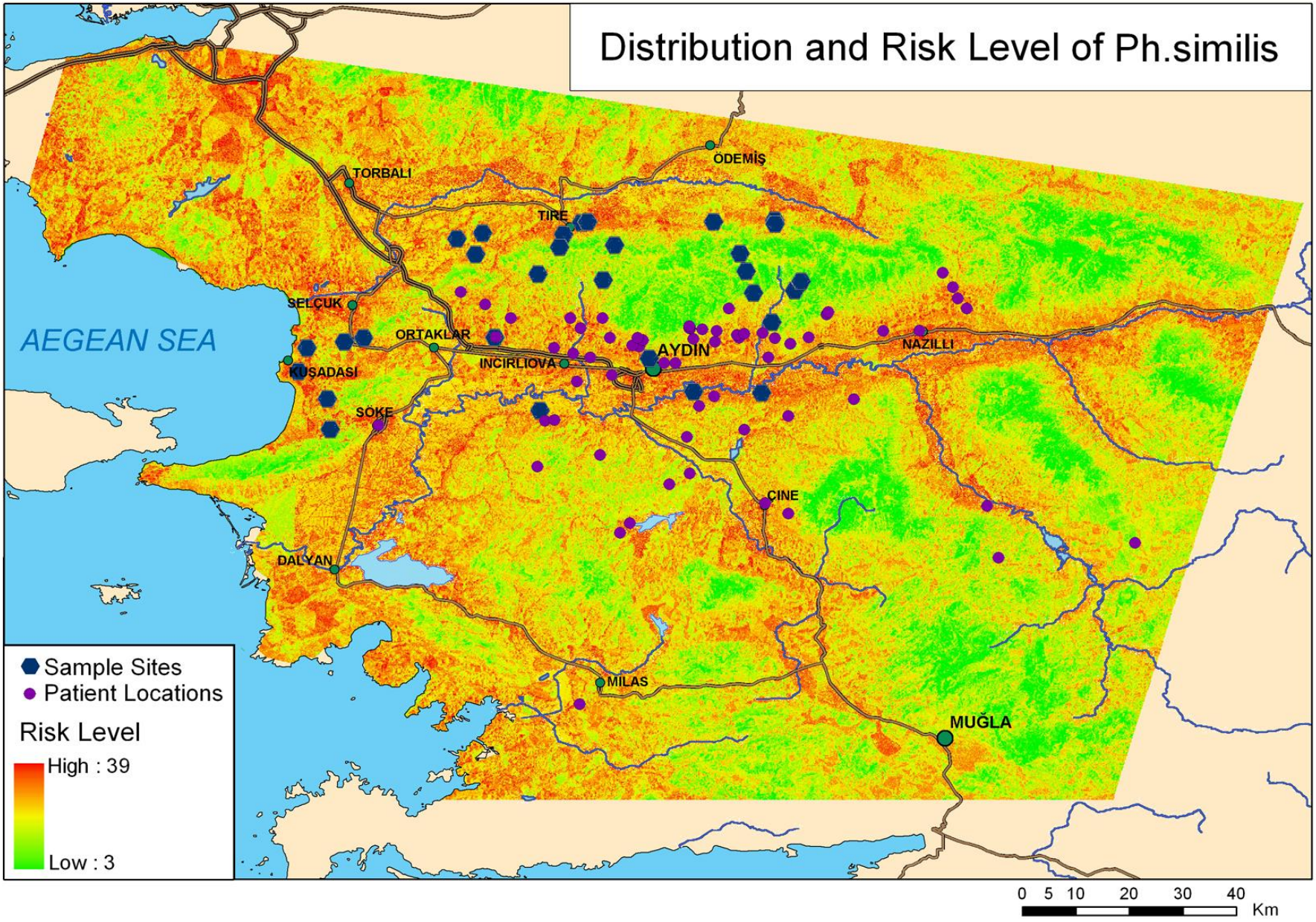
Useful for understanding the gaps in the region


P. (Larroussius) ariasi
Current known distribution: January 2014
Present
Antic. Absent
Obs. Absent
No data
Unknown
Outermost regions
Azores (PT)
Canary Islands (ES)
Madeira (PT)
Svalbard/Jan Mayen (NO)
ECDC 2014/VBORNET


P. (Phlebotomus) papatasi
Current known distribution: January 2014
Present
Antic. Absent
Obs. Absent
No data
Unknown
Outermost regions
Azores (PT)
Canary Islands (ES)
Madeira (PT)
Svalbard/Jan Mayen (NO)
ECDC 2014/VBORNET


Distribution and Risk Level of Ph.similis
AEGEAN SEA
TORBALI
ÖDEMİŞ
TİRE
SELÇUK
ORTAKLAR
AYDIN
NAZILLI
İNCİRLİOVA
KUŞADASI
SÖKE
ÇİNE
DALYAN
MİLAS
MUĞLA
Sample Sites
Risk Level
High : 39
Low : 3
0 5 10 20 30 40
Km

Validation of Risk Map



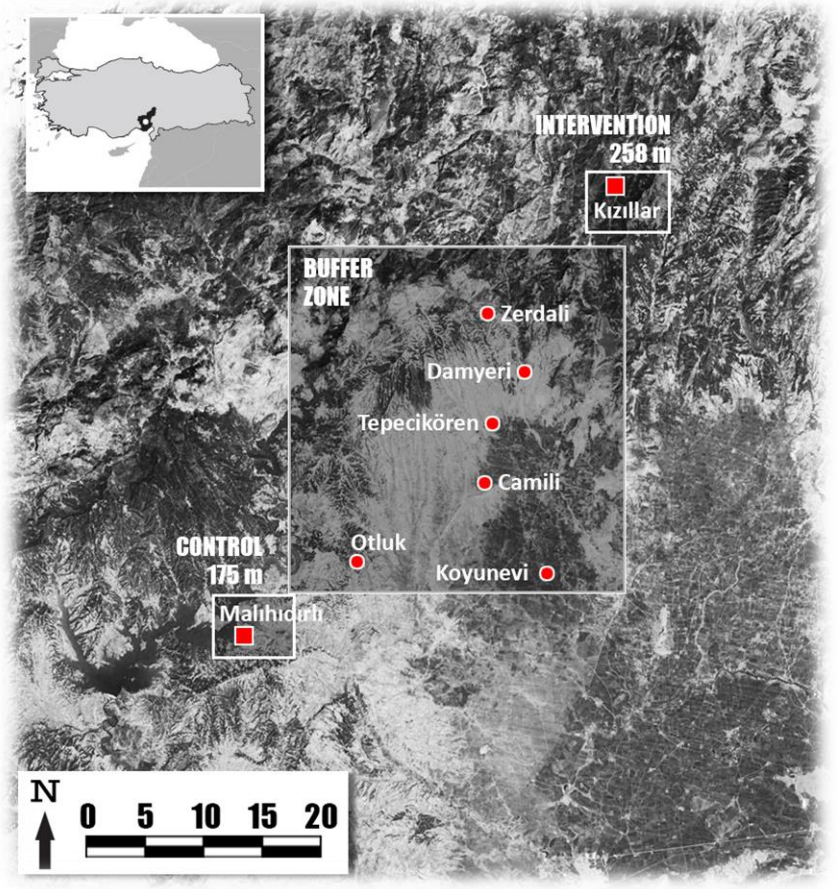
Conclusion (Surveillance)

- One shot is not enough (think for statisticians)
- International guideline for sand fly surveillance is necessary
- Datasets needs to be standardized for comparing or combining data in European and global level, if possible
- We cannot know everything, multidisciplinary studies are important

Examples of Control Measures

Impregnated Bed nets

- Eight villages endemic for CL were divided into three operational zones:
  - Intervention village:
    LLIN- Olyset® Plus (Sumitomo Co Ltd)
  - Control village:
    No bed net
  - Buffer Zone:
    Six villages between the above two villages



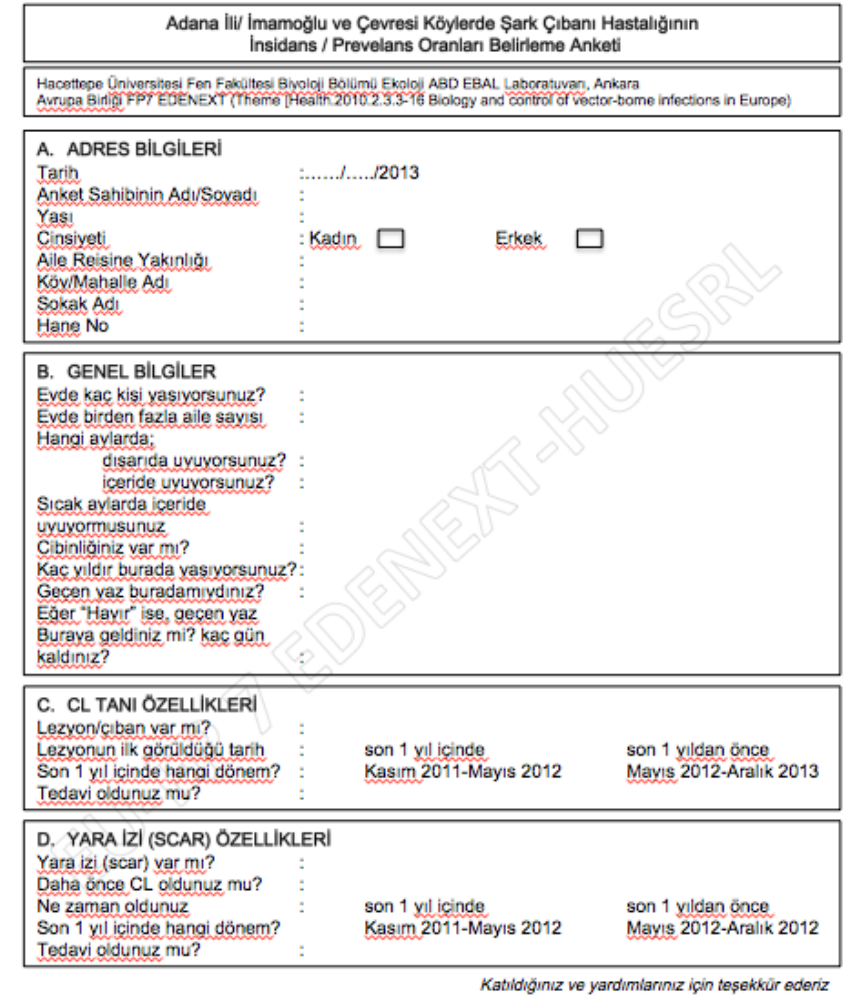
Impregnated Bednets - Questionnaire

Information about Address

How many people lives in same house
Any bednet usage history?

Any CL case before ?
Questions about treatment (if any)

Place of the CL scar
When first seen ?



Adana İli/ İmamoğlu ve Çevresi Köylerde Şark Çıbanı Hastalığının İnsidans / Prevelans Oranları Belirleme Anketi

Hacettepe Üniversitesi Fen Fakültesi Biyoloji Bölümü Ekoloji ABD EBAL Laboratuvarı, Ankara
Avrupa Birliği FP7 EDENEXT (Theme [Health.2010.2.3.3-1] Biology and control of vector-borne infections in Europe)

A. ADRES BİLGİLERİ

Tarih :...../...../2013
Anket Sahibinin Adı/Soyadı :
Yaşı :
Cinsiyeti : Kadın ☐ Erkek ☐
Aile Reisine Yakınlığı :
Köy/Mahalle Adı :
Sokak Adı :
Hane No :

B. GENEL BİLGİLER

Evde kaç kişi yaşıyorsunuz? :
Evde birden fazla aile sayısı :
Hangi avlarda:
dışarıda uyuyorsunuz? :
içeride uyuyorsunuz? :
Sıcak avlarda içeride uyuyormusunuz :
Cibinliğiniz var mı? :
Kaç yıldır burada yaşıyorsunuz? :
Geçen yaz buradamıydınız? :
Eğer "Hayır" ise, geçen yaz Buraya geldiniz mi? kaç gün kaldınız? :

C. CL TANI ÖZELLİKLERİ

Lezyon/çıban var mı?	:		
Lezyonun ilk görüldüğü tarih	:	son 1 yıl içinde	son 1 yıldan önce
Son 1 yıl içinde hangi dönem?	:	Kasım 2011-Mayıs 2012	Mayıs 2012-Aralık 2013
Tedavi oldunuz mu?	:		

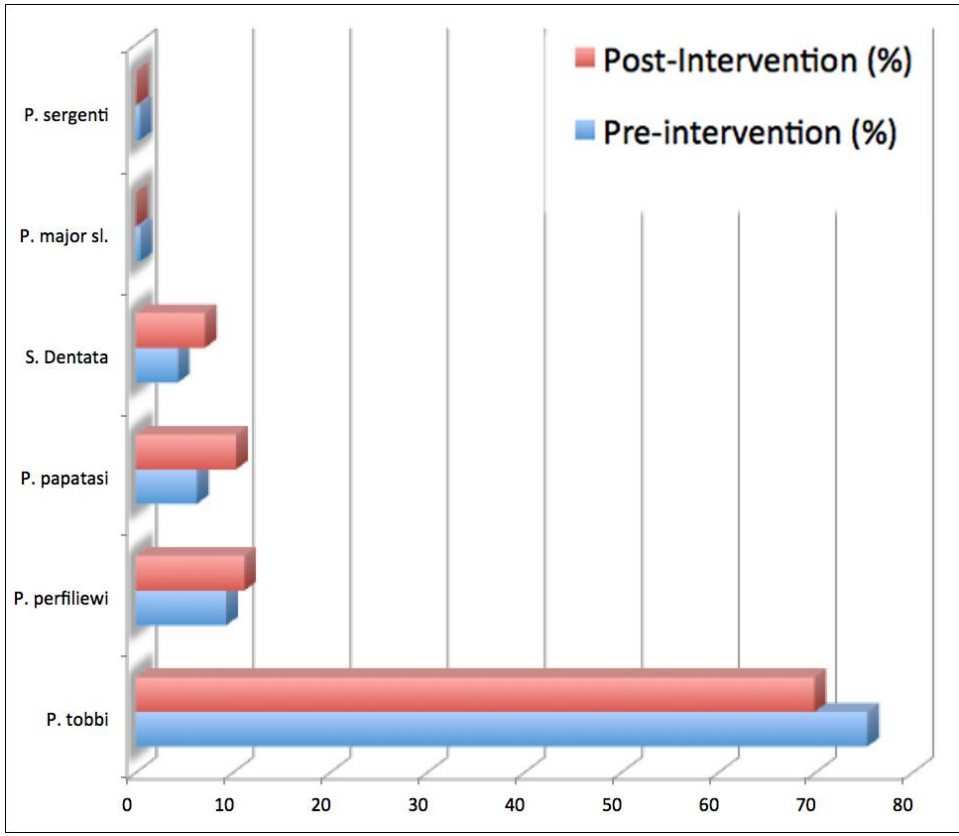
D. YARA İZİ (SCAR) ÖZELLİKLERİ

Yara izi (scar) var mı?	:		
Daha önce CL oldunuz mu?	:		
Ne zaman oldunuz	:	son 1 yıl içinde	son 1 yıldan önce
Son 1 yıl içinde hangi dönem?		Kasım 2011-Mayıs 2012	Mayıs 2012-Aralık 2012
Tedavi oldunuz mu?	:		

Katıldığınız ve yardımlarınız için teşekkür ederiz

Impregnated Bed nets - Entomology

- Entomological study:
  - CDC light traps
  - sticky paper traps
- The use of impregnated bed nets **did not effect on reducing the density of P. tobbi** in the intervention area compared to the control area



Impregnated Bed nets – Efficacy on CL prevalance

- More than 1800 inhabitants were included in this study
- 145 of them (7.25%) were infected before our study: Potentially immune to reinfection!
- Before the study, CL prevalence was remarkably high in both villages.

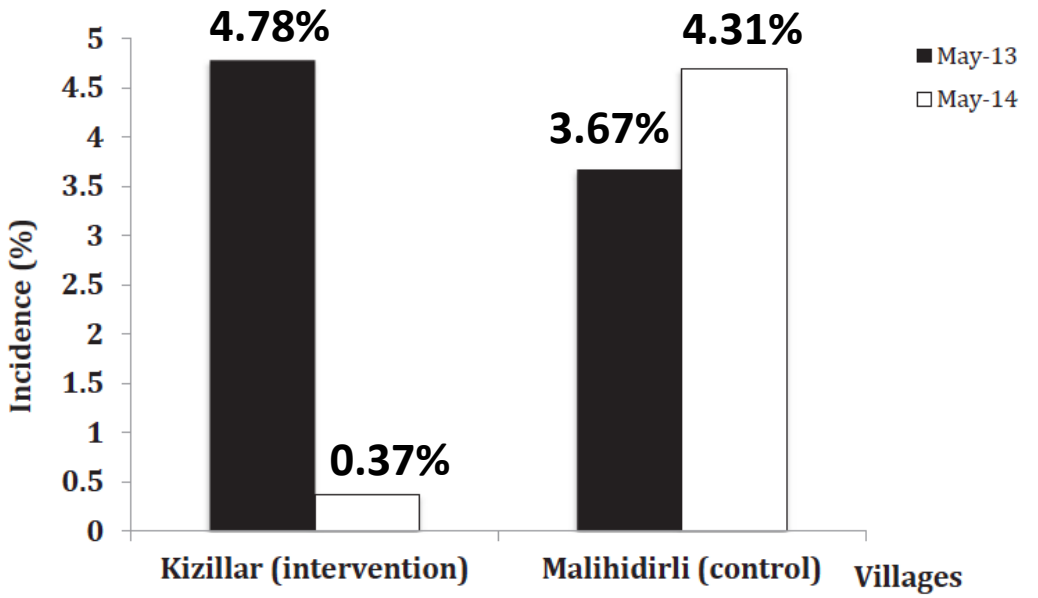


Figure 2. The annual changes of cutaneous leishmaniasis incidence (%) in intervention and control villages between May, 2013 and May, 2014.

The reduction rate of yearly CL cases was 92.2% !
It is reducing risk of CL infection

Evaluation of the efficacy of Olyset® Plus in a village-based cohort study in the Cukurova Plain, Turkey, in an area of hyperendemic cutaneous leishmaniasis

Filiz Gunay[1], Mehmet Karakus[2], Gizem Oguz[1], Mert Dogan[1], Yasemen Karakaya[1], Gokhan Ergan[1], Sinan Kaynas[3], Ozge Erisoz Kasap[1], Yusuf Ozbel[2], and Bulent Alten[1]✉

[1]*Hacettepe University, Faculty of Science, Department of Biology, Ankara, Turkey, kaynas@hacettepe.edu.tr*
[2]*Ege University, Faculty of Medicine, Parasitology Department, Bornova Izmir, Turkey*
[3]*Mehmet Akif Ersoy University, Veterinary Faculty, Burdur, Turkey*

Received 20 June 2014; Accepted 21 August 2014

chemical analysis

- The average permethrin and PBO contents were determined using the CIPAC method (www.cipac.org)

- There was no significant decrease of both permethrin and PBO content of Olyset® Plus under normal conditions of use over a one-year period

Larvacides

- Larvacides are using in various water sources for mosquito control
- **This is not applicable for sand flies**
- Insecticides (low dose) can be used in places where soil and organic wastes remain in the same place for >50 days
- This application is using for house flies

Indoor thermal fogging

- For increasing effectiveness, the application can be done in the time that sand flies are active

Outdoor fogging

- Can be applied for sand flies
- The application should be carried out at active hours of sand flies, when possible, to include resting sites

Outdoor cold fogging

- Cold fogging (ULV) can be done in the evening or night during summer months

Physical/Mechanical Measures

- Sand flies need to rest and hide for two reasons
  - For developing the eggs after sucking blood and mating
  - For protecting themselves from unsuitable climate conditions such as sun light, rain, strong wind, etc

- The resting places need to be minimized
- Improving the surfaces is also useful if any kind of insecticides are used
  - Insecticides stay longer if the surface is flat

Physical/Mechanical Measures

- In rural places, it is recommended that the trees where the sand flies are resting are lime-ridden up to 1-1.5 meters in height
- Because, sand flies climb to trees by hoping from the ground
- Pesticides used in agricultural areas also affect sand fly populations

Training workshops for Leishmaniasis

A new approach for controlling sand fly populations

Microbiome

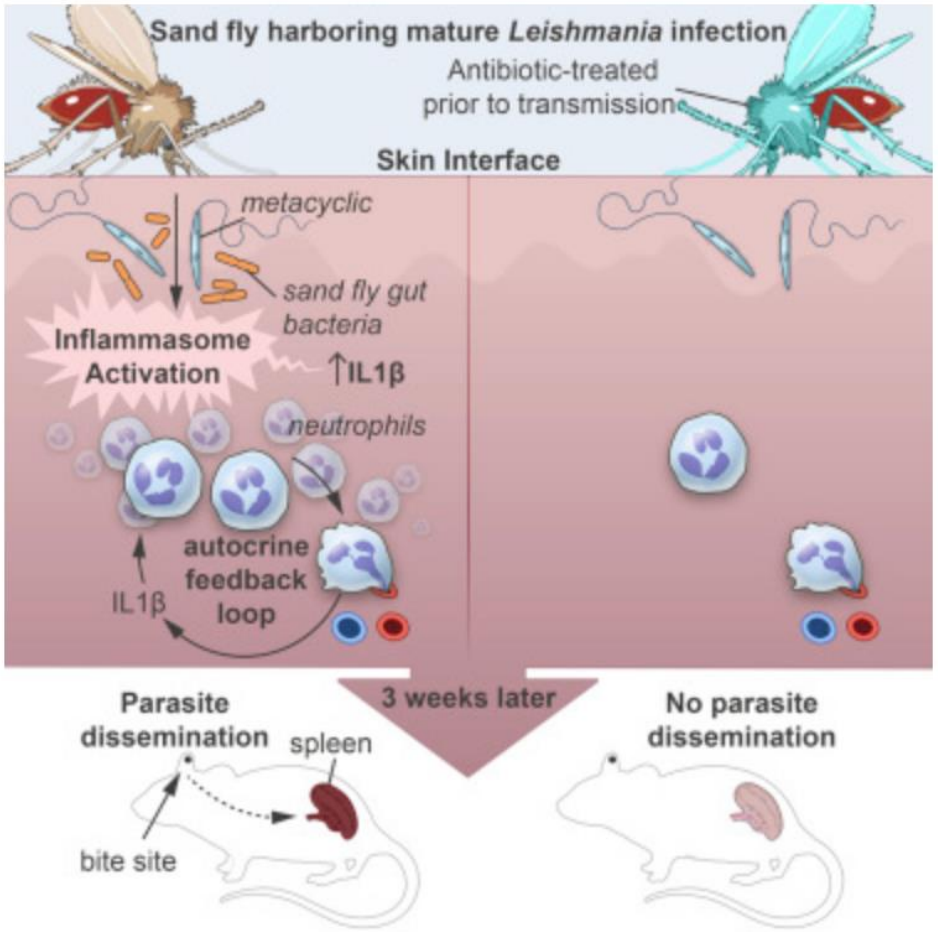
(bacterial microbiota)

Sand fly gut microbiota

- Microbiota studies in human and insects are getting increase
- Both sexes of sand flies feed on natural sugars, and only females feed on blood
- The interaction between midgut flora of sand fly and Leishmania has an importance, because metacyclogenesis takes place in the midgut and bacterial composition may either enhance or inhibit the parasitic activity
- We need to look for "**paratransgenic bacteria**" for controlling sand fly populations
  - **genetically transformed microbes to express anti-parasitic molecules for reducing the transmission**
- For this, suitable candidate bacteria need to be identified in the vector species and host-bacteria interactions need to be clarified

Sand fly gut microbiota are also transferred to the bite site, promoting neutrophil recruitment and parasite dissemination to distal organs
(*Aksoy S, Cell Host Microbe 2018, 23:8*)

- Sand fly gut microbes are egested into host skin alongside *Leishmania* parasites
- It triggers to start host inflammasome
- It means, vehicles come to biting site to carry Leishmania
- Giving antibiotics to sand flies before transmission abolishes neutrophil infiltration
- Abolishing neutrophil infiltration at bite sites impairs Leishmania dissemination



(Dey et al., 2018, Cell Host & Microbe 23:134)

www.nature.com/scientificreports

OPEN

Midgut Bacterial Diversity of Wild Populations of *Phlebotomus (P.) papatasi*, the Vector of Zoonotic Cutaneous Leishmaniasis (ZCL) in Turkey

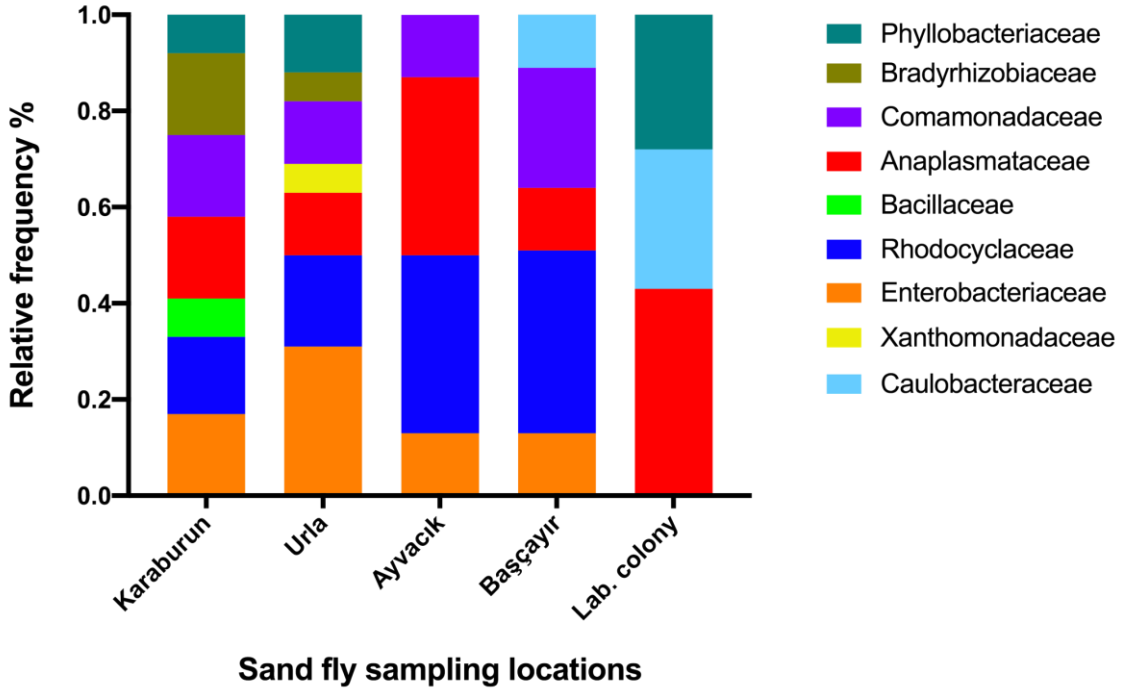
Received: 18 July 2017
Accepted: 3 October 2017
Published online: 01 November 2017

Mehmet Karakuş[1,4], Burçin Karabey[2], Şaban Orçun Kalkan[2], Güven Özdemir[2], Gizem Oğuz[3], Özge Erişöz Kasap[3], Bülent Alten[3], Seray Töz[1] & Yusuf Özbel[1]

- To identify the microbiome of different natural populations wild-caught and laboratory reared P. papatasi specimens
- To identify the possible paratransgenic bacteria candidates, which could be used in the control of sand flies/leishmaniasis
- Optimizing Denaturating Gradient Gel Electrophoresis (DGGE) for this analysis

Results

- 13 different bacteria belonging to nine families were identified from DGGE band sequences
- All specimens (from field and lab), including males, bear bacteria in their gut
- **Wolbachia** sp. was the most abundant, presented in all studied pools
- Bosea sp. and Variovorax sp. not detected only in field-collected specimens

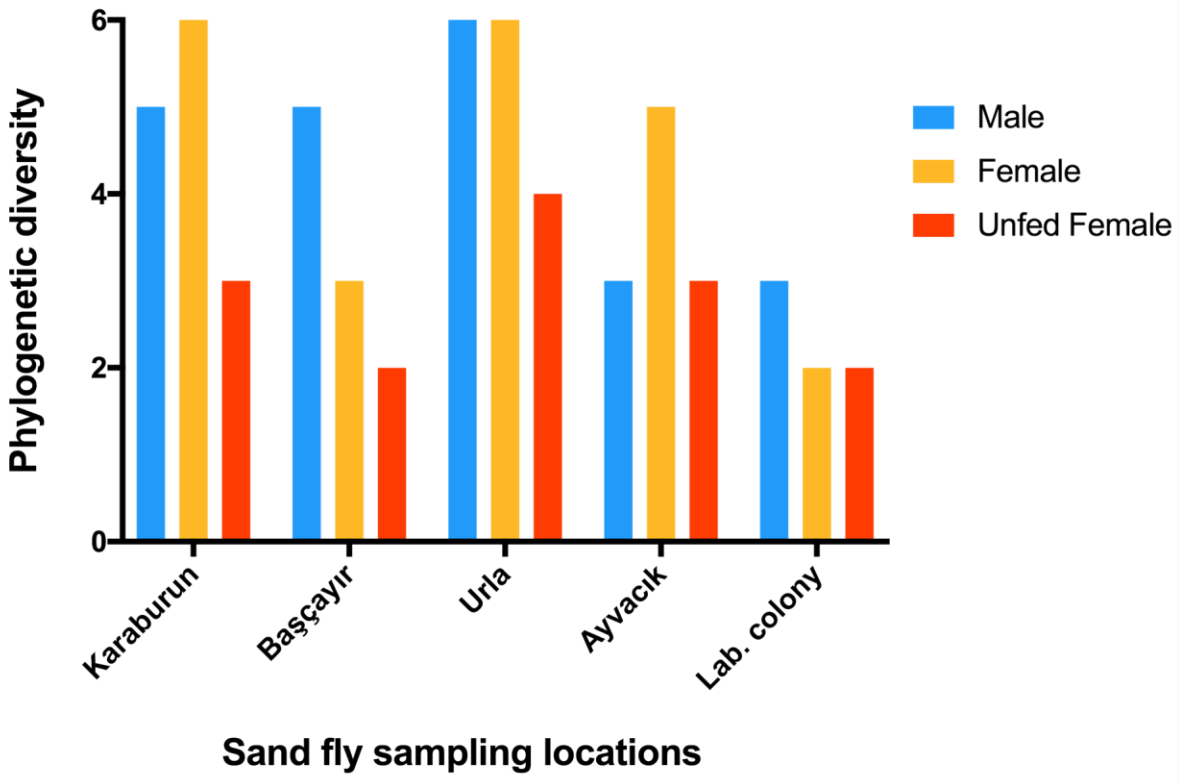


Relative abundance of gut bacteria of different P. papatasi populations

- Variovorax sp.
- Bosea sp.
- Brevundimonas sp.
- Ochrobactrum sp.
- Pantoea sp.
- Thauera sp.
- Wolbachia sp.
- Klebsiella sp.
- Bacillus cereus
- Erwinia aphidicola
- Serratia marcescens
- Candidatus ishikawaella capsulata
- Stenotrophomonas maltophilia

Results

Phylogenetic diversity of gut bacteria (in genus level) of male, unfed female and blood-fed female of P. papatasi populations



Female specimens have more bacterial diversity in their guts

<u>Thauera</u> sp. (10 isolates) was the most frequent bacteria among the field collected specimens

Lab colony has lowest diverse group of bacteria (3 taxa)

Conclusion

- Microbiome of four different populations of P. papatasi was not exactly similar
  - Same species – similar vegetation – different bacterial diversity
  - For SF collection in the field: different ecological environment & altitude needs to be selected
- Presence of sand fly symbiont bacteria, Wolbachia, in natural populations of Turkey and possible control agents were identified
- Further studies are needed to identify bacterial diversity of wild populations of other vector SF species
- Our next studies will aim the paratransgenic use of these identified bacteria as a vector control agent

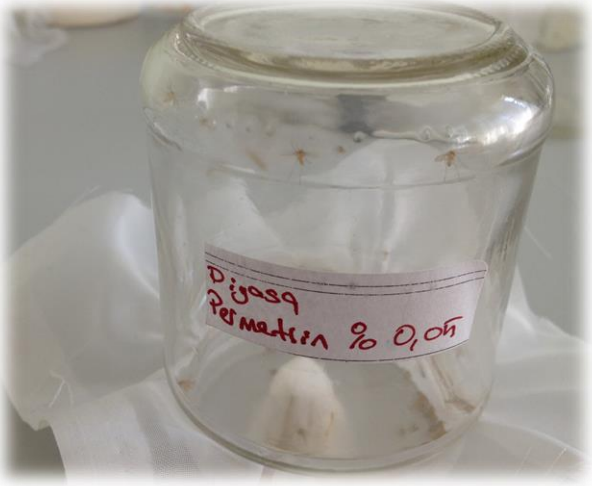
Problems in Insecticide Tests

Disadvantages of WHO's tube test

- **Not suitable for sand flies** (because of their size – physical damage)
- Not designed for the flying habit of the sand flies
- Impregnated papers can only be used up to 5 times

Disadvantages of CDC's Bottle Assay

- Not suitable for field work
- Droplets may occur inside the bottle because of the changing humidity
- The sand fly specimens can easily adhere to the drops



Newly designed insecticide test kits are necessary for sand flies

Acknowledgements

10th International Symposium On Phlebotamine Sandflies

Thank you very much for your ATTENTION