

The *Leishmania* collection of Montpellier (France), a tool for studying taxonomy, phylogeography and epidemiology.

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Backgrounds

- **1956** : starting work on leishmaniasis in Montpellier
- **1971** : starting strain collection (Laboratoire d'Ecologie Médicale et pathologie parasitaire, Pr J.A. Rioux)
- **1979** : starting isoenzymatic identification
- **1990** : Rioux's classification of *Leishmania* based on isoenzymes
- **1998** : Creation of the Centre National de Référence des *Leishmania* (French Ministry of Health)
- **1998** : starting molecular identification
- **2002** : Emerging Biological Resource Center
- **2004-2008** : WHO Collaborative Centre for Leishmaniasis

Fully equipped technical capacity

- **Security laboratories** for handling *Leishmania* strains

level 2

level 3



1. The *Leishmania* collection of Montpellier

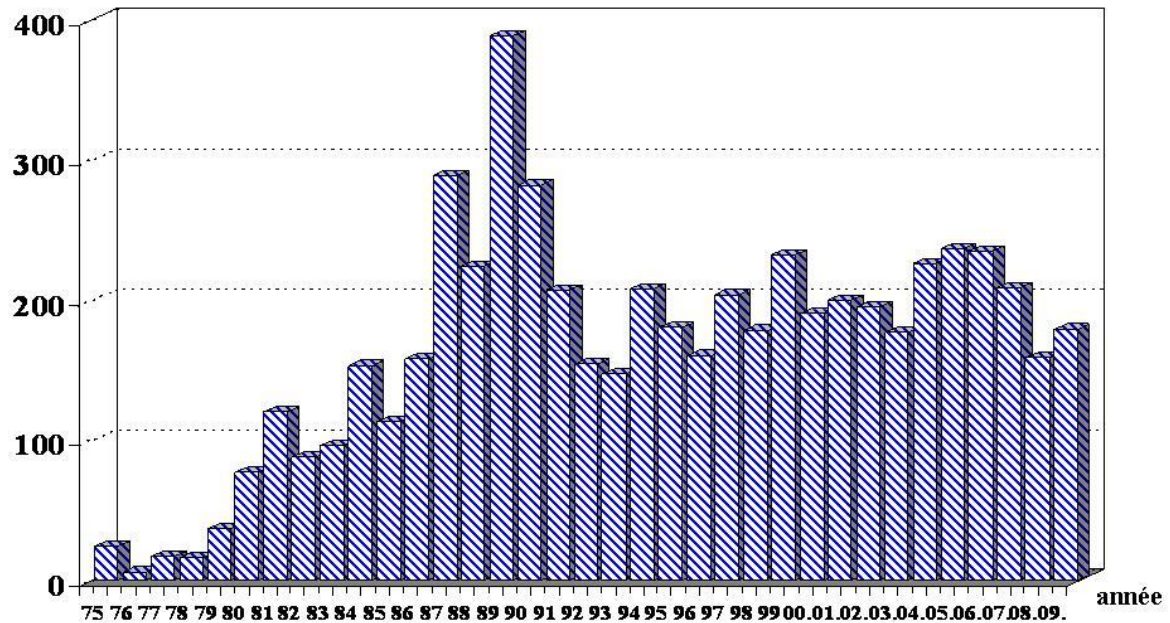
- **Missions :**
 - **Cryopreservation (collection)**
 - **Distribution of (reference) strains**
 - **Training and technology transfers**

A specifically equipped room for cryopreservation in liquid nitrogen



- **a unique collection, started in 1971**
- which contains at the present time :
 - **6,053 *Leishmania* strains**
 - **from 72 countries**
 - **in 4 continents**

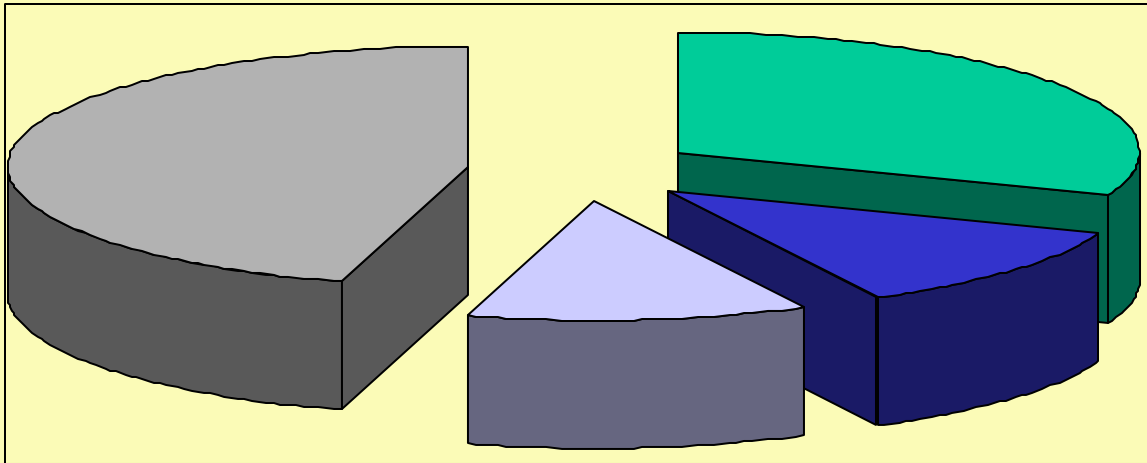
Centre National de référence des *Leishmania*.
Nombre de souches stockées annuellement de 1975 à 2009



Geographic origin of the strains

Europe : 45.5%

Africa : 30.7%



Asia : 12.1%

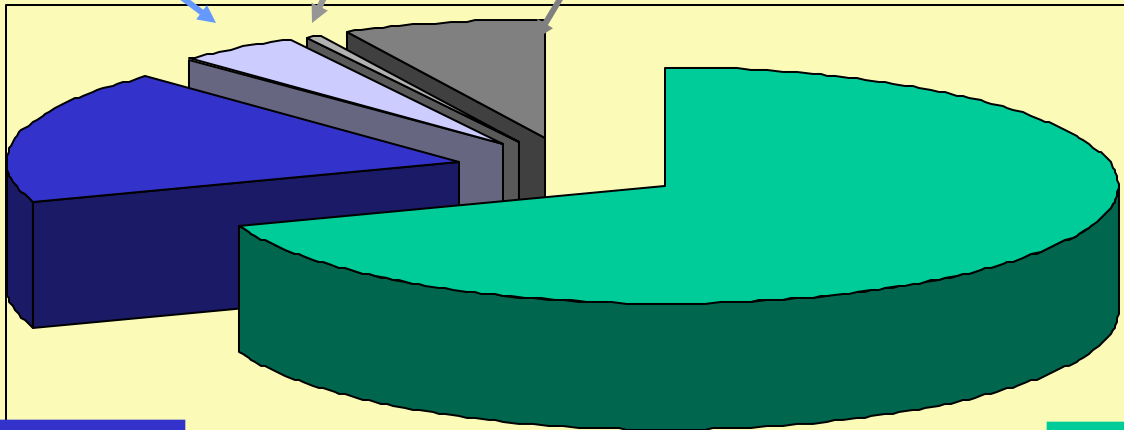
America : 11.7%

Origin host of the strains

Other mammals : 0.4%

Rodents : 4.6%

Sandflies : 7.2%



Dogs : 18.3%

Humans : 69.5%

Barcode-based management for computer processing of

- culture tubes or flasks
- storage cryotubes



Quality management system

Distribution of strains

- **Reference strains** (WHO reference strains, zymodeme markers, others) on request
- **Curator (Dr F. Pratlong) helps for strain choice**
- **Between 1998 and 2009 :**
 - **1,326 strains were provided**
 - 907 to French labs
 - 419 to foreign labs

Training

- **56 trainees received (1985-2009)**
- **from 20 countries**
 - **Europe** : Albania, France, Greece, Hungary, Italy, Spain, Portugal, UK
 - **Africa** : Algeria, Burkina Faso, Cameroon, Morocco, Tunisia, Sudan
 - **Asia** : Jordan, Syria, West-Bank
 - **America** : Brazil, Peru, USA

Technology transfert

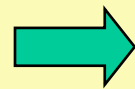
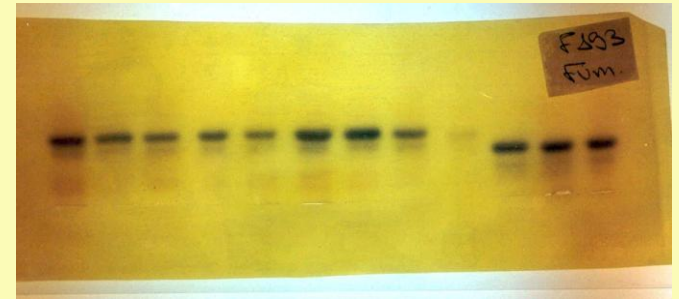
- **Help in establishing National and Regional *Leishmania* Cryobank and Identification Centres :**
 - Instituto de Salud Carlos III (Madrid, Spain)
 - Istituto Superiore di Sanita (Roma, Italy)
 - Institut Pasteur d'Algérie (Algiers, Algeria)
 - Lab. Parasitology, Faculty of Pharmacy (Monastir, Tunisia)
 - Lab. Parasitology, Faculty of Pharmacy (Barcelona, Spain)

2. Identification techniques

- **Isoenzymatic identification**
- **Molecular identification**

Isoenzymatic identification (MLEE)

- **Current technique :**
 - **Starch gel electrophoresis using** 15 enzymatic systems (Rioux et al., 1990)
- **Complémentary technique :**
 - **Iso-electrofocusing**



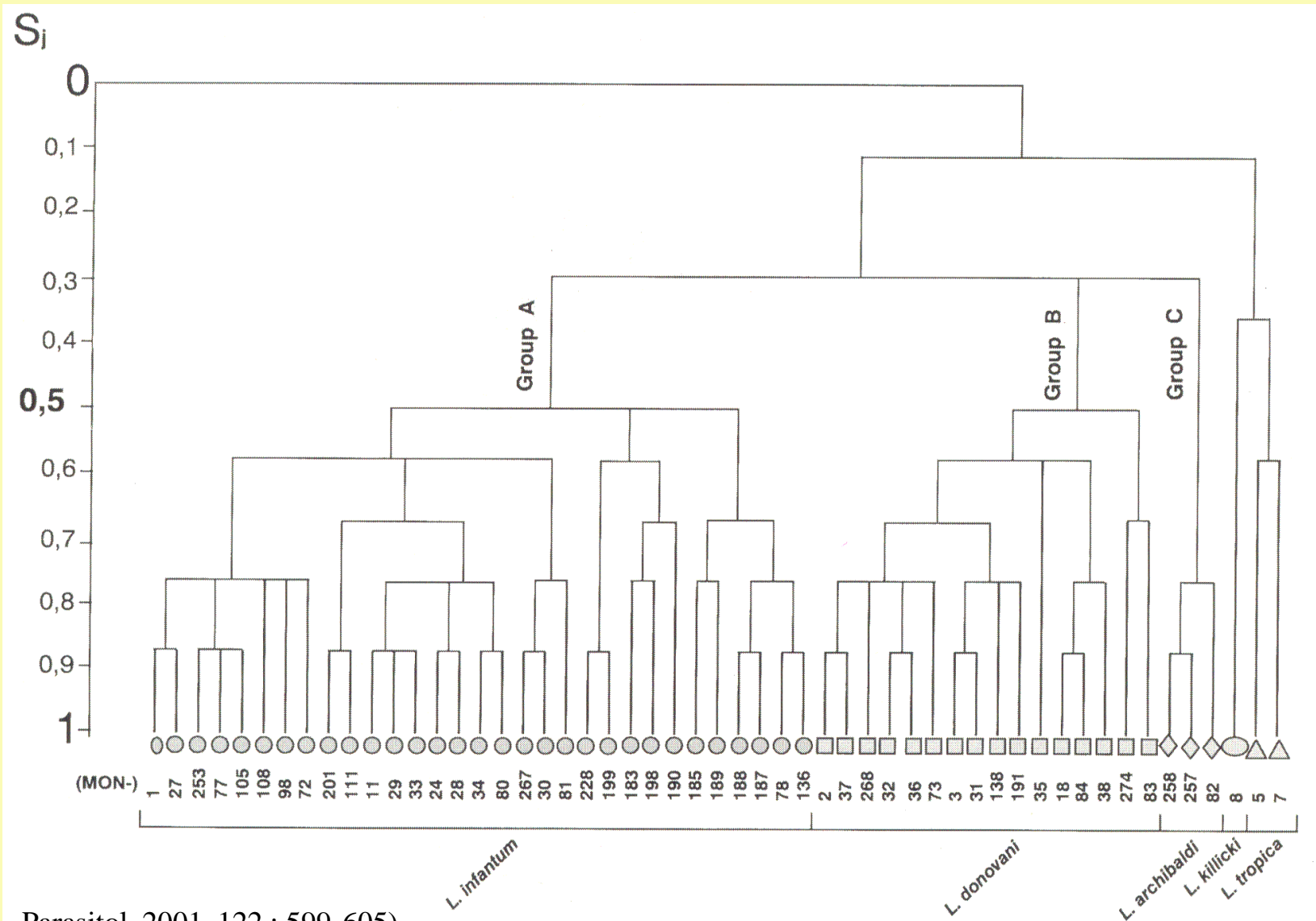
Taxa, Zymodeme MON-

(time limit : 1 to 1.5 months)

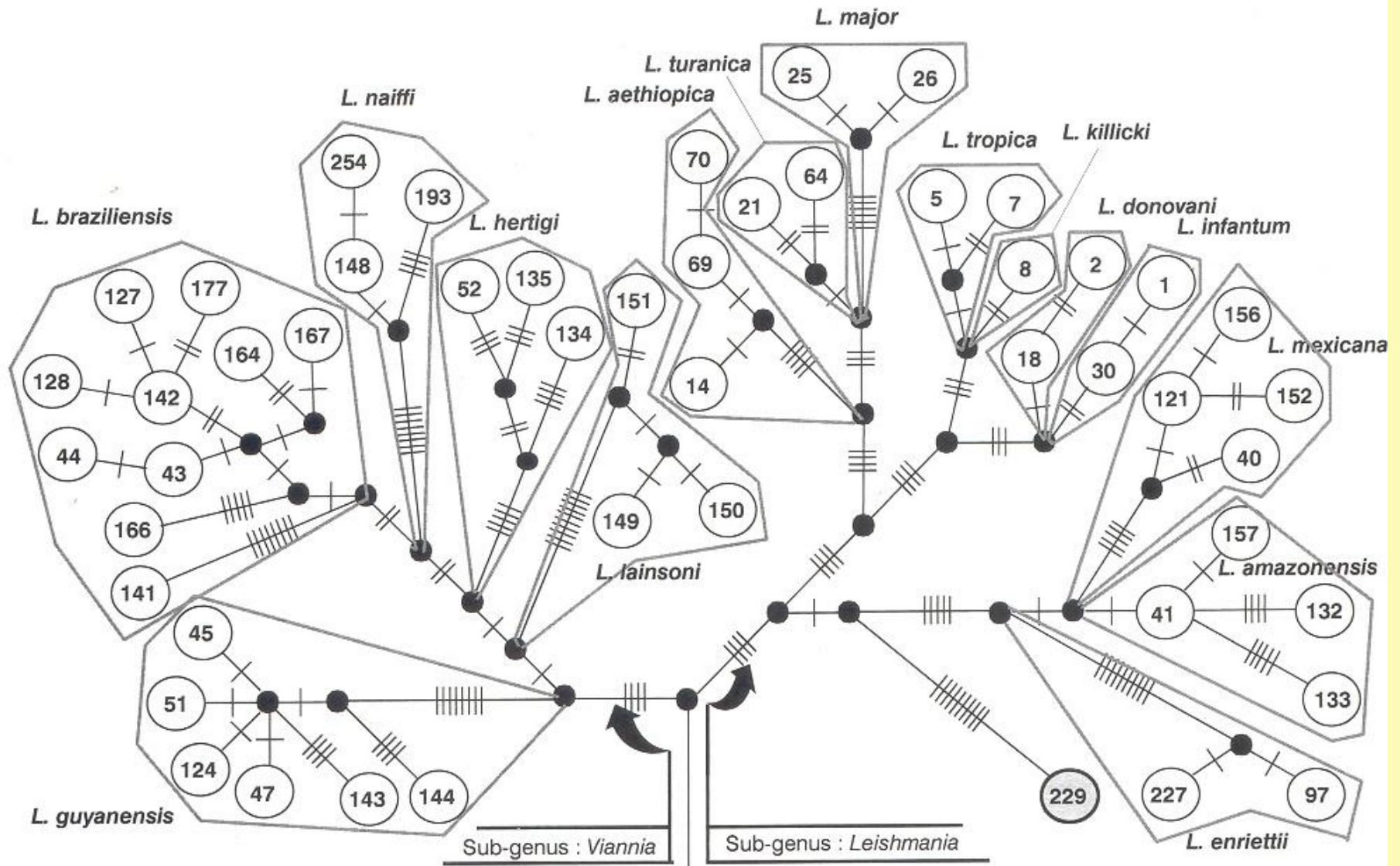
Applications :

- **Taxonomic studies**
- **Eco-epidemiological studies of foci**

Numerical taxonomy : phenetic tree of Old World viscerotropic species



The phylogenetic tree of the *Leishmania* genus

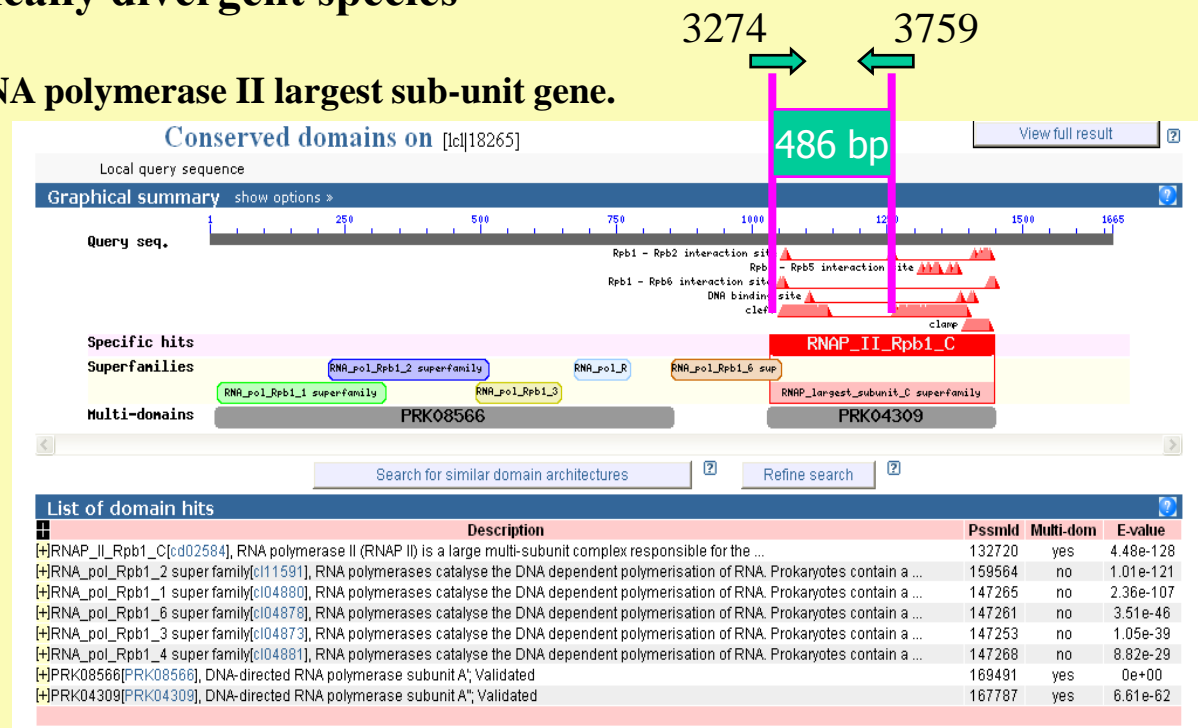


Molecular identification

MLST (Multilocus Sequence Typing)

- Single copy sequences
- Genetically independent
- Usable on genetically divergent species

RNA polymerase II largest sub-unit gene.



MLST

7 genetically independent loci analysed

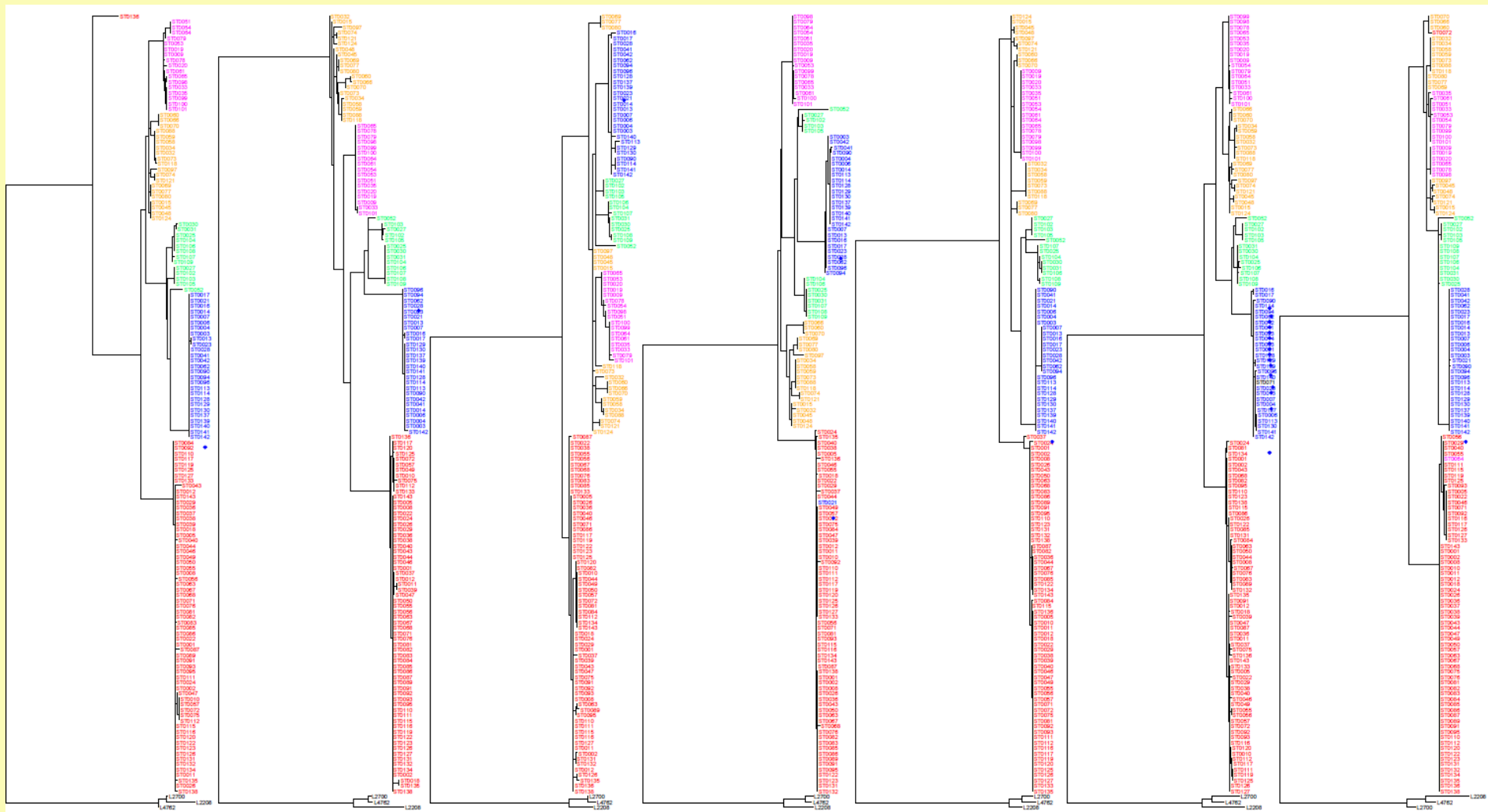
Locus	CDS	Size analysed
03.0980	Elongation initiation factor 2 alpha subunit	678 bp
04.0580	Spermidine synthase I	711 bp
10.0560	Zinc binding dehydrogenase-like protein	636 bp
12.0010	Translation initiation factor eIF-2B alpha subunit	714 bp
14.0130	Nucleoside hydrolase like protein	642 bp
31.0280	Hypothetical protein	810 bp
31.2610	RNA polymerase II largest subunit	486 bp

Total 4677 pb

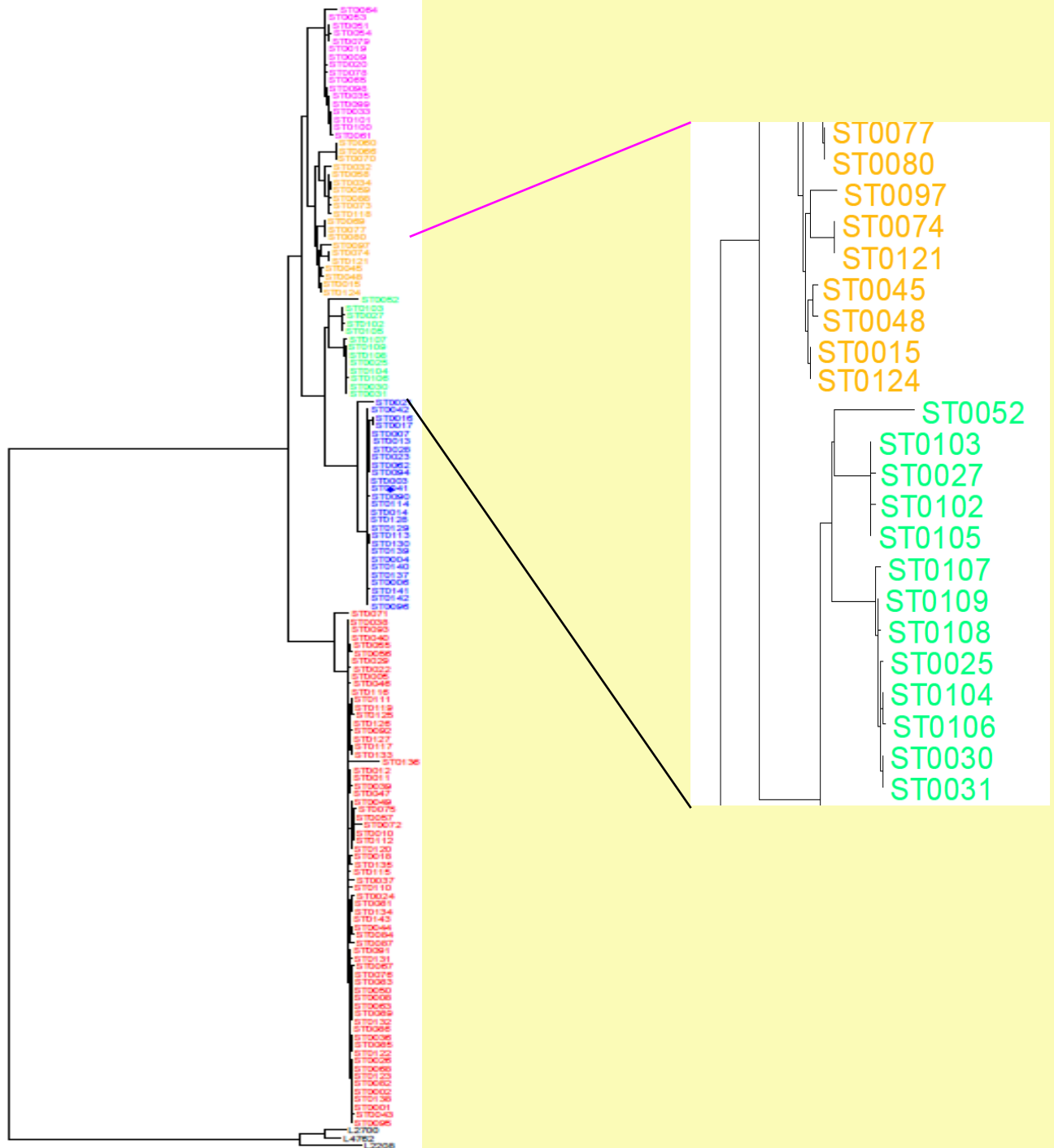
About 240 Leishmania strains (24 species) analysed

MLST

Clustering on 7 loci (Old World strains)



Concatenated sequences (Old World strains)



3. Biogeographical epidemiology of Old World *Leishmania*

Large sample : 3,324 strains, 34 years, 47 countries

Tropical Medicine and International Health

doi:10.1111/j.1365-3156.2009.02336.x

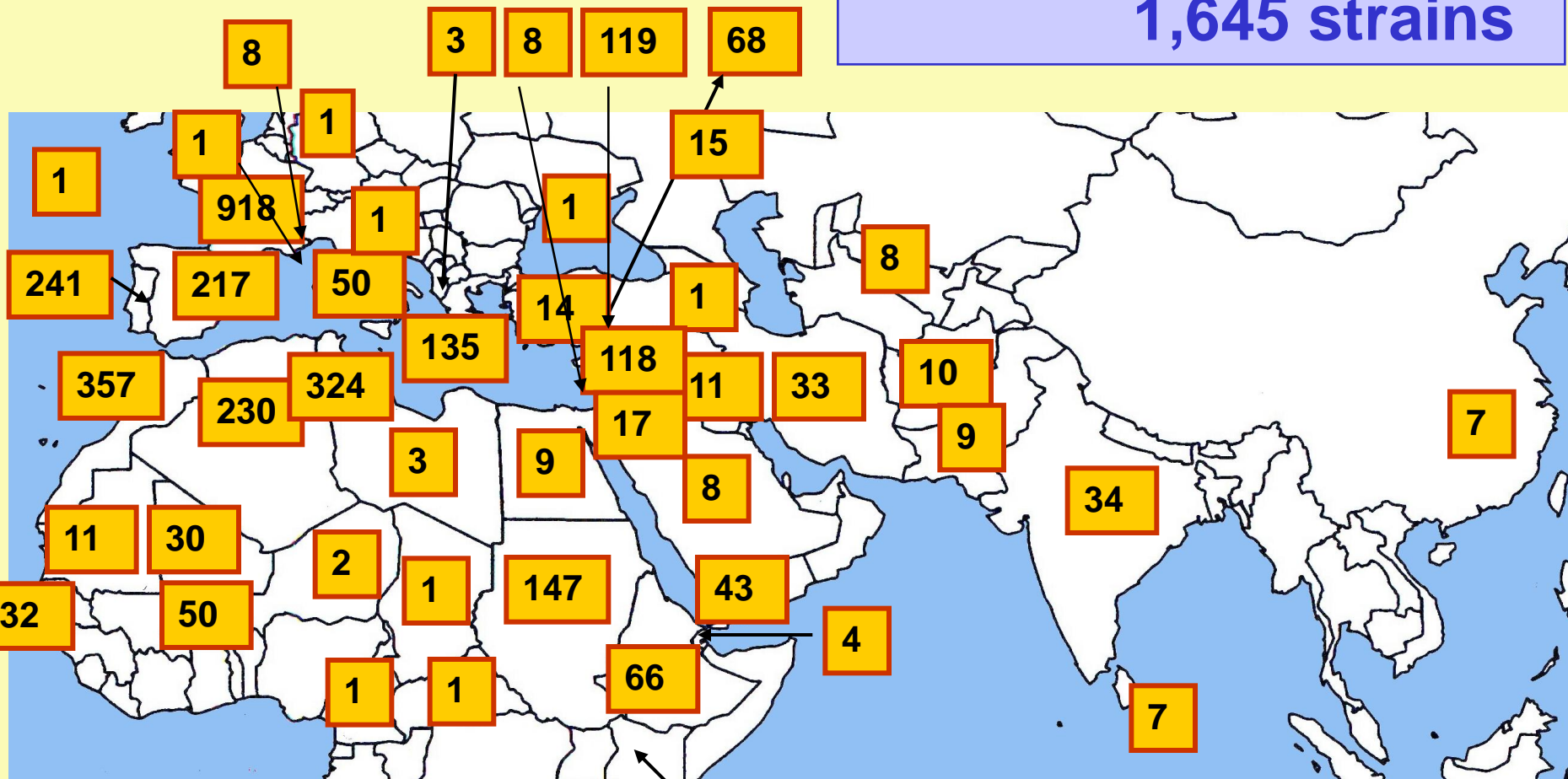
VOLUME 14 NO 9 PP 1071-1085 SEPTEMBER 2009

Geographical distribution and epidemiological features of Old World cutaneous leishmaniasis foci, based on the isoenzyme analysis of 1048 strains

Francine Pratlong, Jacques Dereure, Christophe Ravel, Patrick Lami, Yves Balard, Ghislaine Serres, Geneviève Lanotte, Jean-Antoine Rioux and Jean-Pierre Dedet

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Europe 13 countries
1,645 strains



Africa 17 countries
1,289 strains

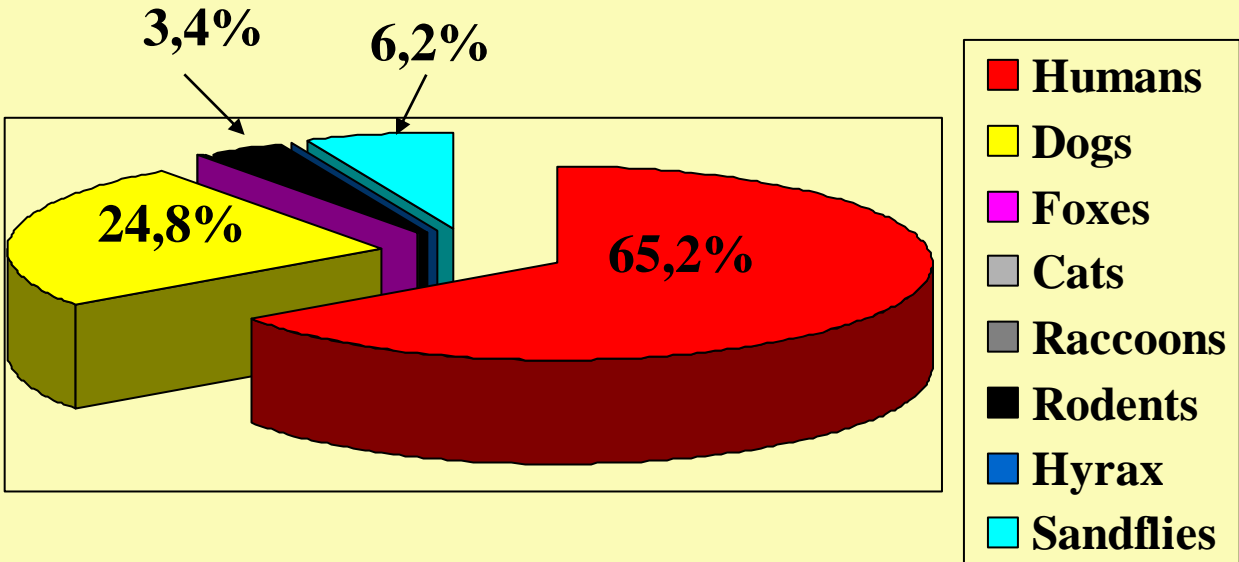
Asia 17 countries
390 strains

Leishmania major

- wild zoonosis (Cricetid rodents)
- arid and desertic climate



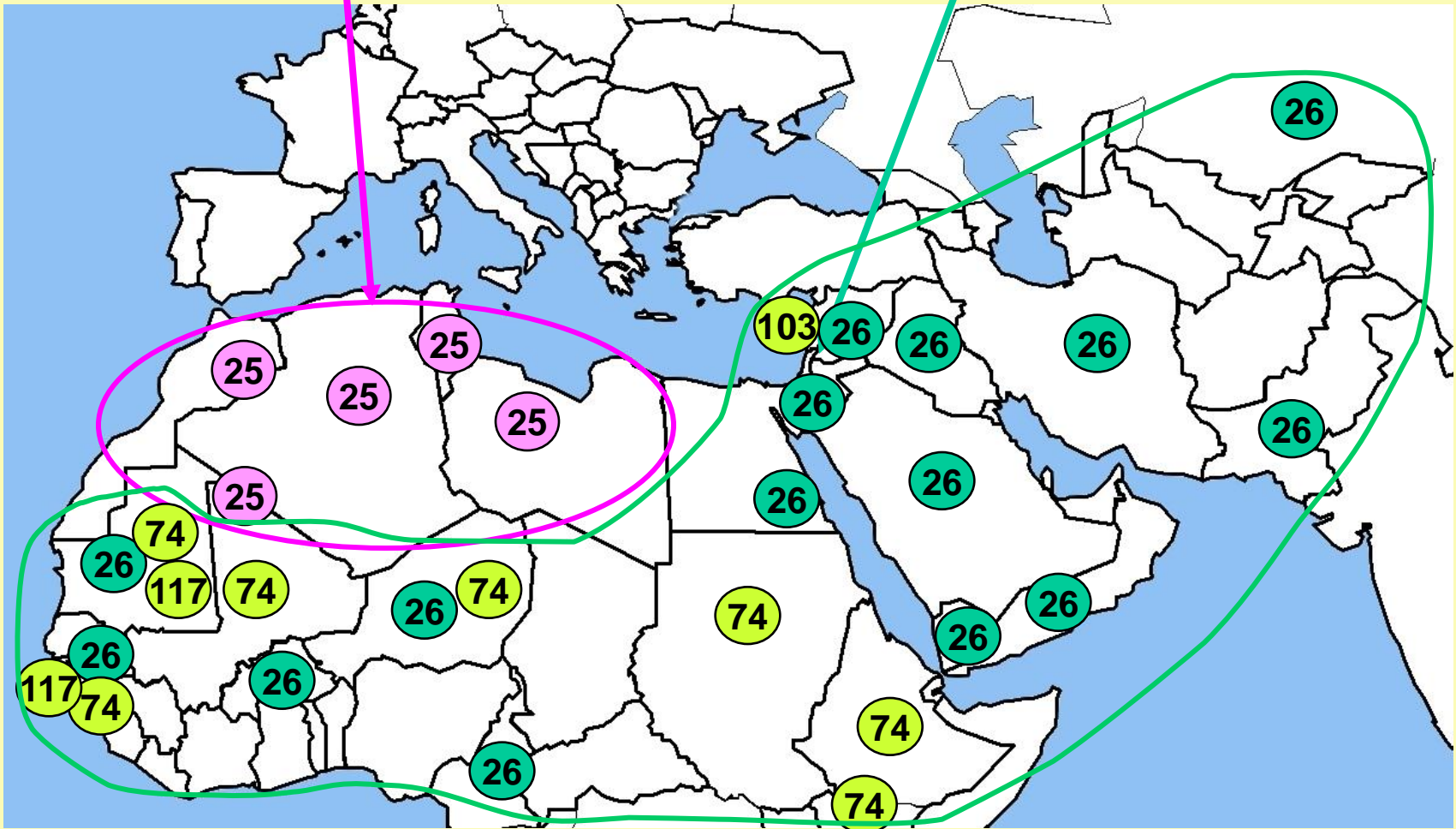
% *Leishmania* strains (n = 638) per host (25 countries)



Distribution of the 12 *L. major* zymodemes

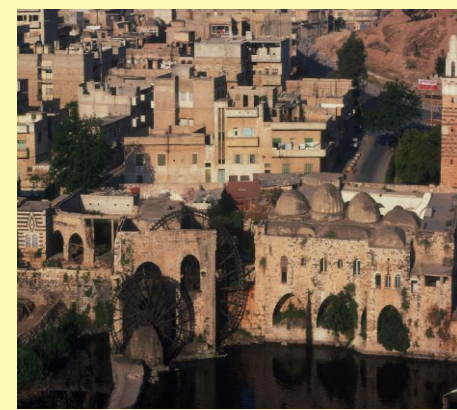
Maghreb : 1 zymodeme MON-25

Israël/Palestine : 5 zymodemes



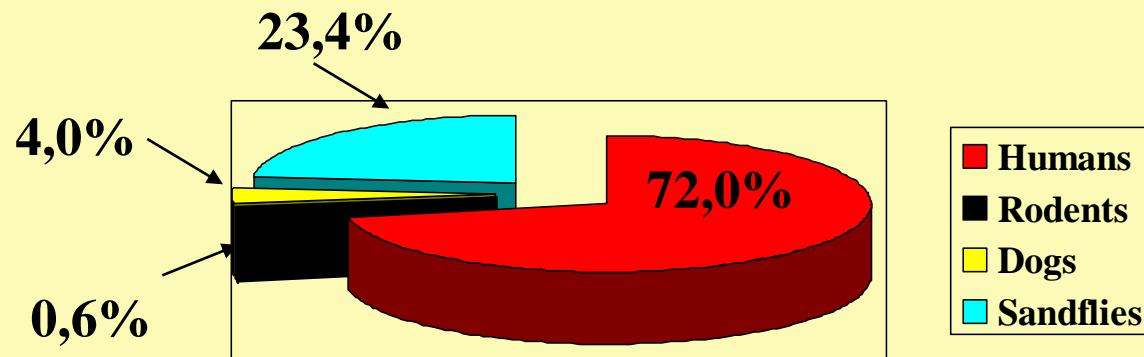
Leishmania tropica

- anthroponotic cutaneous leishmaniasis
- urban, endemic, possible epidemic outbreaks

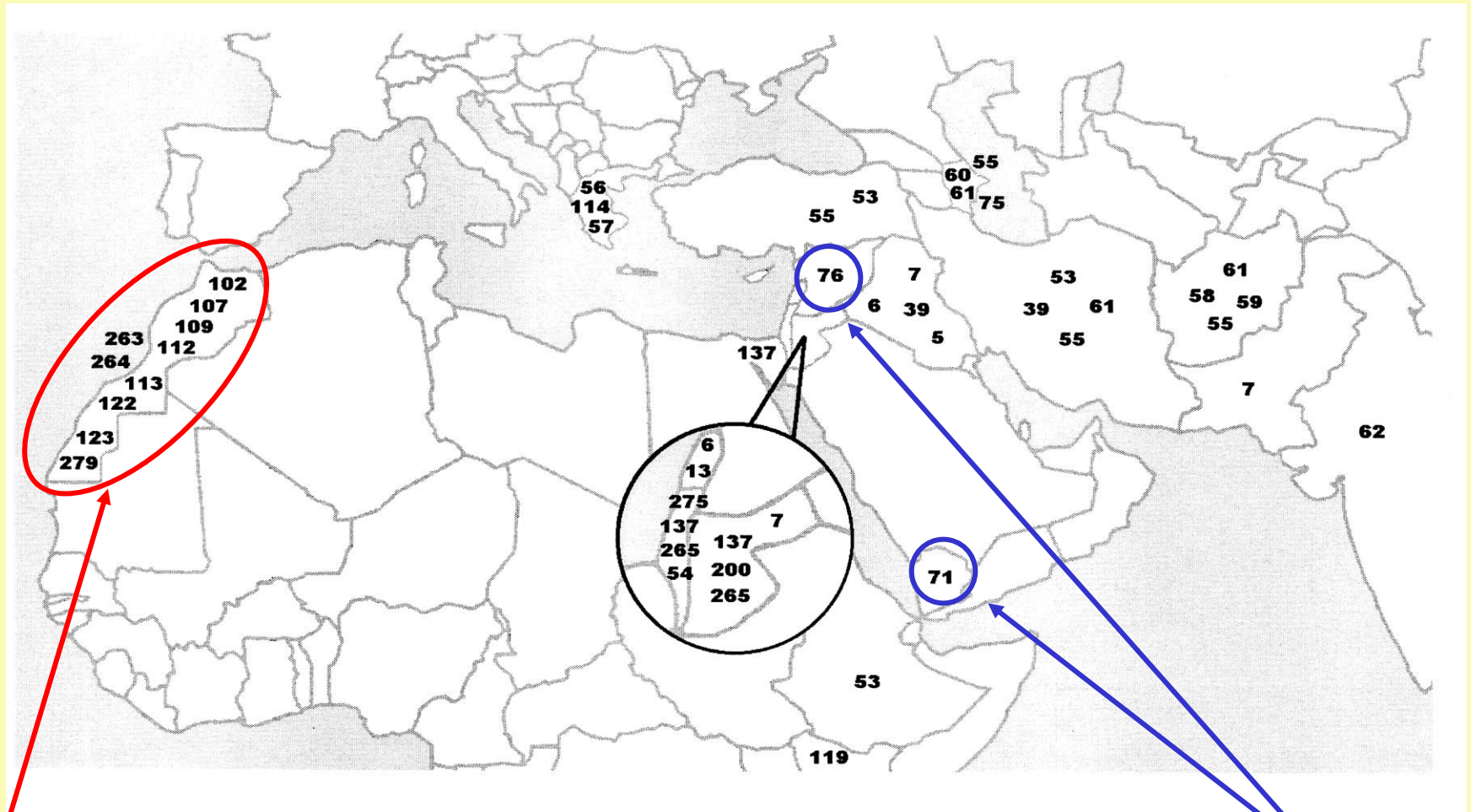


% *Leishmania* strains (n = 329) per host

(17 countries)



Distribution of the 35 *L. tropica* zymodemes (highly diversified electromorphs)



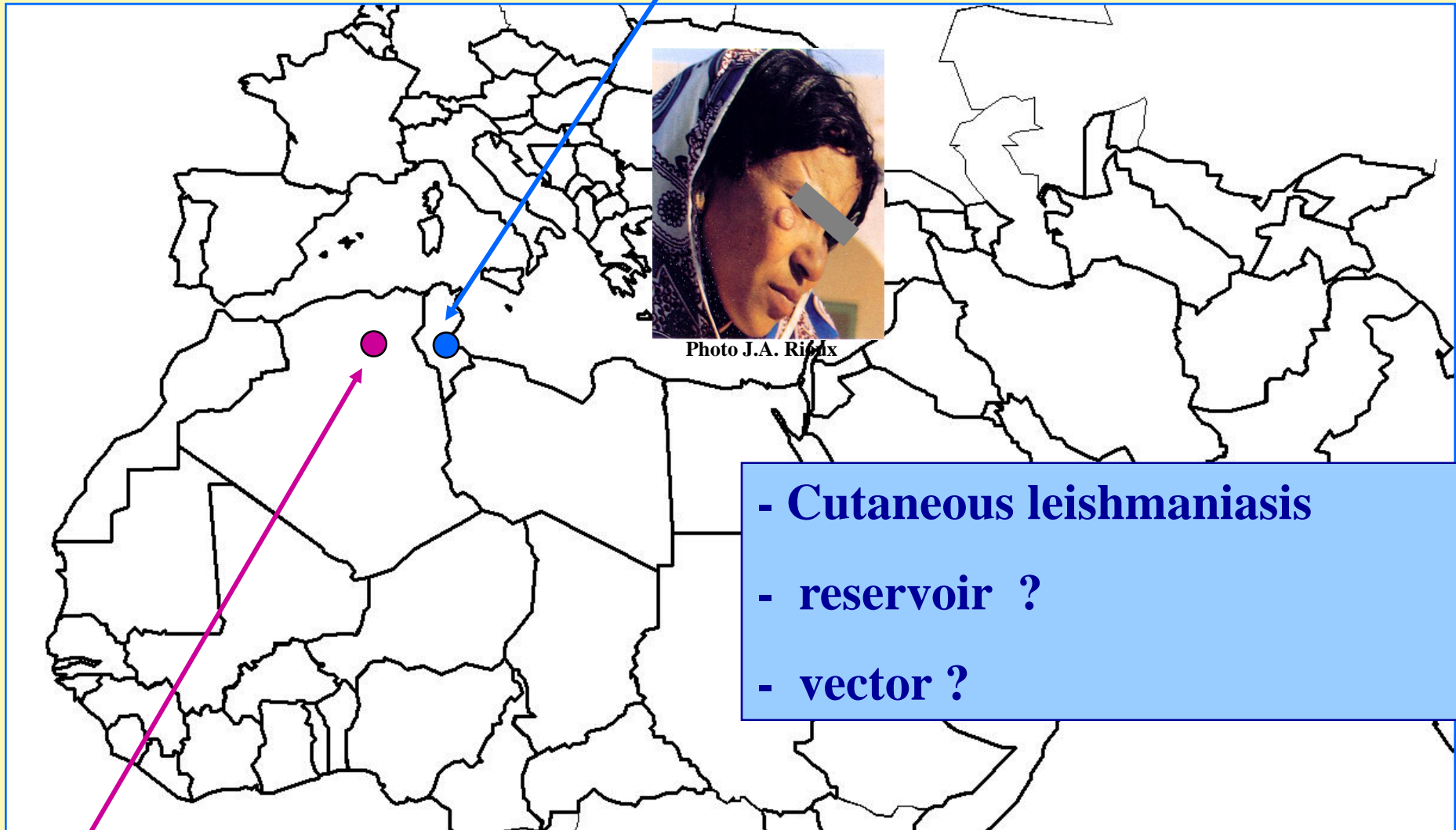
Highly variable enzyme polymorphism according to countries

10 zymodemes

1 zymodeme *

***Leishmania killicki* MON-8**

(39 strains)



- **Cutaneous leishmaniasis**
- **reservoir ?**
- **vector ?**

Leishmania* MON-301 close to *L. killicki

(2 strains)

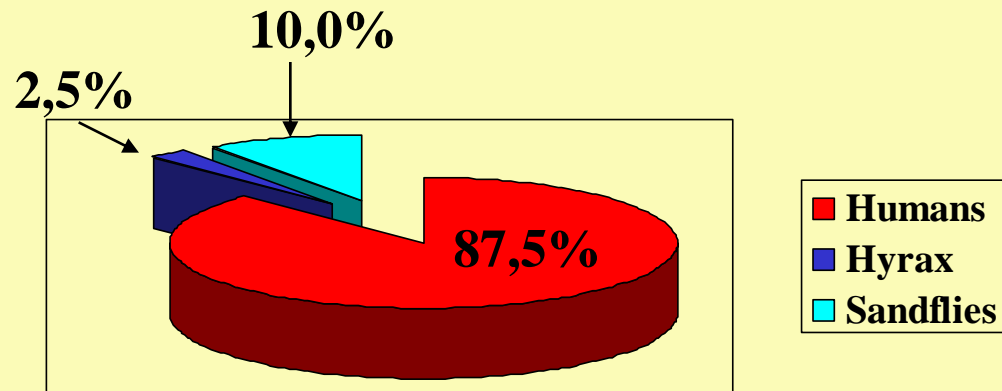
(Harrat et al 2009 in press)

Leishmania aethiopica

- zoonotic cutaneous leishmaniasis
- rural, Ethiopian highlands



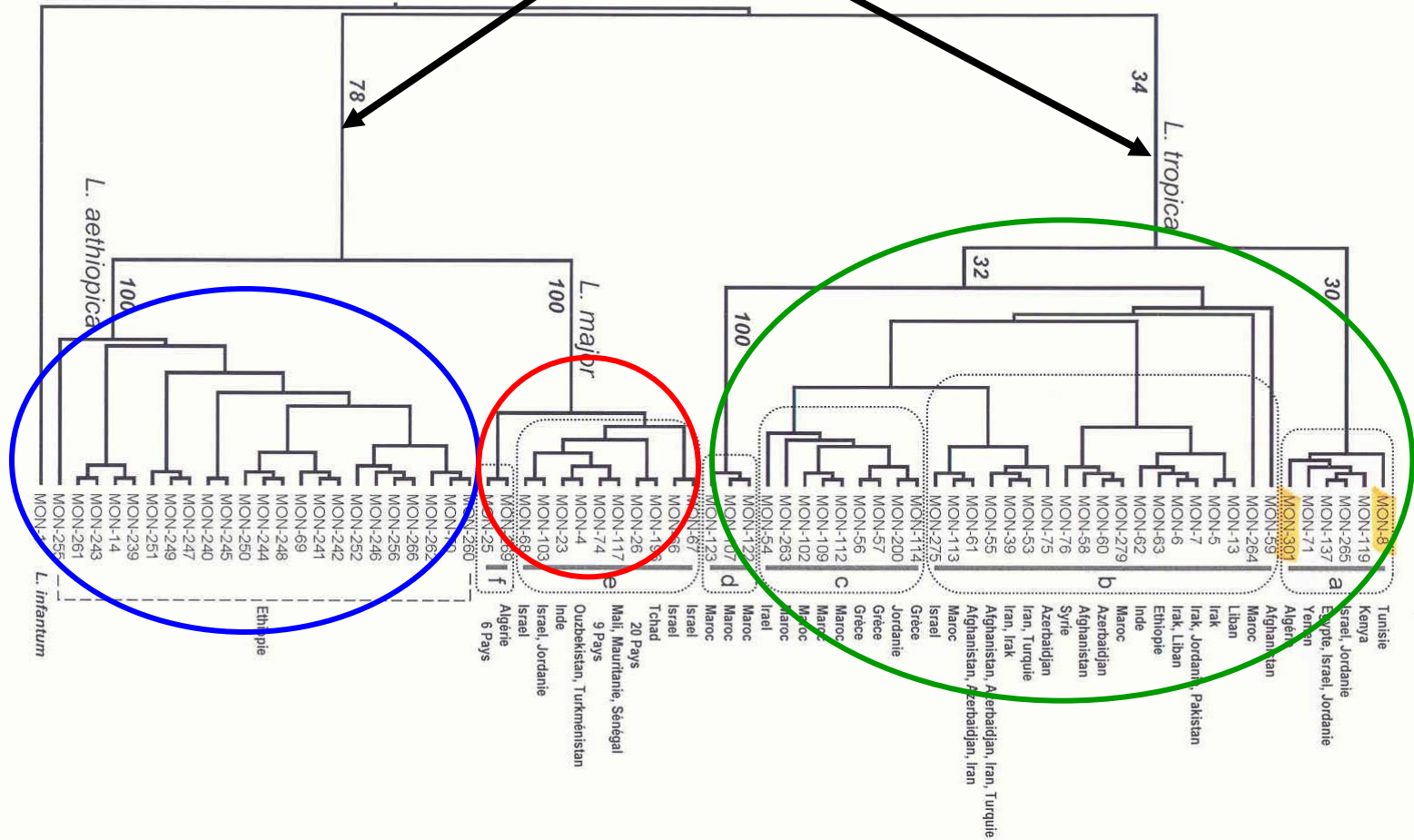
% *Leishmania* strains (n = 40) per host



23 different zymodemes

Clusters *L. aethiopica*, *L. major* and *L. tropica*

2 main groups



L. major

- Low polymorphism (12 zymodemes)
- Large geographic distribution of zymodemes MON-25, MON-26
→ Weak structuration of populations

L. tropica

- High polymorphism (35 zymodemes)
- Polymorphism highly variable according to the foci : Morocco 10 zymodemes, Syria and Yemen 1 zymodeme
→ Strong structuration of populations

L. aethiopica

- High polymorphism (23 zymodemes) with numerous intraspecific hybrids
- Localised geographic distribution

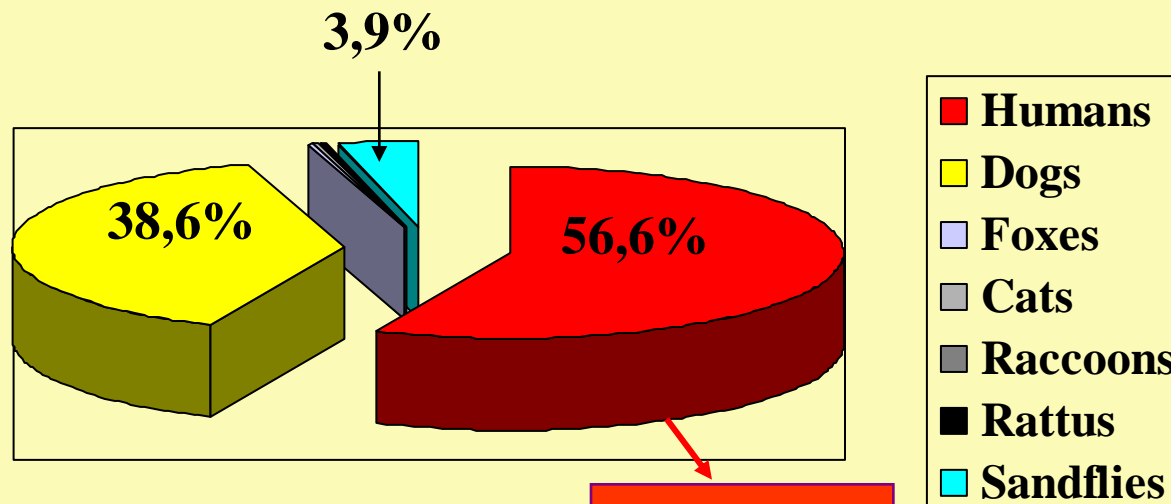
L. infantum

- zoonotic (Canids) VL
- Rural disease (Old & New Worlds)



% *Leishmania* strains (n = 2,061) per host

(28 countries)

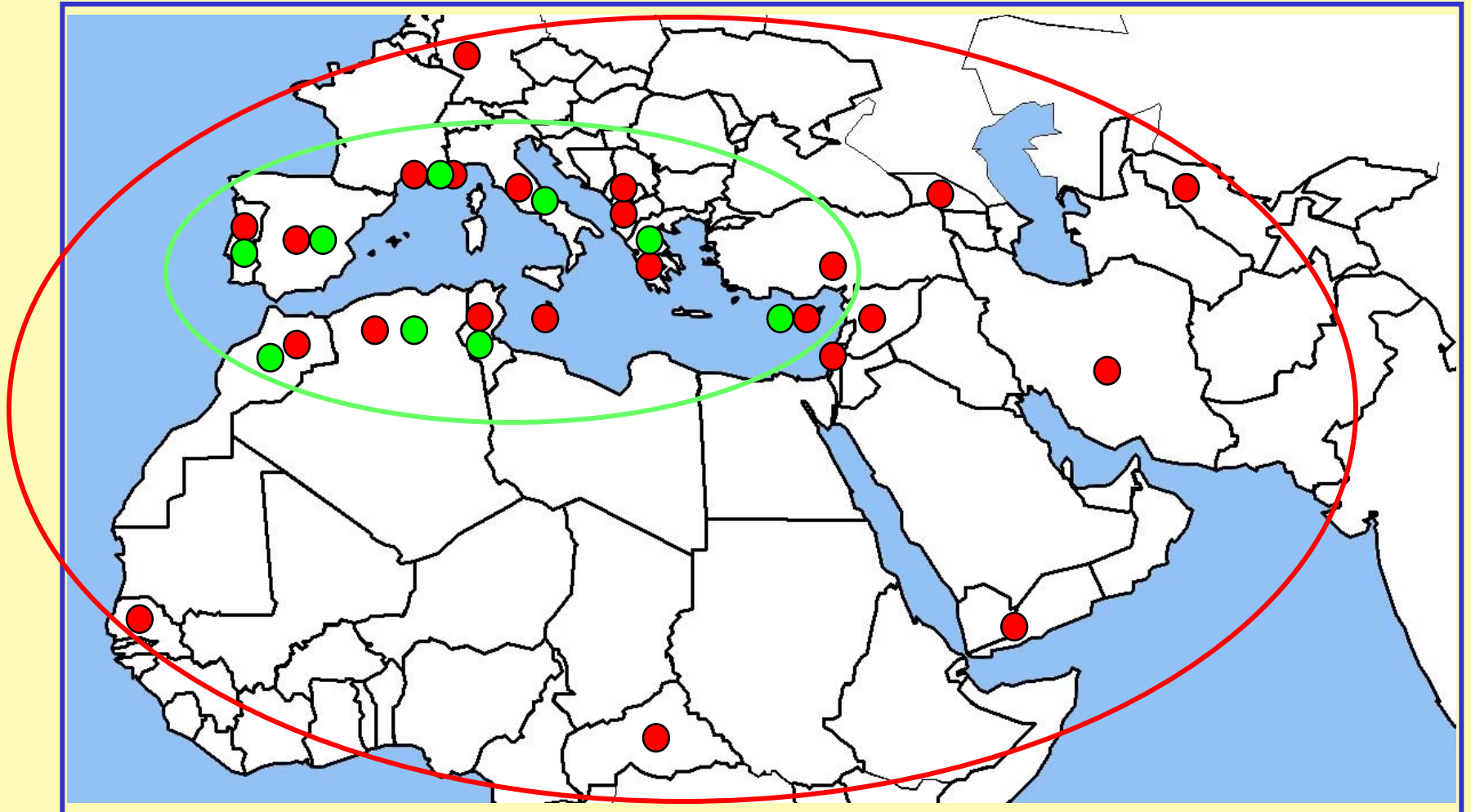


VL : 77.3 %

CL : 20.8 %

ML : 0.4 %

Distribution of *L. infantum* zymodemes (n=37)



Zymodemes MON-1 ● → 61.7 % of strains
MON-24 ●

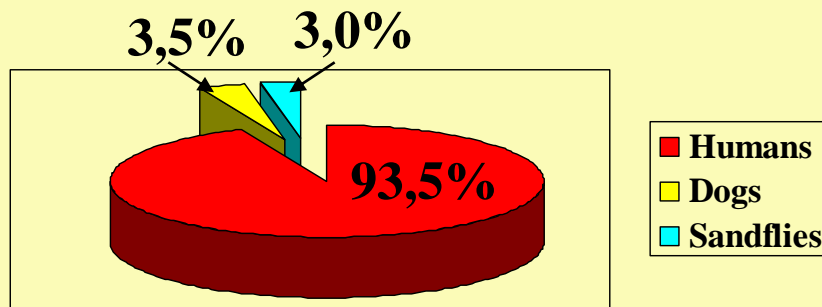
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L. donovani

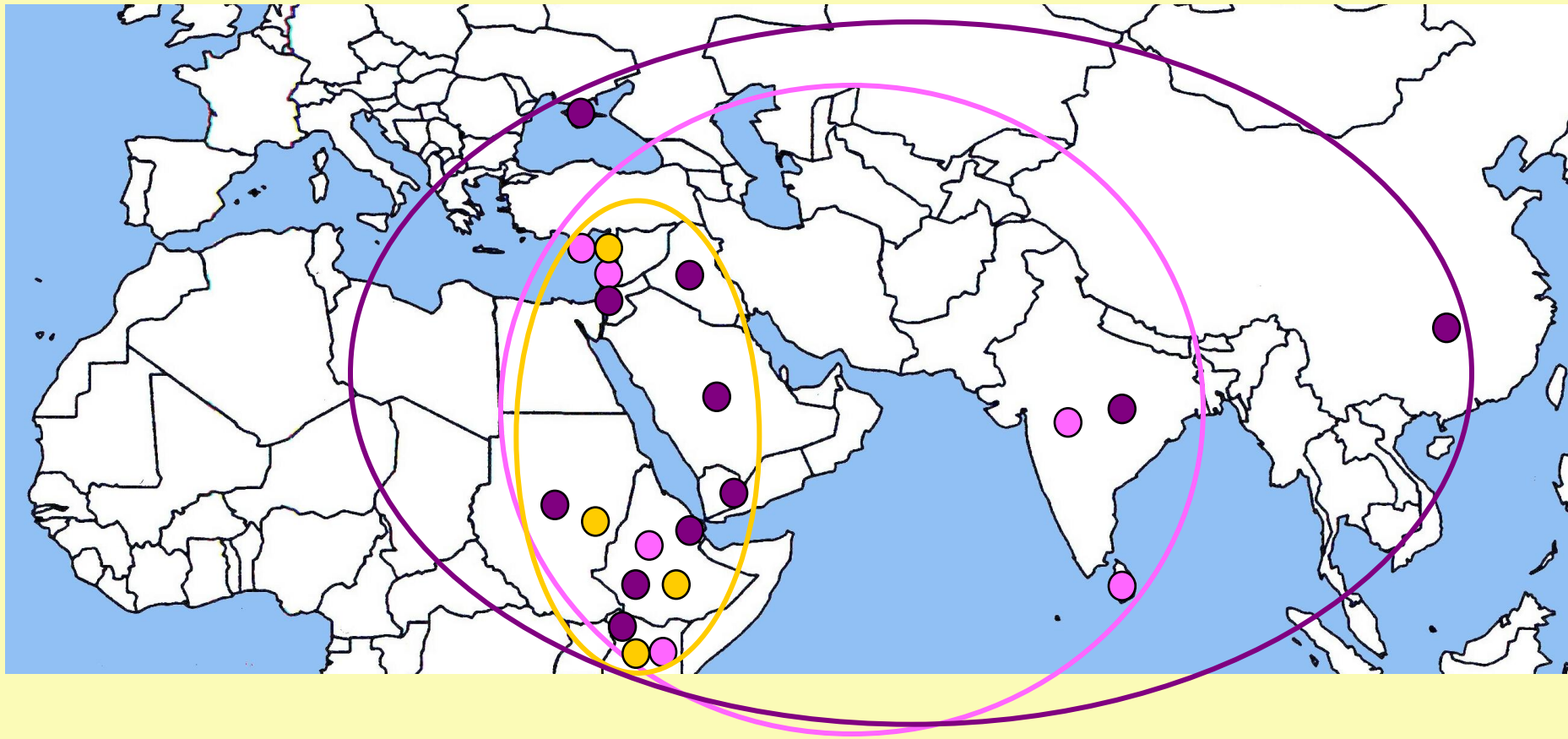
- anthroponotic VL (+PKDL)
- rural disease, epidemics



% *Leishmania* strains (n = 169) per host



Distribution of 23 *L. donovani* ● and *archibaldi* ○ zymodemes



L. donovani zymodeme MON-37 ●

L. infantum

- High polymorphism (37 zymodemes) varying according to the foci (Spain 19 zymodemes, Italy 11 zymodemes)
- 2 classes of populations :
 - large geographic distribution of MON-1, MON-24
—————→ Weak structuration
 - numerous zymodemes restricted to localised areas
—————→ Strong structuration

L. donovani including *L. archibaldi*

- Polymorphism (23 zymodemes)
 - *L. donovani* : 1 to 5 zymodemes per focus
 - Only *L. donovani* MON-37 as a large distribution
 - *L. archibaldi* mainly located in east Africa, poorly differentiated (3 zymodemes)

CNRL

Montpellier,

France

Thank you for attention

Muito obrigado pela atenção

We acknowledge all the Laboratories and
Recherch groups which provided *Leishmania*
strains, since more than 20 years

