

# Diagnostika syfilis pomocí PCR a typování jejího původce:

## pokroky v molekulární epidemiologii syfilis

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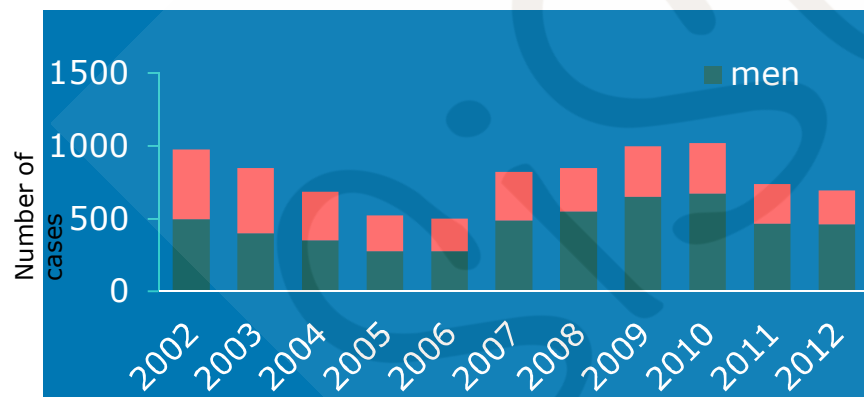
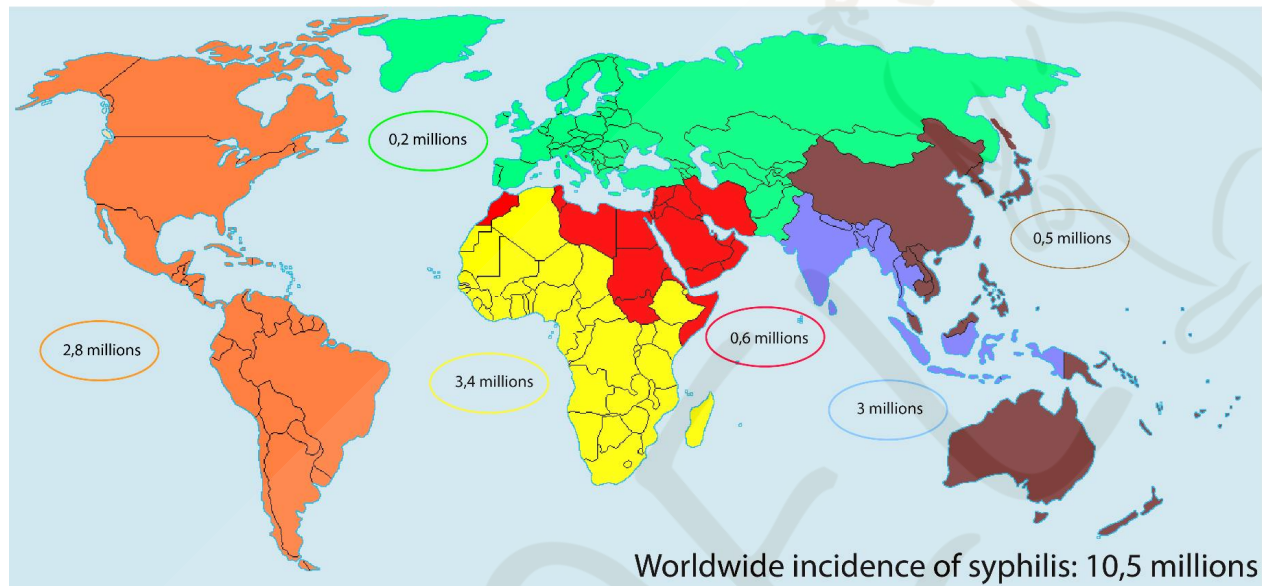
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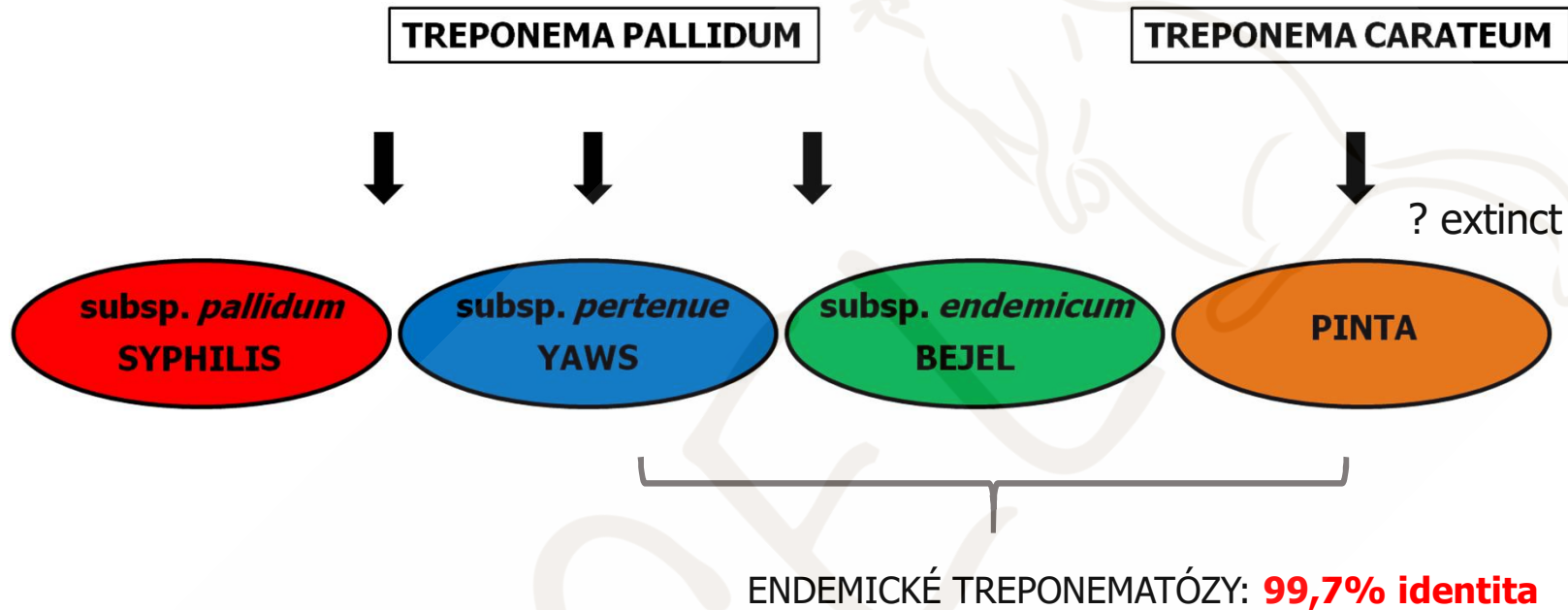
## Incidence syphilis



Incidence syphilis v ČR



## Syphilis způsobuje *T. pallidum* ssp. *pallidum* (TPA)



### Charakteristika poddruhů *T. pallidum*

#### DIFFERENCES

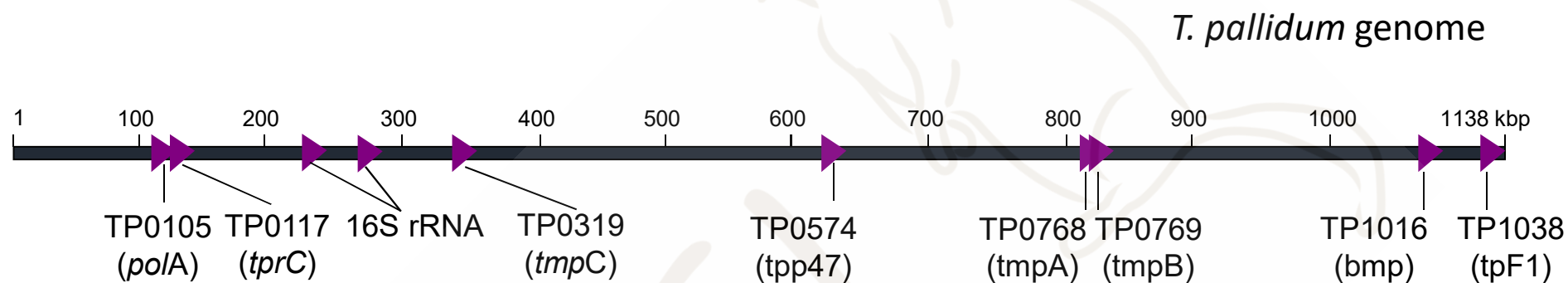
- invasiveness and pathogenicity
- clinical manifestation
- mode of transmission
- congenital infection
- age distribution
- geographical distribution

#### SIMILARITIES

- obligate human pathogens
- uncultivable
- serologically and morphologically indistinguishable
- small genome size (~1.14 Mbp)
- *T. pallidum* subspecies >99.8% identical at DNA level
- genetically monomorphic bacterial pathogens



## Molekulární diagnostika syfilis

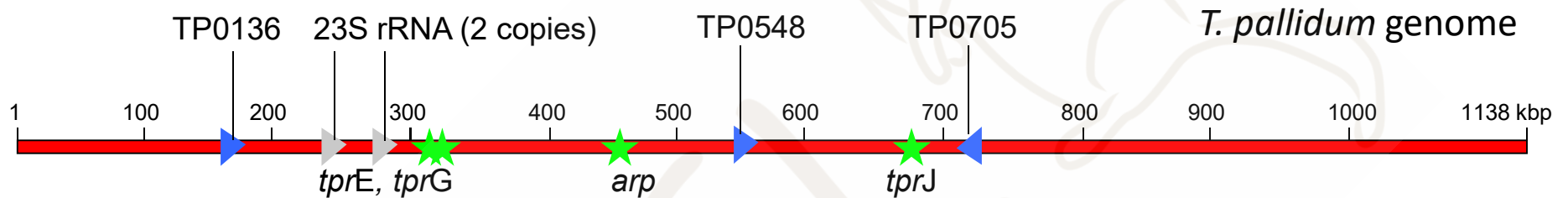


► Diagnostic loci

### Diagnostic loci

Gene	Gene function	Reference
TP0105	DNA polymerase I	Liu et al., 2006
TP0117	Tpr protein C	Rajan et al., 2006
TP0319	membrane lipoprotein	Flasarová et al., 2006
TP0574	carboxypeptidase	Burstain et al., 1991
TP0768	antigen	Hay et al., 1990
TP0769	antigen	Hay et al., 1990
TP1016	membrane protein	Noordhoek et al., 1991
TP1038	antigen	Hay et al., 1990
16S rRNA		Centurion-Lara et al., 1997

## Typovací systémy *T. pallidum*



### **TP0136/TP0548/TP0705** typing

- MLST
- additional detection of macrolide resistance
- Grillová *et al.*, 2018

### **CDC typing** (*arp/tprEGJ* genes)

- *arp* gene: number of tandem repeats (60 bp)
- *tprEGJ* genes: *MseI* restriction digest
- Pillay *et al.*, 1998

### **Enhanced CDC typing system** (*arp/tprEGJ* genes/TP0548)

- *arp* gene: number of tandem repeats (60 bp)
- *tprEGJ* genes: *MseI* restriction digest
- sequencing of 86 bp of TP0548
- Marra *et al.*, 2010

## Detekce DNA v klinických vzorcích

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### PCR

- + easy to perform
- lower sensitivity

### Multiplex PCR

- + detection of causative agents of genital ulcers in one reaction (*T. pallidum*, *Haemophilus ducreyi*, Herpes simplex virus)
- lower sensitivity

### Real-time PCR

- + quantitative results
- + fast method
- + low risk of contamination
- hard to use for molecular typing purposes

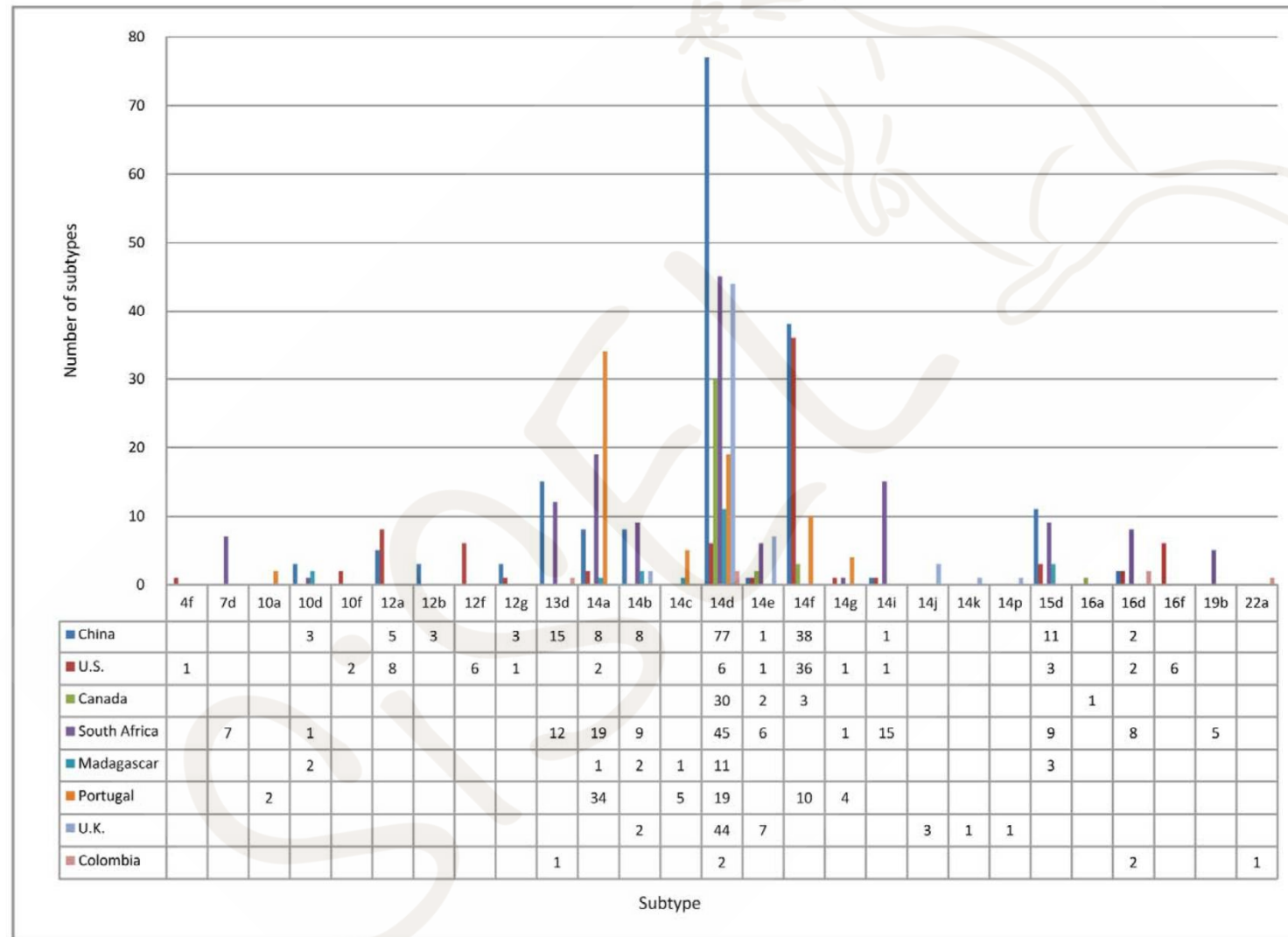
### Nested-PCR

- + high sensitivity
- contamination
- time-consuming

- results depend on sample type and syphilis stage



## Typování pomocí CDC

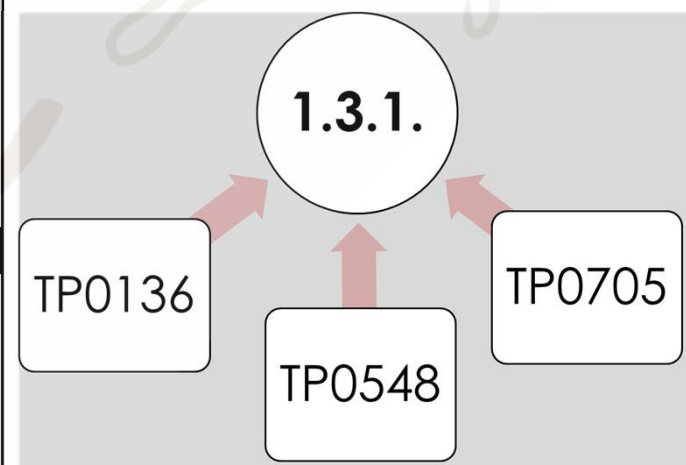


# MLST *T. pallidum*



## Alelické profily klinických izolátů

Coordinates according SS14	154	155	158	163	167	448	485	488	491	503	516	772	773	775	965
TPA_SS14	G	G	G	A	G	T	C	G	G	A	G	A	A	G	A
TP_0548_1	G	G	G	A	G	T	C	G	G	A	G	A	A	G	A
TP_0548_3	A	G	A	A	G	T	C	G	G	A	G	A	A	G	A
TP_0548_4	A	G	A	A	G	T	C	A	G	A	G	A	A	G	A
TP_0548_5	A	G	A	G	G	T	C	G	G	A	G	A	A	G	A
TP_0548_26	A	G	A	A	G	T	C	G	G	A	G	G	A	G	A
TP_0548_27	A	G	A	A	G	T	C	G	G	A	G	G	A	G	G
TP_0548_28	A	G	A	A	G	T	C	G	G	G	G	A	G	G	A
TP_0548_29	A	G	A	A	A	T	C	G	G	A	G	A	A	G	A
TP_0548_30	A	G	A	A	G	T	C	G	G	A	G	A	A	G	A
TP_0548_31	A	G	A	A	G	T	C	G	G	A	G	A	A	T	A
TP_0548_32	G	G	A	A	G	T	C	G	G	A	G	A	A	G	A
TP_0548_33	G	G	G	A	G	C	C	G	G	A	G	A	A	G	A
TP_0548_34	G	G	G	A	G	T	C	G	G	A	T	A	A	G	A
TP_0548_35	G	G	G	A	G	T	A	G	A	A	G	A	A	G	A
TP_0548_36	G	A	G	A	G	T	C	G	G	A	G	A	A	G	A





# A public database for the new MLST scheme for *Treponema pallidum* subsp. *pallidum*: surveillance and epidemiology of the causative agent of syphilis

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## ABSTRACT

*Treponema pallidum* subsp. *pallidum* is the causative agent of syphilis, a sexually transmitted disease with worldwide prevalence. Several different molecular typing schemes are currently available for this pathogen. To enable population biology studies of the syphilis agent and for epidemiological surveillance at the global scale, a harmonized typing tool needs to be introduced. Recently, we published a new multi-locus sequence typing (MLST) with the potential to significantly enhance the epidemiological data in several aspects (e.g., distinguishing genetically different clades of syphilis, subtyping inside these clades, and finally, distinguishing different subspecies of non-cultivable pathogenic treponemes). In this short report, we introduce the PubMLST database for treponemal DNA data storage and for assignments of allelic profiles and sequencing types. Moreover, we have summarized epidemiological data of all treponemal strains ( $n = 358$ ) with available DNA sequences in typing loci and found several association between genetic groups and characteristics of patients. This study proposes the establishment of a single MLST of *T. p. pallidum* and encourages researchers and public health communities to use this PubMLST database as a universal tool for molecular typing studies of the syphilis pathogen.

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## Detekce DNA v klinických vzorcích

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**klinický materiál:** stěr kožní léze, plná krev, mozkomíšní mok, synoviální tekutina

**izolace DNA** na přístroji Qiacube (Qiagen)

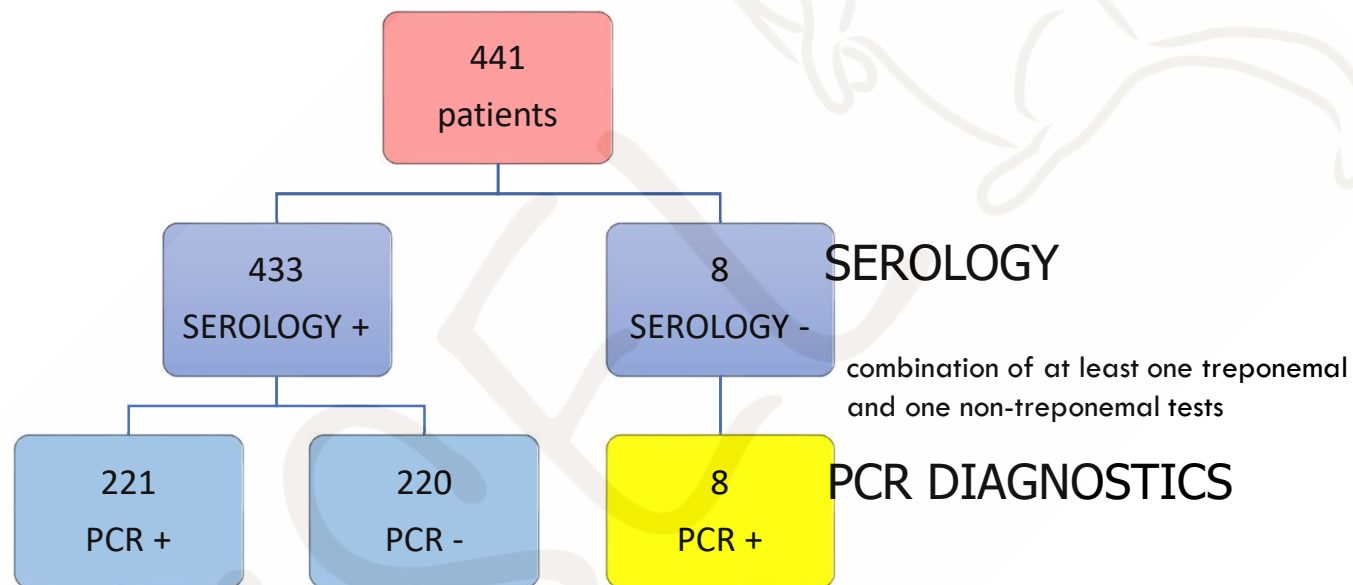
plně automatizovaná izolace pomocí kolonek

2-12 vzorků

200 mikrolitrů klinického vzorku => 100 mikrolitrů eluátu



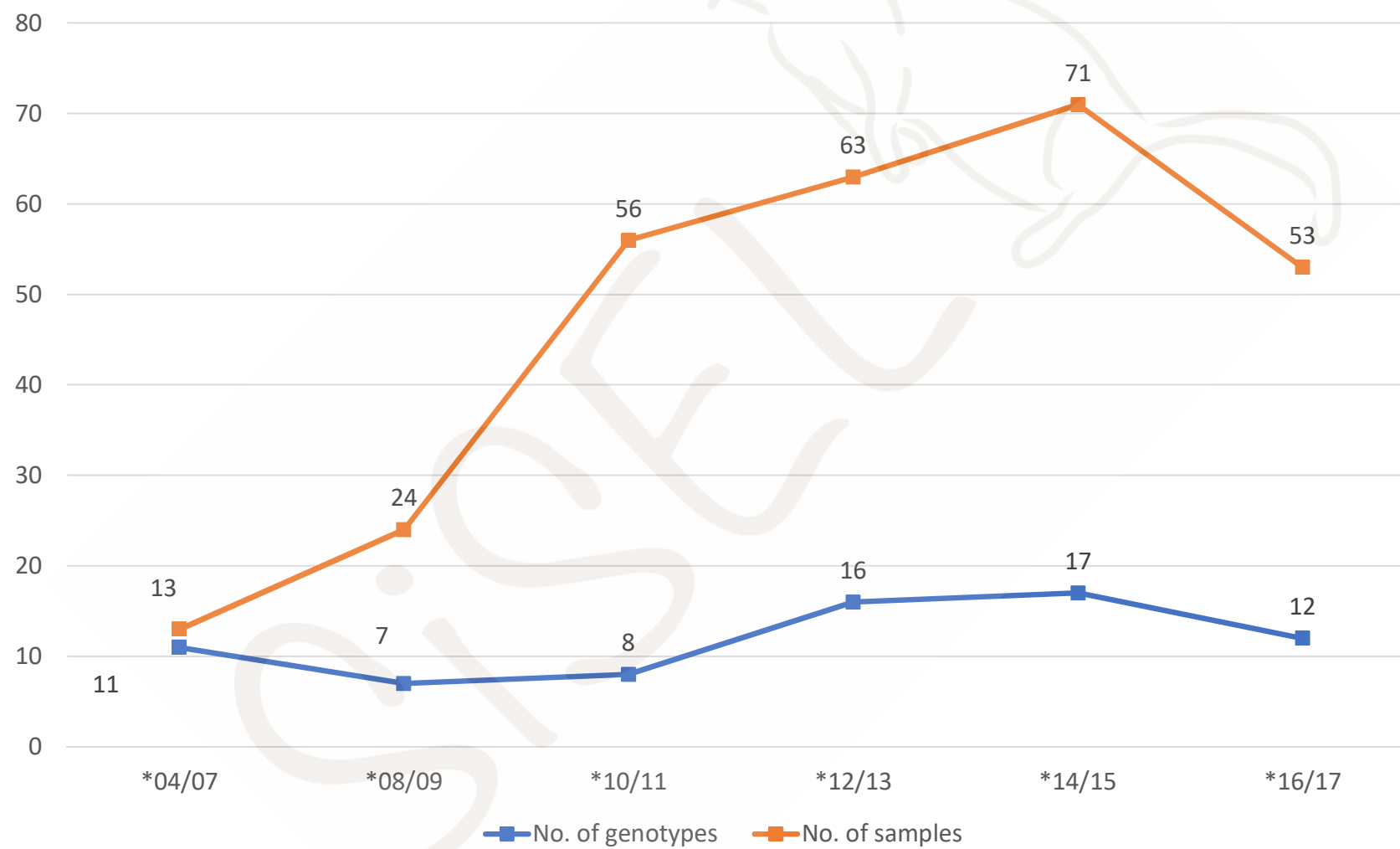
## Detekce DNA v klinických vzorcích



Success rate of treponemal DNA detection: 51%

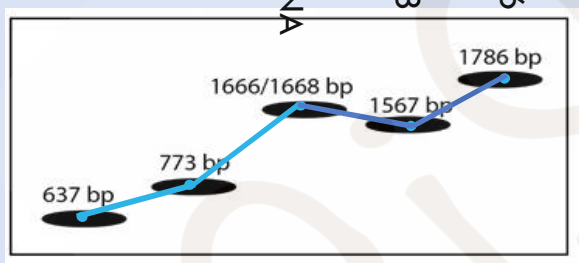
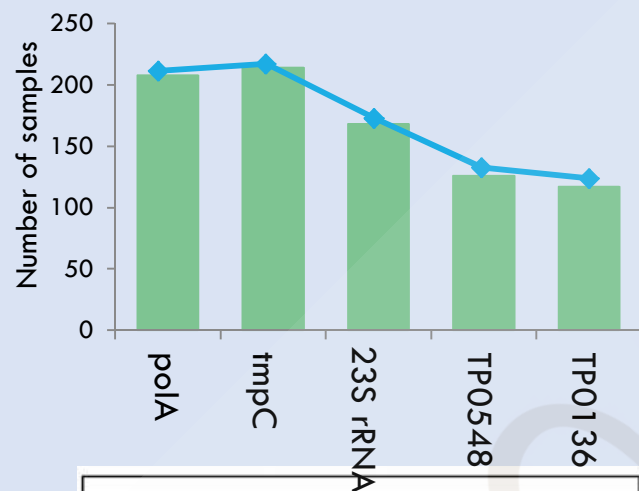


## Alelické profily klinických izolátů



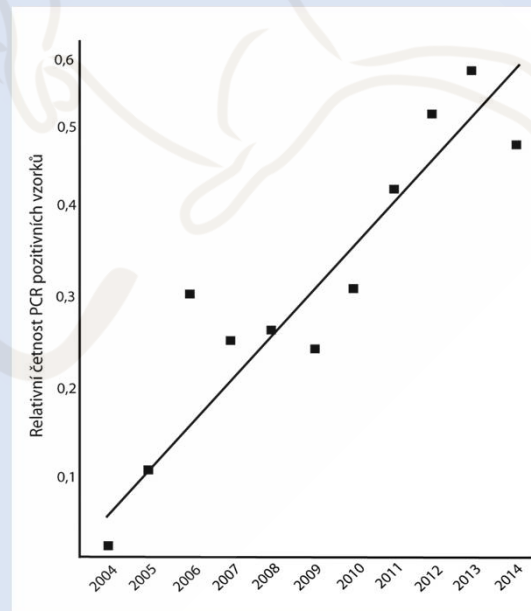
## PCR pozitivita klinických vzorků

### Success rate of amplification



Shorter genes are amplified with higher success rate

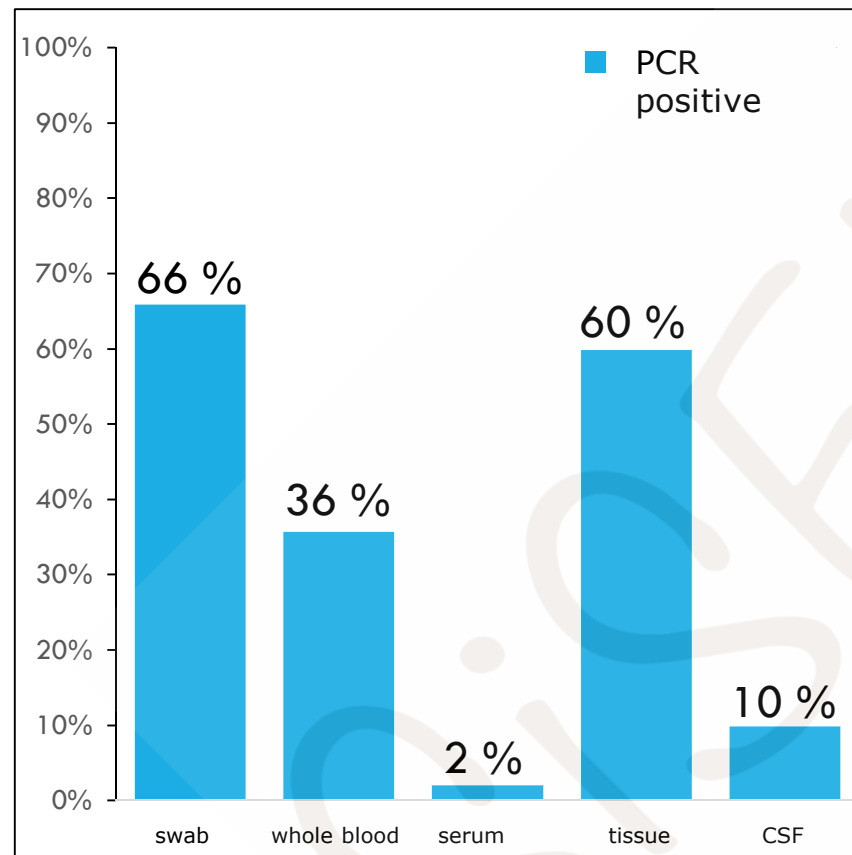
### Optimization of amplification



Optimization of PCR conditions led to higher relative frequency of PCR positive samples ( $p=0,0003$ )



## PCR pozitivita klinických vzorků



Number of samples tested

swab	256	169	66%
whole blood	338	121	36%
serum	187	4	2%
tissue	5	3	60%
CSF	10	1	10%

## PCR pozitivita klinických vzorků

### Comparison of PCR-positivity

#### PCR-positive swab

52.3% positive blood vs. 47.6% negative blood

#### PCR-positive blood

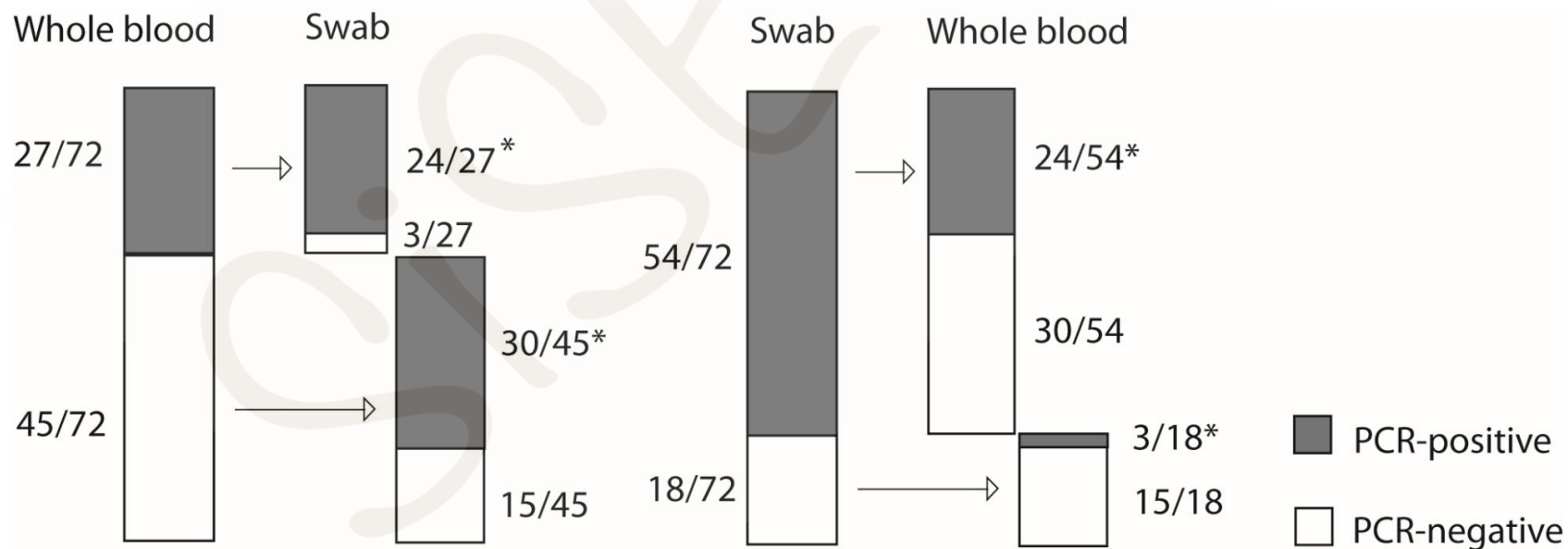
84.9% positive swab vs. 15.1% negative swab

#### PCR-negative swab

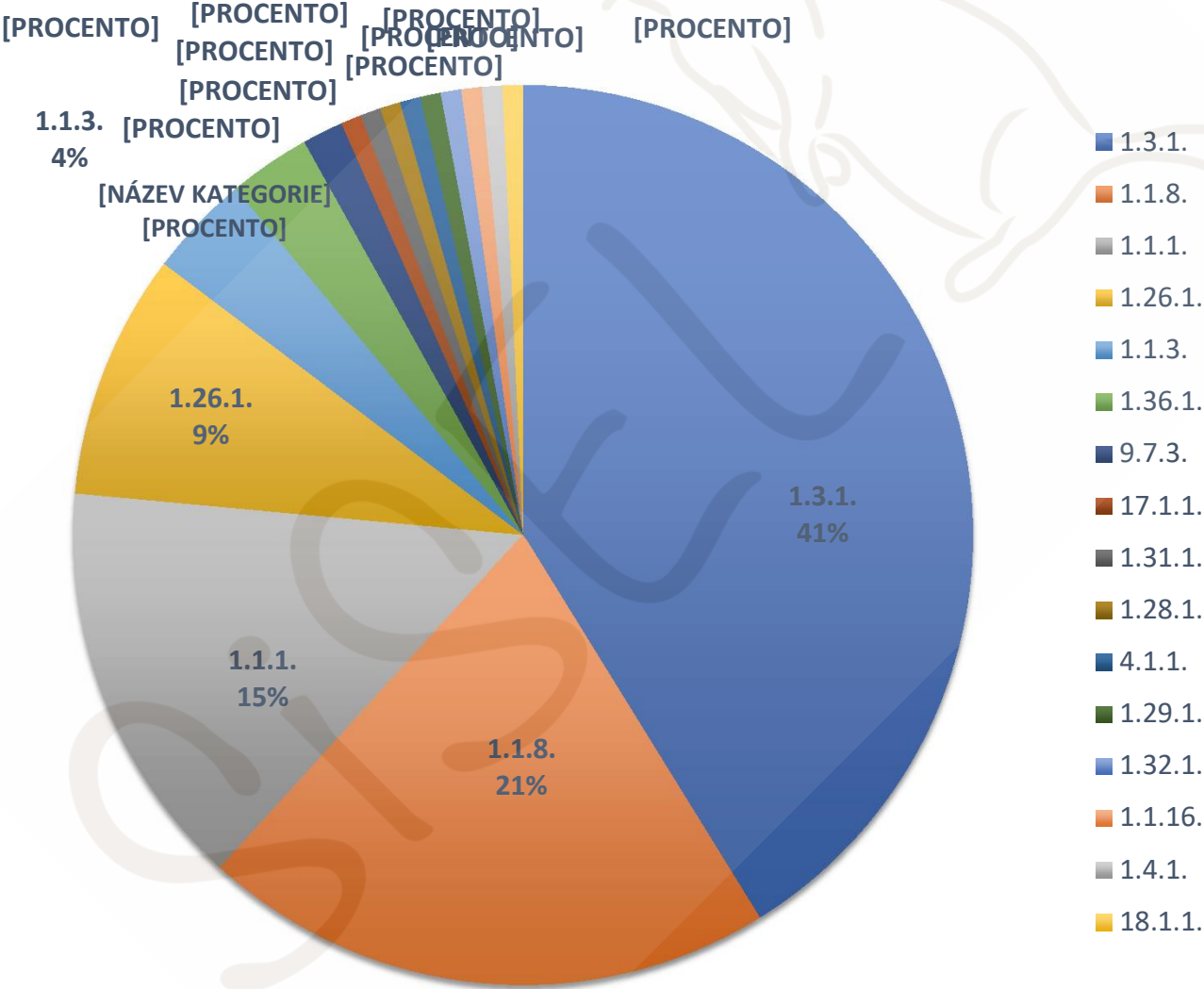
23.5% positive blood vs. 76.5% negative blood

#### PCR-negative blood

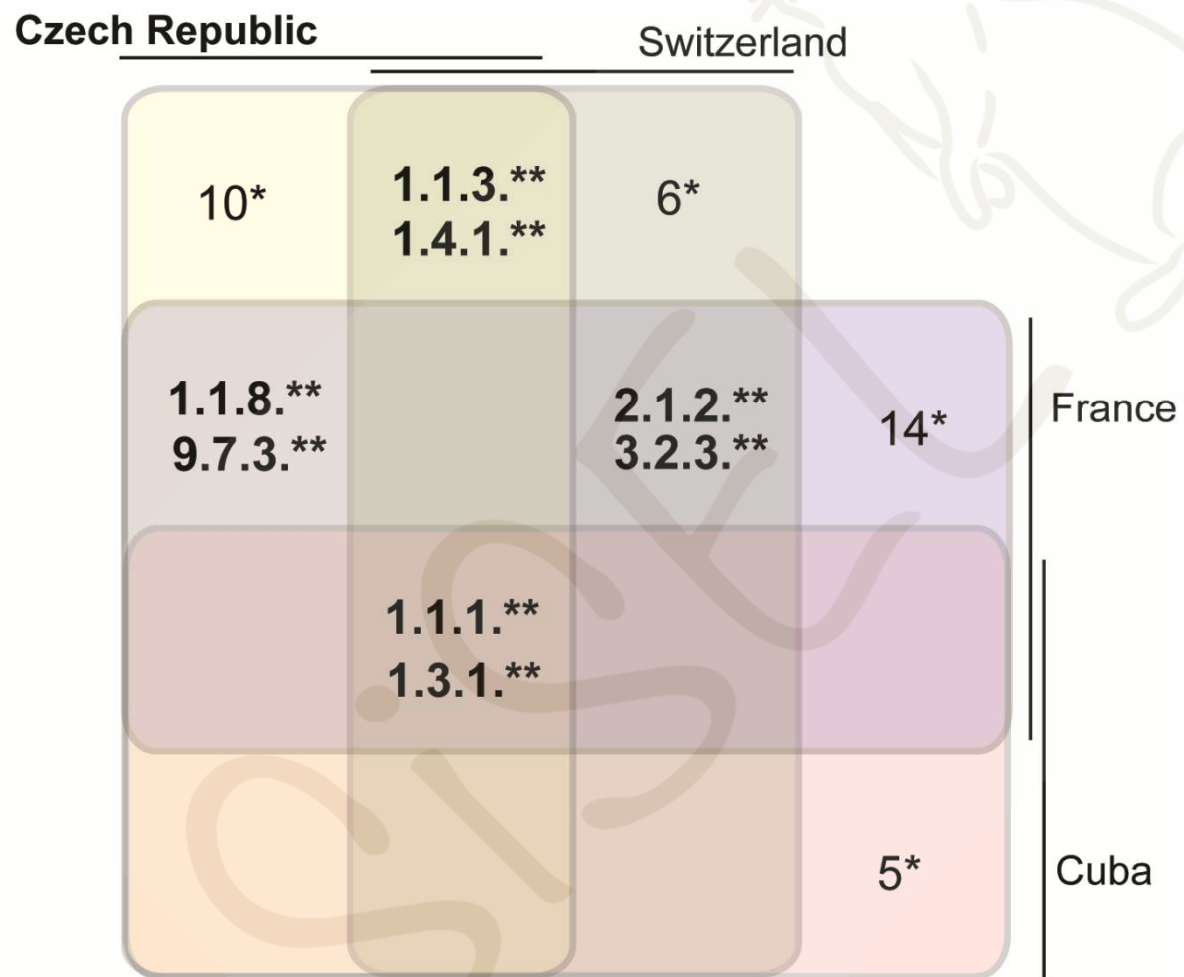
61.2% positive swab vs. 38.8% negative swab



# Alelické profily klinických izolátů



## Alelické profily klinických izolátů

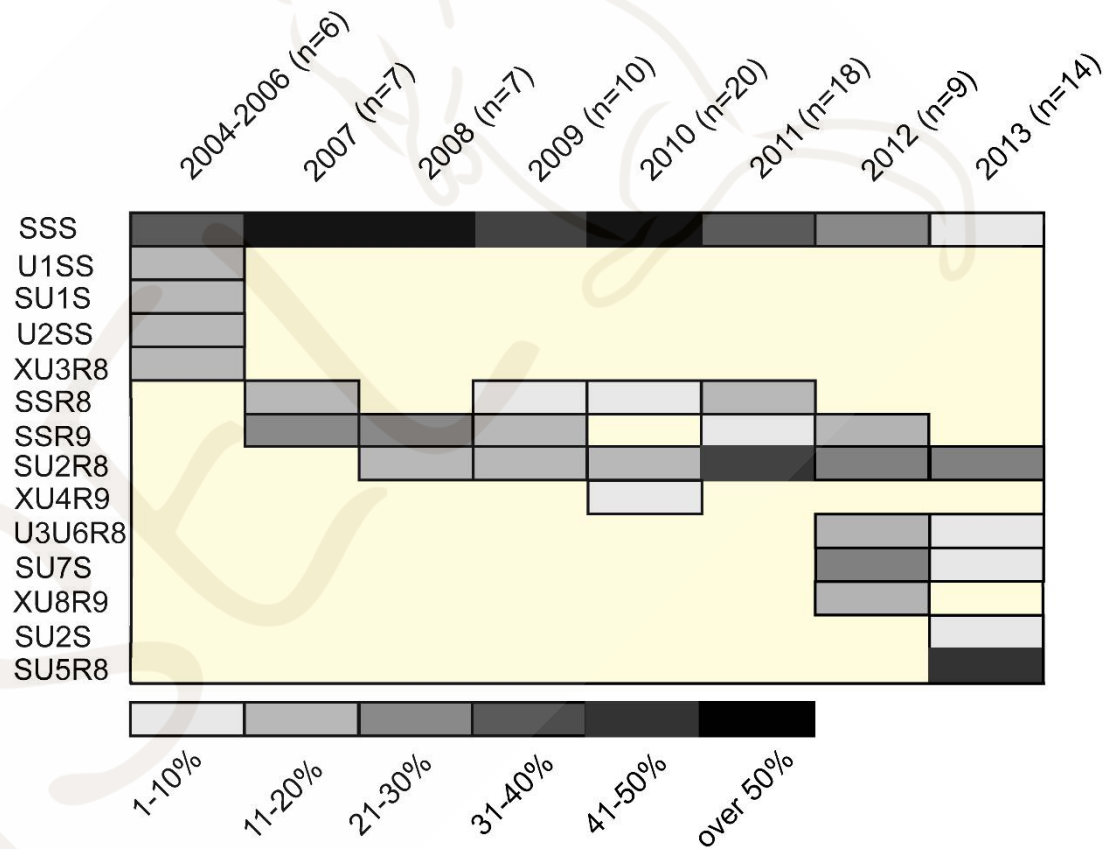


\*Number of unique allelic profiles found in a single country

\*\*Alelic profiles shared by two or four countries

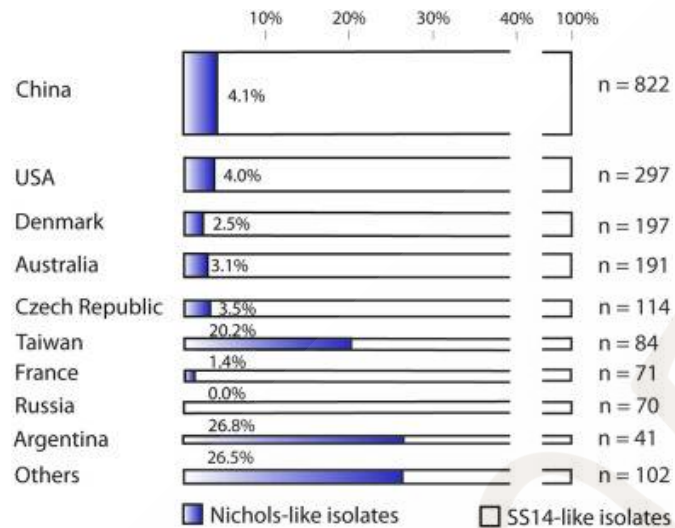
## Alelické profily klinických izolátů

- 16 different genotypes
- Most genotypes showed genetic relatedness to TPA SS14 strain, only one genotype was similar to TPA Nichols
- Dynamic spectra of TPA strains in population

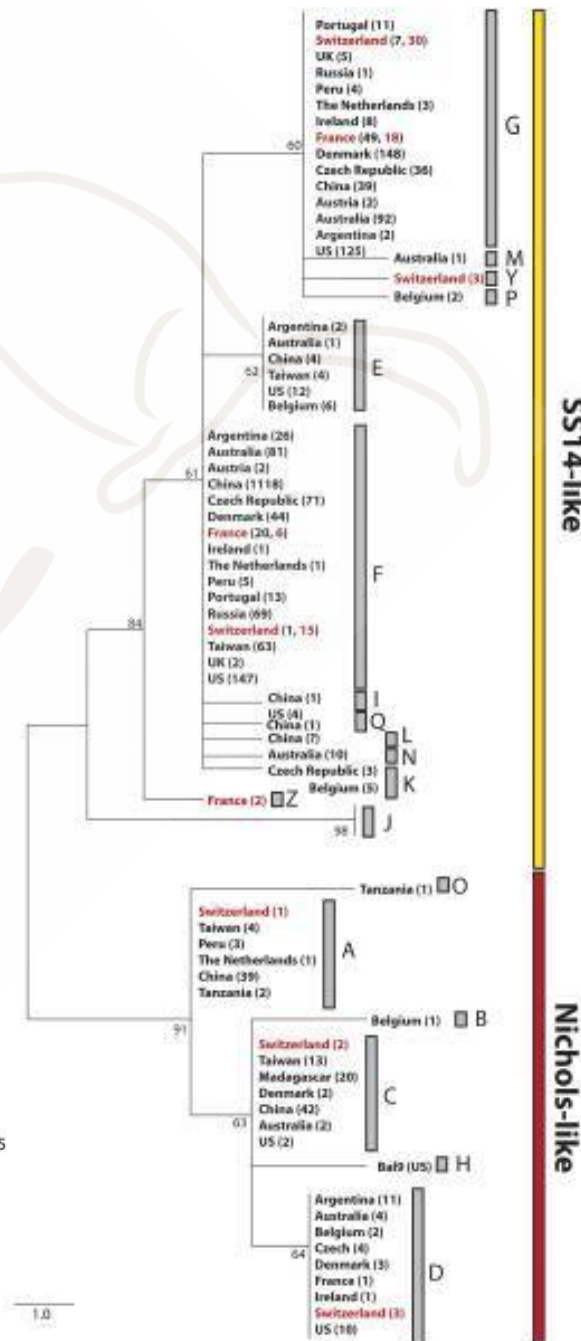
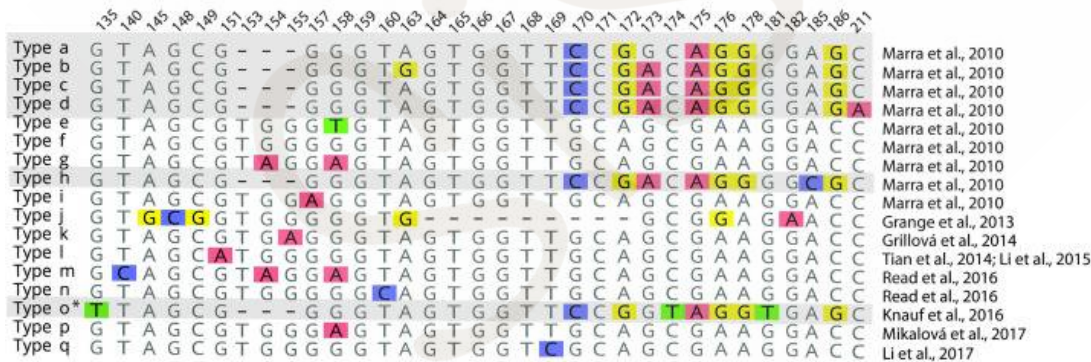


Relative frequency of genotypes according to the color code.

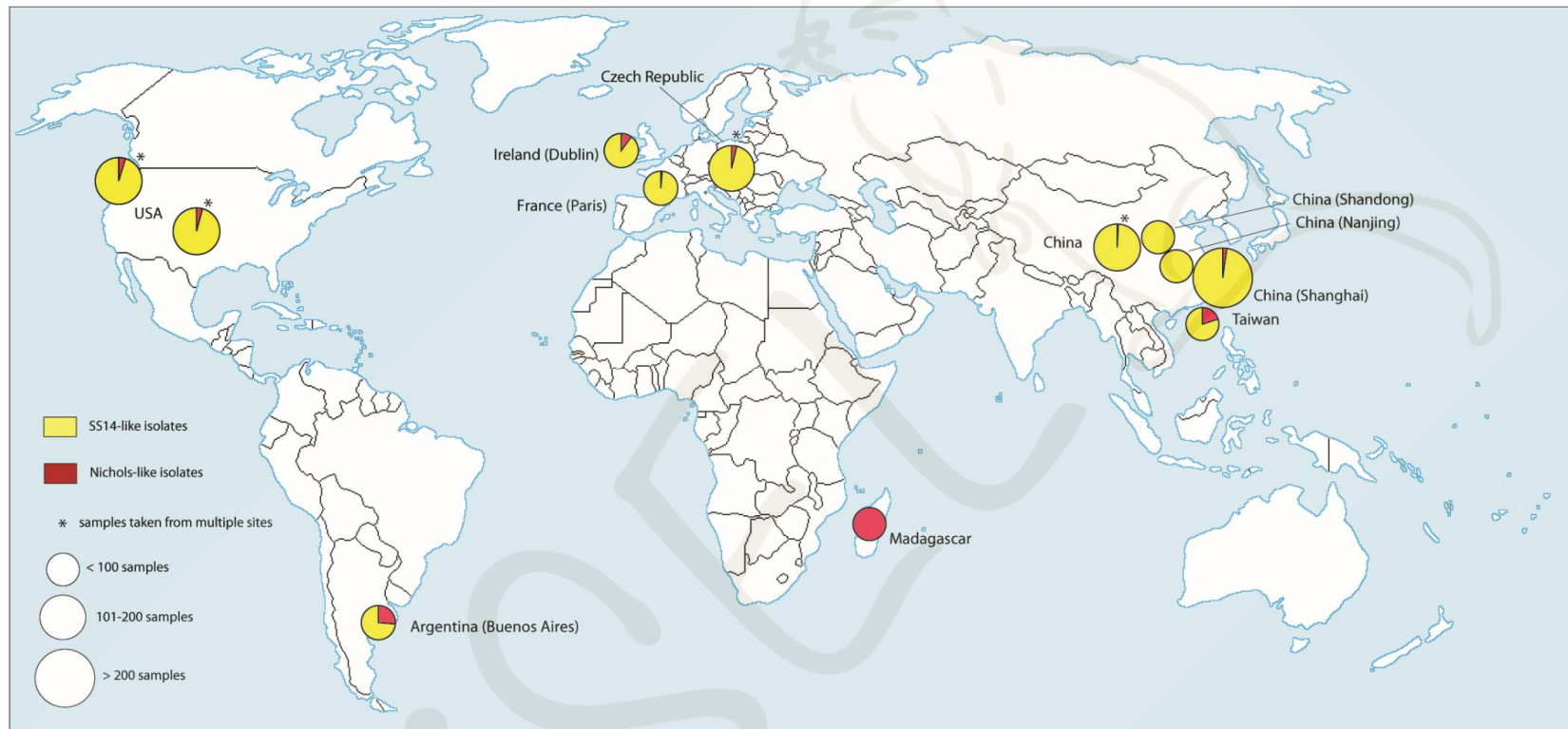
## SS14-like and Nichols-like kmeny



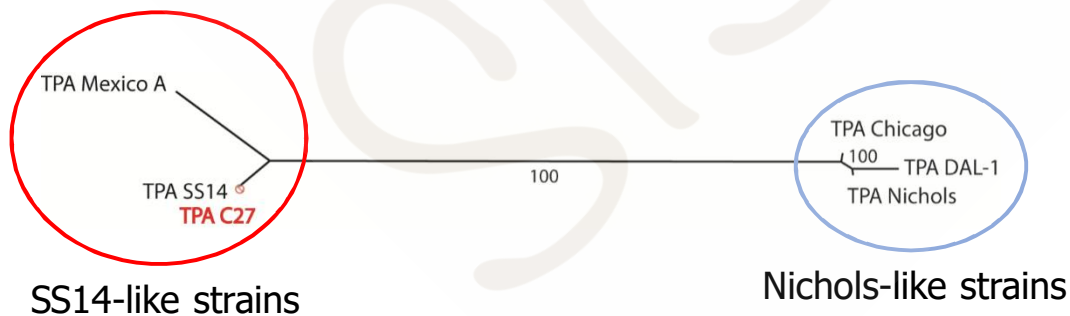
TPA SS14 TP0548 (CP004011.1), coordinates 130 - 215 within TP0548



## *T. pallidum* tvoří dvě skupiny: SS14-like kmeny a Nichols-like kmeny



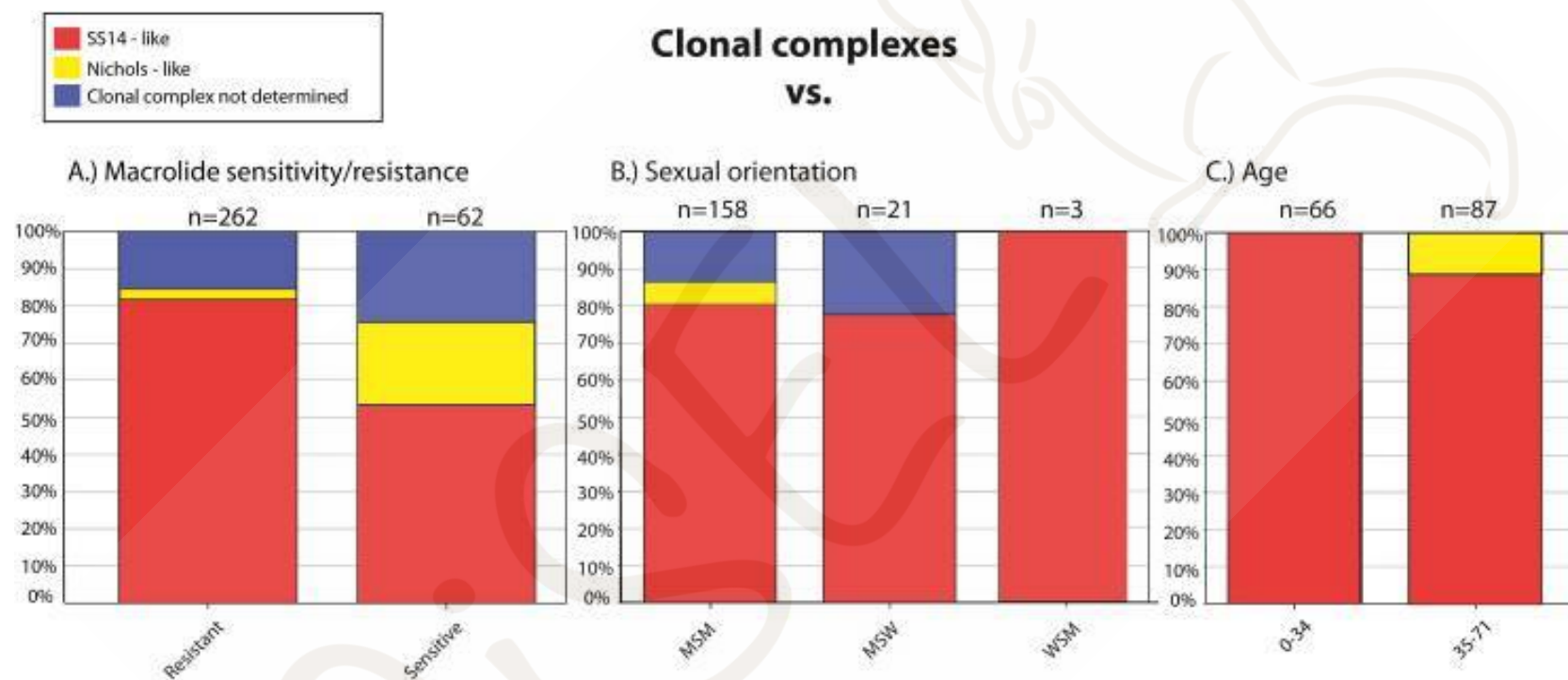
970 worldwide clinical isolates characterized by sequencing of short gene fragment of TP0548.



SS14-like : Nichols-like  
907 : 63

94% of worldwide TPA clinical isolates belong to SS14-like strains although most reference strains belong to Nichols-like strains

## Alelické profily klinických izolátů



Clonal complexes associated with the macrolide sensitivity/resistance (A), sexual orientation (B), and age of the patients (C).

## Alelické profily klinických izolátů

**Resistance to macrolides**

**1.3.1., 1.1.3., 1.26.1.**

**Sensitivity to macrolides**

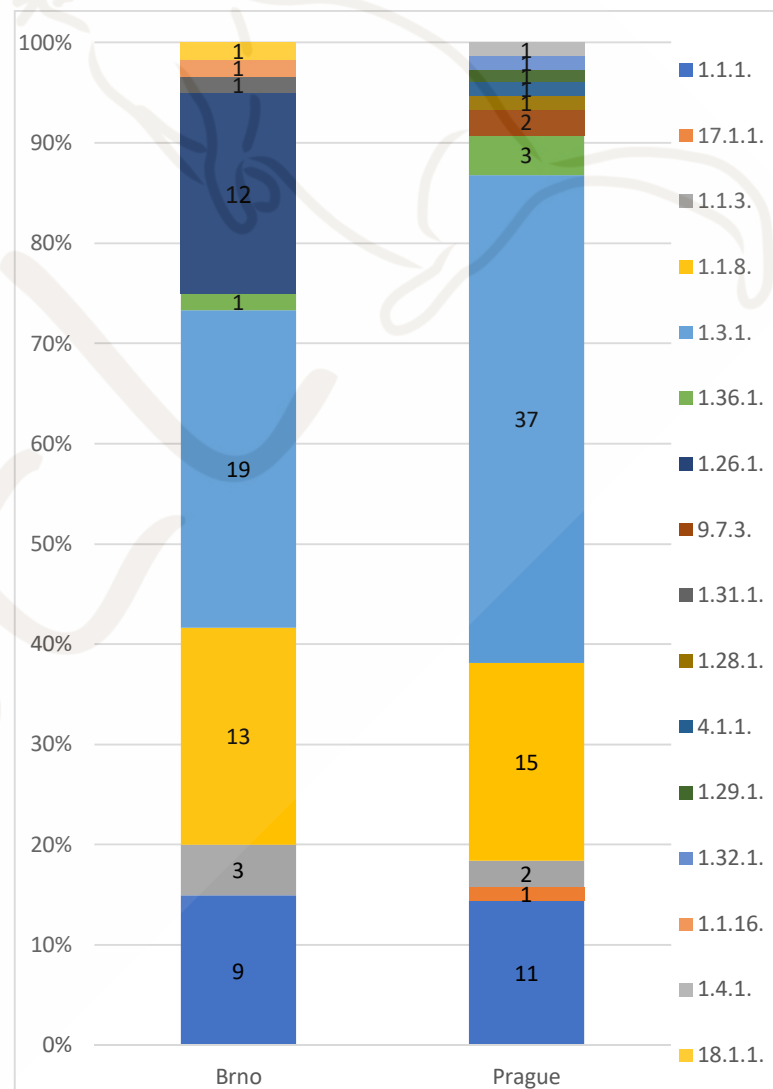
**1.1.8.**

**Sex**

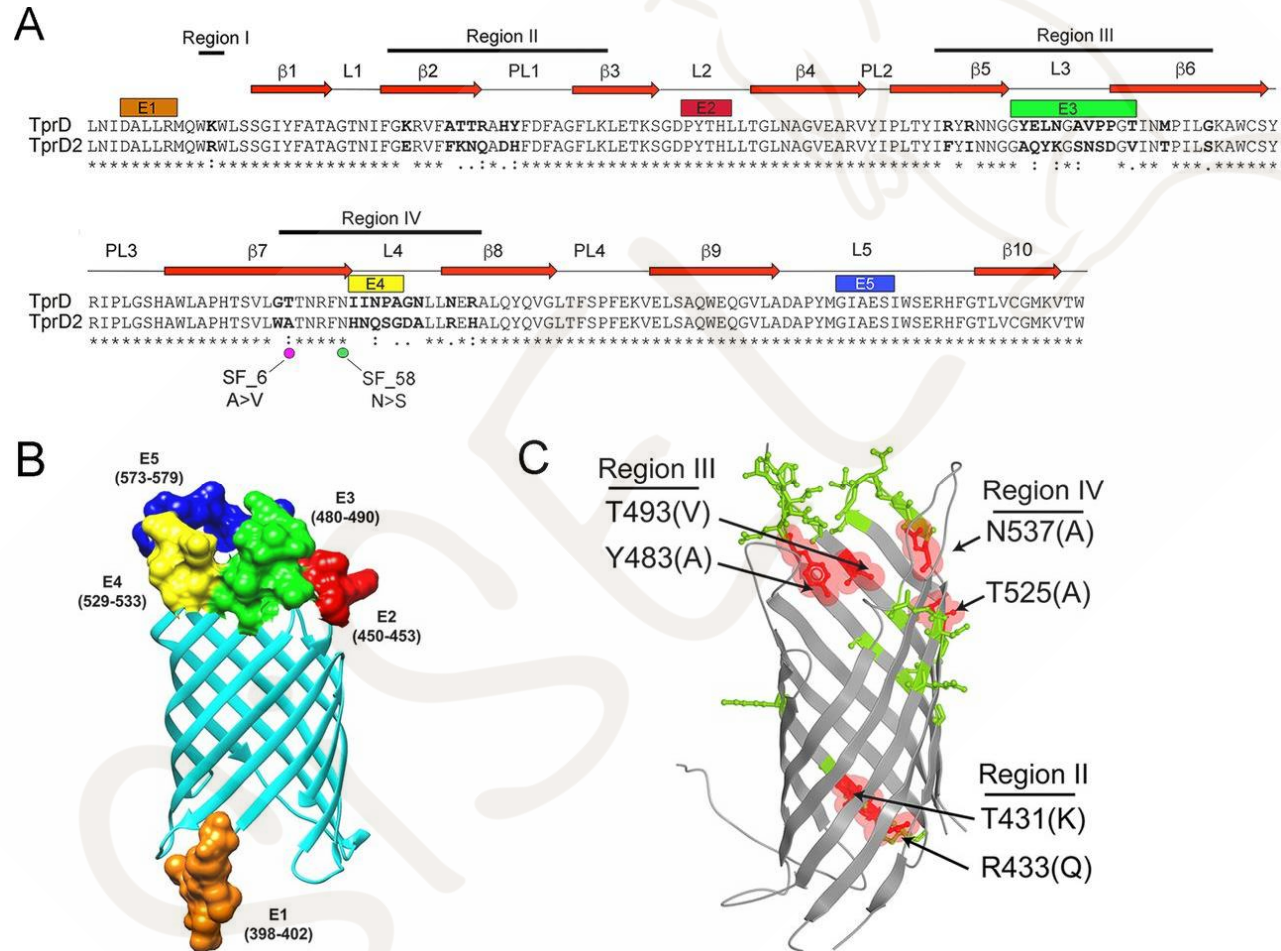
**1.1.8. in women**

**Locality**

**1.26.1. in Brno**

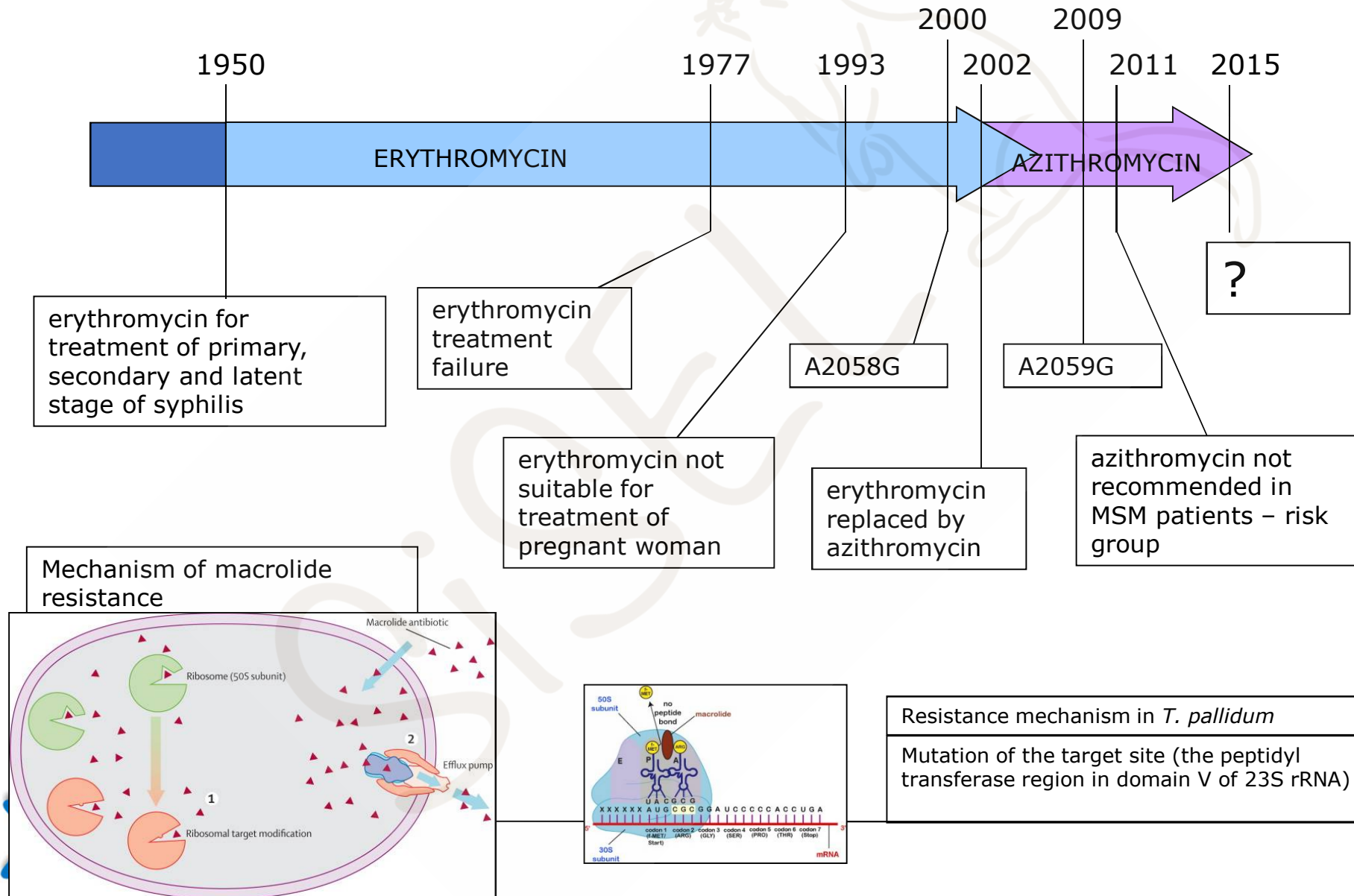


## Klinické izoláty TPA z Cali, San Francisca a ČR a jejich rozdíly v *tprD* alelách

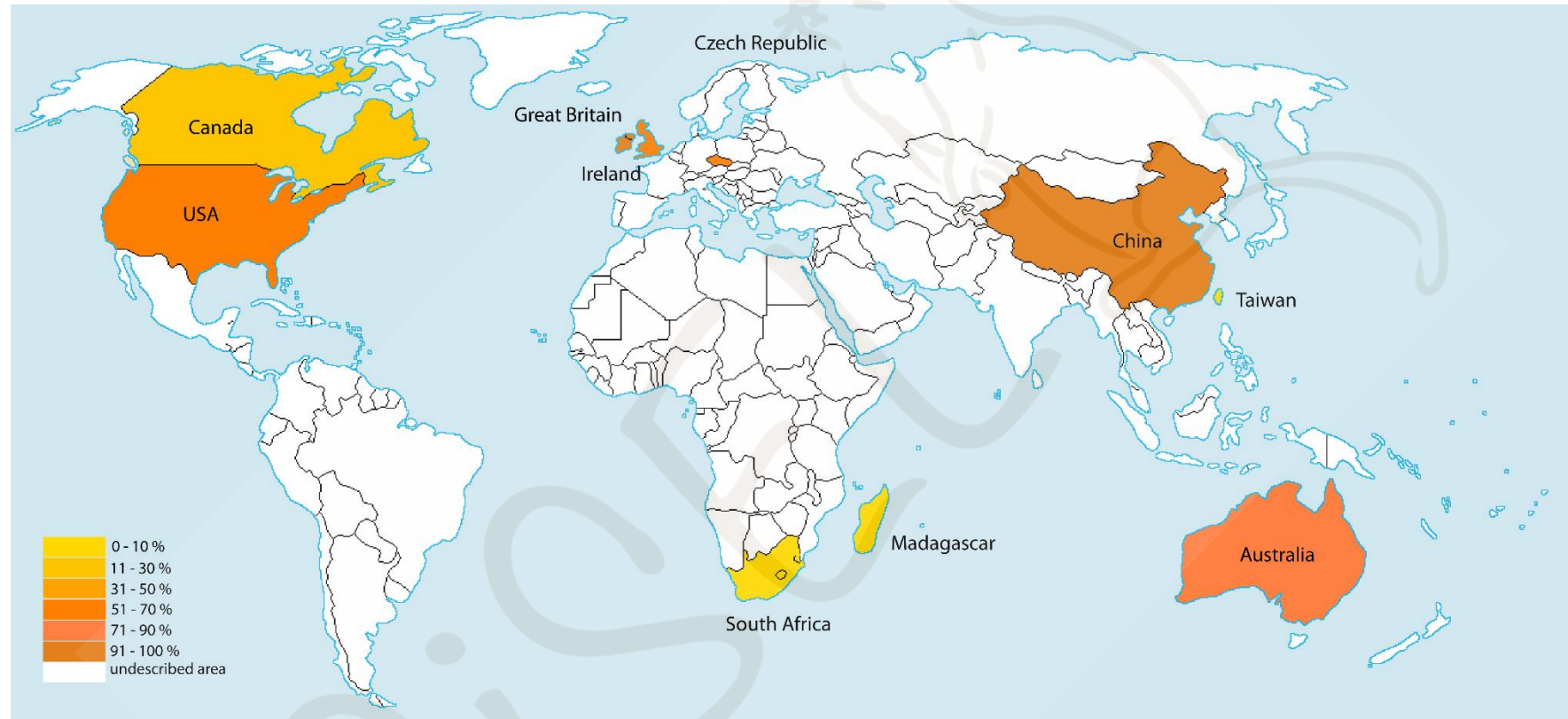


Sanjiv Kumar et al. mBio 2018; doi:10.1128/mBio.01006-18

## Detekce makrolidové rezistence v klinických vzorcích

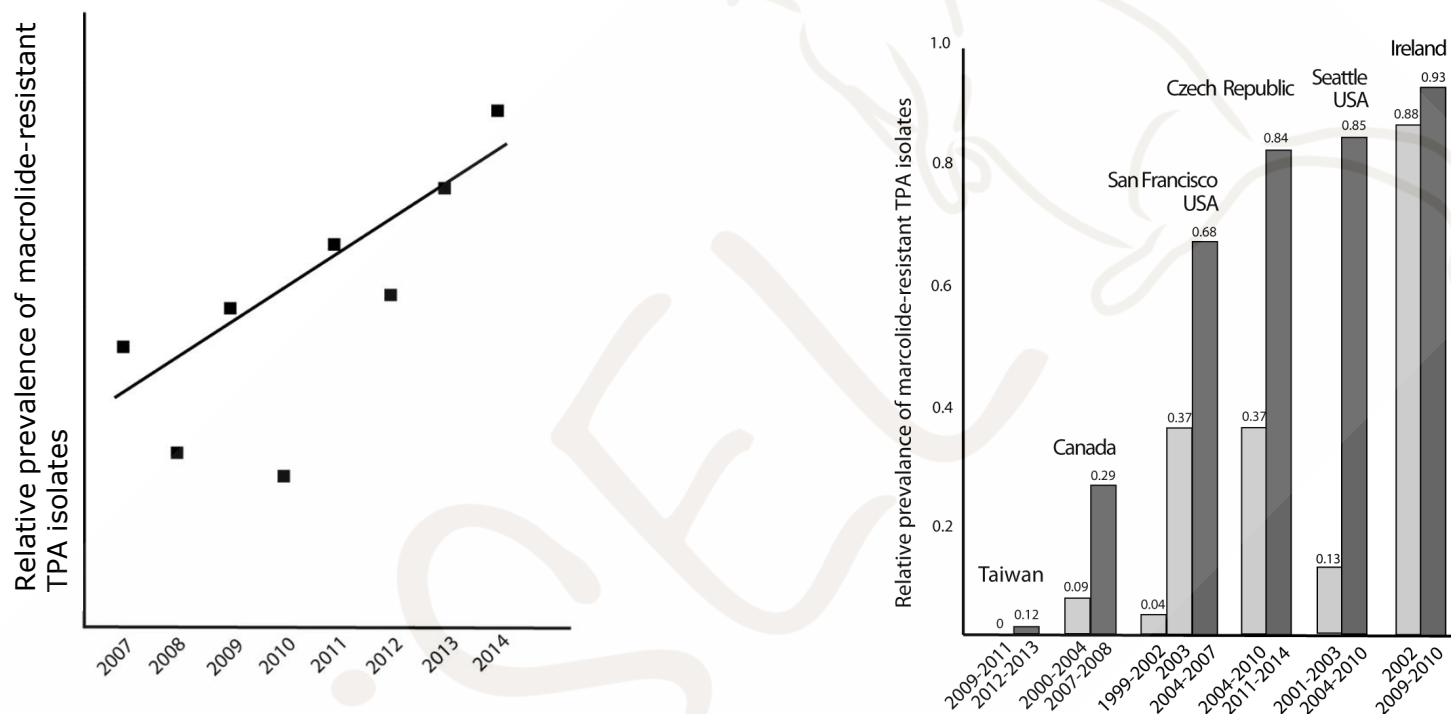


## Detekce makrolidové rezistence v klinických vzorcích



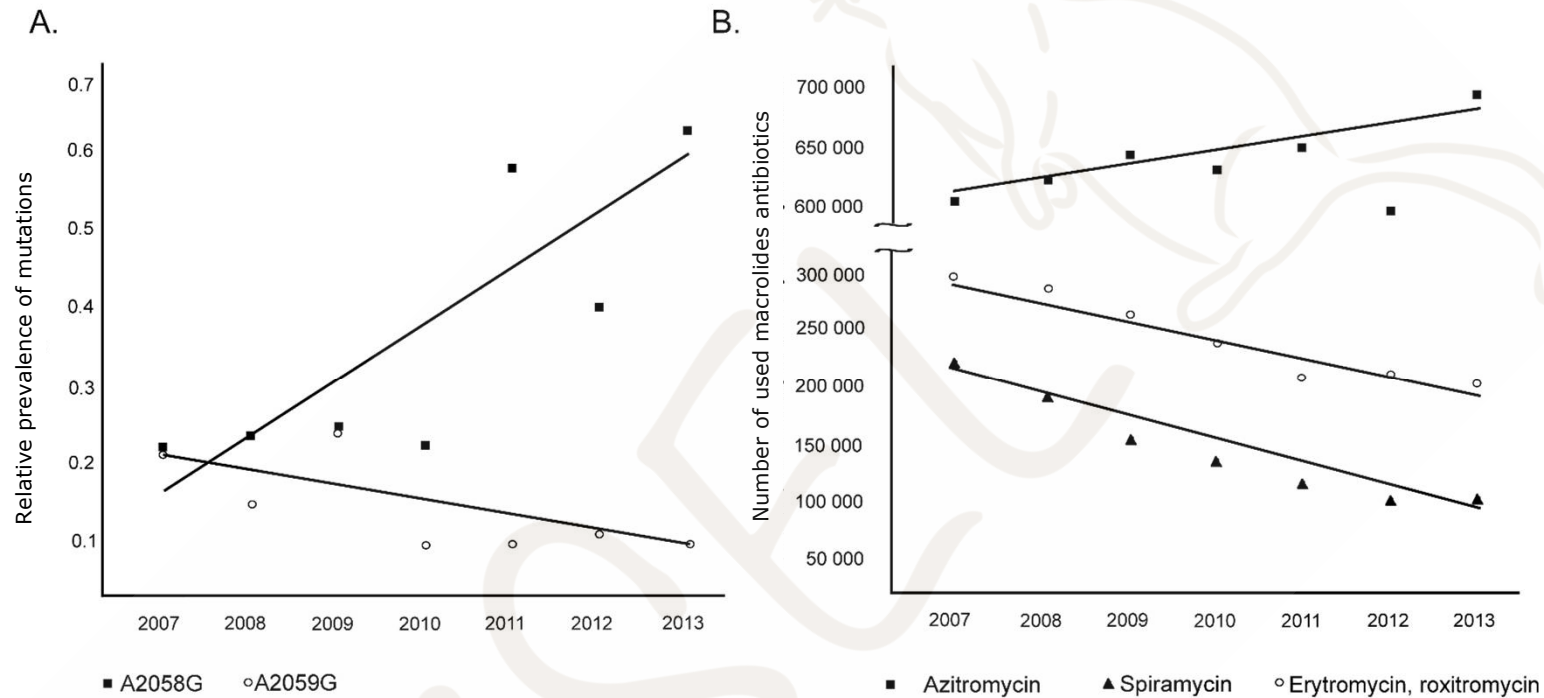
- The prevalence of macrolide-resistant TPA isolates appears to show geographical differences with lower prevalence on islands (Madagascar, Taiwan) and relatively remote areas (Northwest Territories, Canada; Lesotho, South Africa), while the highest prevalence was in large cities with a high level of tourism and business travel.
- Macrolide resistant TPA strains – spread of resistance due to selection pressure and appearance of de novo mutation or spread of already existing resistant strains?

## Detekce makrolidové rezistence v klinických vzorcích



- Macrolide resistance-causing mutation A2058G was identified in 2000. In the Czech Republic, the A2058G mutation was firstly detected in 2006. Second mutation causing macrolide resistance in *T. pallidum* A2059G was firstly identified and described in the Czech Republic in 2007.
- Mutations A2058G or A2059G were always detected in both copies of 23S rRNA.
- The number of macrolide-resistant isolates increased over time.
- In 2014, the number of macrolide resistant strains in the Czech Republic reached 82%.

## Detekce makrolidové rezistence v klinických vzorcích



- Mutation A2058G causes resistance to azithromycin, clarithromycin, erythromycin and roxithromycin
- Mutation A2059G causes resistance to macrolides including spiramycin

## Shrnutí

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1. Molekulární diagnostika syfilis je možná a dosahuje zhruba **polovičního** zachytu oproti sérologii
2. MLST typování má **30%** rozlišovací schopnost ve srovnání s celogenomovými daty
3. Nevhodnějším materiálem jsou **kožní stěry** nebo plná krev
4. Nejcitlivější metoda detekce treponemální DNA je **nested-PCR**
5. Alelické profily TPA jsou buď **často** se vyskytující (1.3.1, 1.1.8, 1.1.1, 3.2.3) a společné mnoha státům nebo **vzácné**, které jsou odlišné v různých státech
6. Klinické izoláty jsou dvojího typu: patří do skupiny **SS14-like** kmenů nebo **Nichols-like** kmenů
7. Alelické profily TPA jsou **dynamické** v čase
8. Alelické profily TPA jsou asociovány s **geografickým původem a s pohlavím**
9. **Makrolidová rezistence** je asociována s některými alelickými profily
10. Zjištění genetické diverzity klinických izolátů je předpokladem pro vývoj **vakcíny** proti syfilis, která je nezbytná pro ev. eradikaci syfilis



**Děkuji za pozornost**



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