Molecular epidemiology of pathogenic *Leptospira* in Thailand

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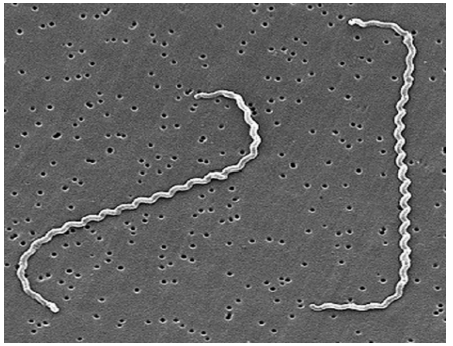
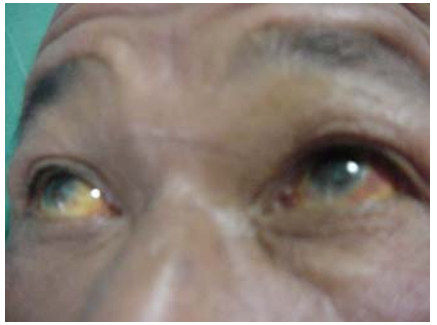
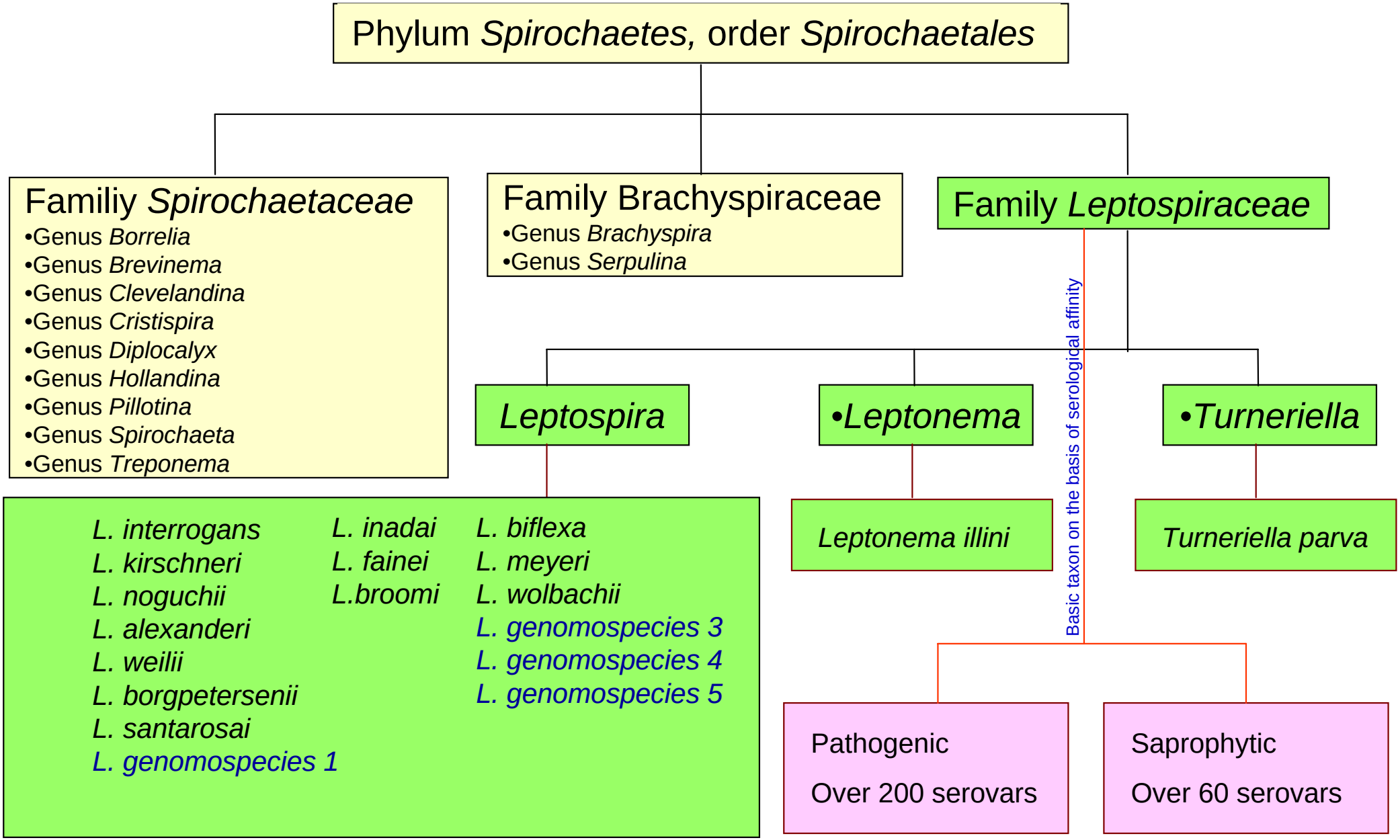
Leptospirosis in human

- **A important worldwide zoonosis caused by pathogenic members of the genus *Leptospira*.**
- **Human disease is acquired following environmental exposure to contaminated soil or water, or via direct contact with bodily fluid from infected animals**
- **Rainy season disease, related to occupation**
- **Acute febrile illness with predominantly non-specific clinical features found about 90%**
- **Severe disease found about 10% leads in multi-organ involvement and death in one quarter of these**

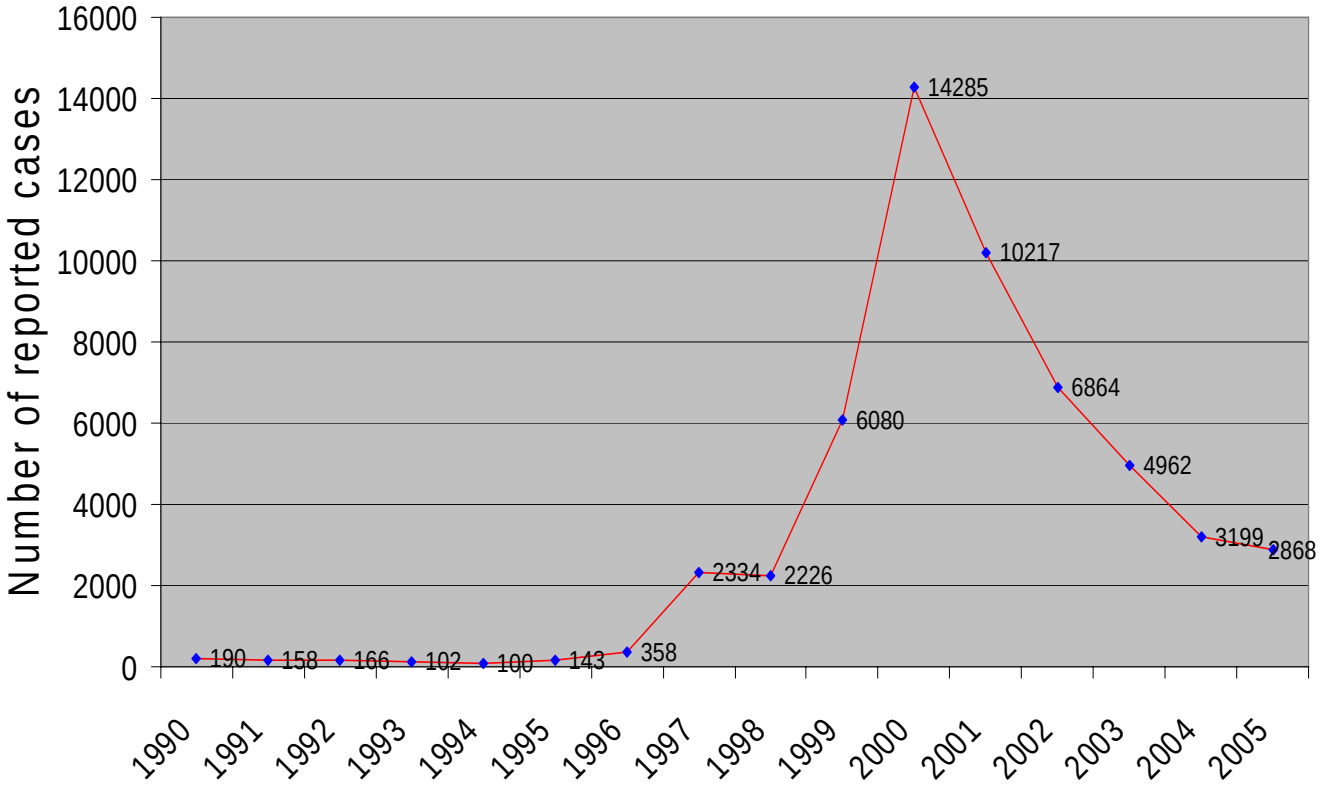





Phylum *Spirochaetes*, order *Spirochaetales*
Familiy *Spirochaetaceae*
•Genus *Borrelia*
•Genus *Brevinema*
•Genus *Clevelandina*
•Genus *Cristispira*
•Genus *Diplocalyx*
•Genus *Hollandina*
•Genus *Pillotina*
•Genus *Spirochaeta*
•Genus *Treponema*
Family Brachyspiraceae
•Genus *Brachyspira*
•Genus *Serpulina*
Family *Leptospiraceae*
Basic taxon on the basis of serological affinity
Leptospira
•*Leptonema*
•*Turneriella*
L. interrogans
L. kirschneri
L. noguchii
L. alexanderi
L. weilii
L. borgpetersenii
L. santarosai
L. genomospecies 1
L. inadai
L. fainei
L.broomi
L. biflexa
L. meyeri
L. wolbachii
L. genomospecies 3
L. genomospecies 4
L. genomospecies 5
Leptonema illini
Turneriella parva
Pathogenic
Over 200 serovars
Saprophytic
Over 60 serovars

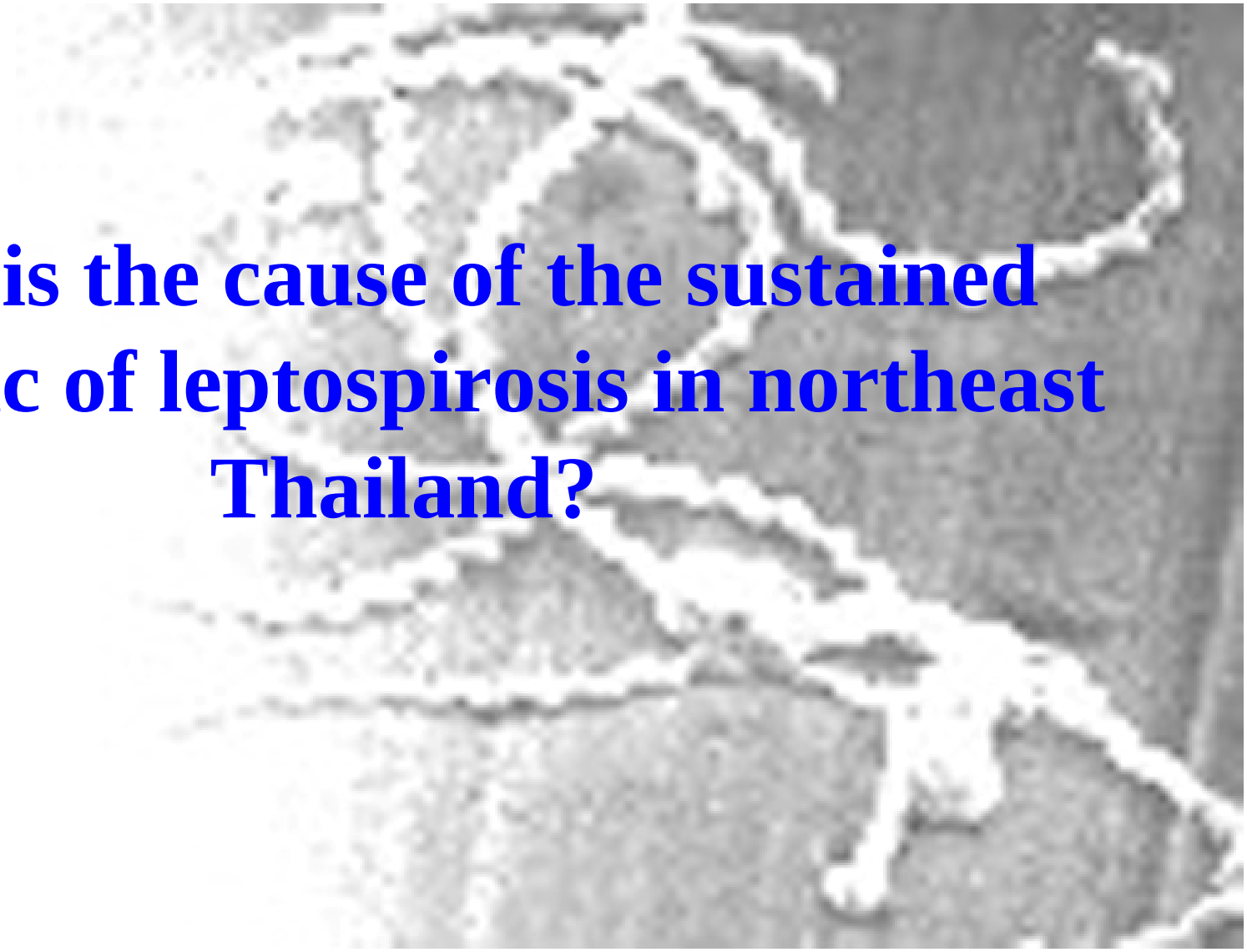
Leptospirosis in Thailand

Number of reported leptospirosis cases in Thailand: 1990-2006
(source:Disease notification report.Ministry of Public Health, Thailand)



- **Reporting is voluntary and probably represents a small proportion of true cases.**
- **The cause of the epidemic was not known.**

What is the cause of the sustained epidemic of leptospirosis in northeast Thailand?



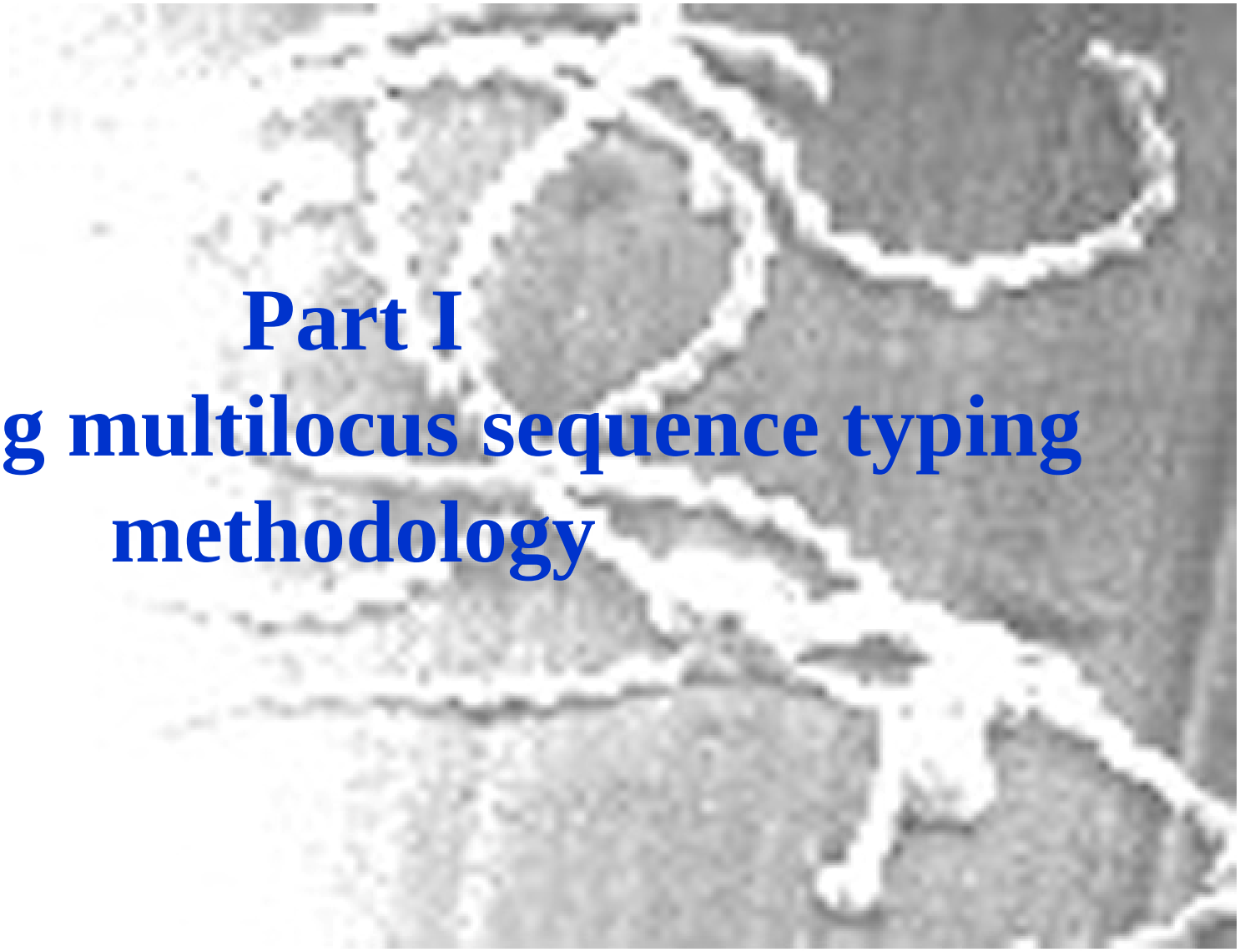
- **Outbreaks in Thailand and elsewhere are often linked to climate events such as flooding and concomitant increase in human exposure to environments contaminated by *Leptospira*.**

- **However, in this case, it could not be explained by persistent climate change or sequential episodes of regional flooding**

We hypothesized that the epidemic was caused by the rapid expansion of a single virulent clone of pathogenic *Leptospira*

Aims of study

To define the molecular epidemiology of *Leptospira* strains isolated from cases of human leptospirosis during the epidemic and to relate this to the maintenance animal hosts



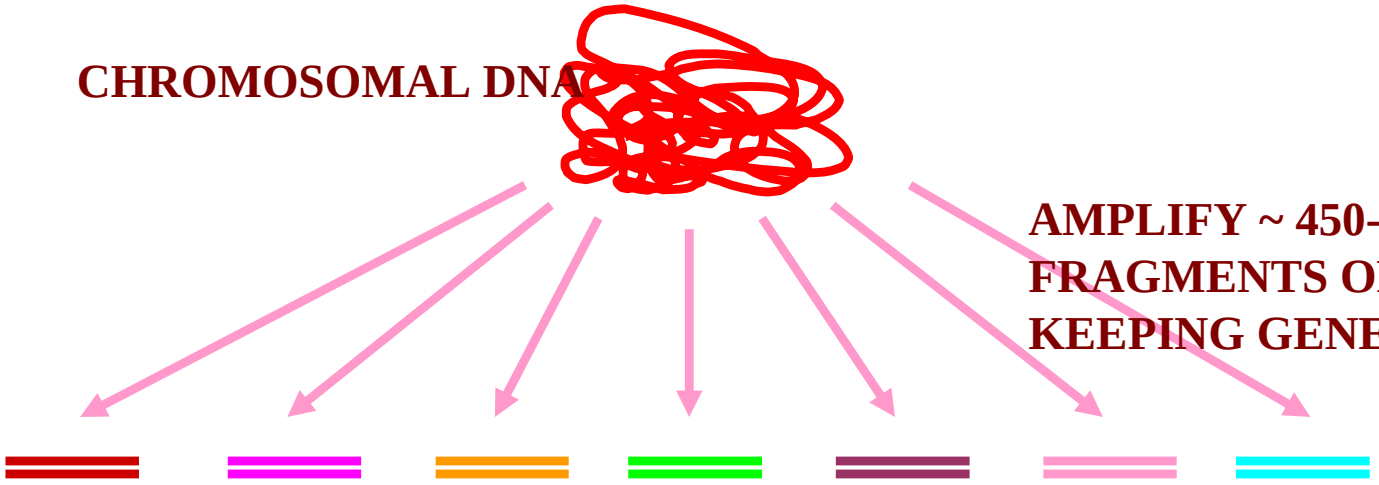
Part I
Developing multilocus sequence typing methodology

Multilocus sequence typing (MLST)

- **Is based on nucleotide sequence which is easy to compare between laboratories via internet.**

- **Already developed for many bacterial pathogens for global epidemiology studies such as *Neisseria meningitides, Streptococcus pneumoniae, Burkholderia pseudomallei, etc.***

CHROMOSOMAL DNA



AMPLIFY ~ 450-BP INTERNAL FRAGMENTS OF SEVEN HOUSE-KEEPING GENES

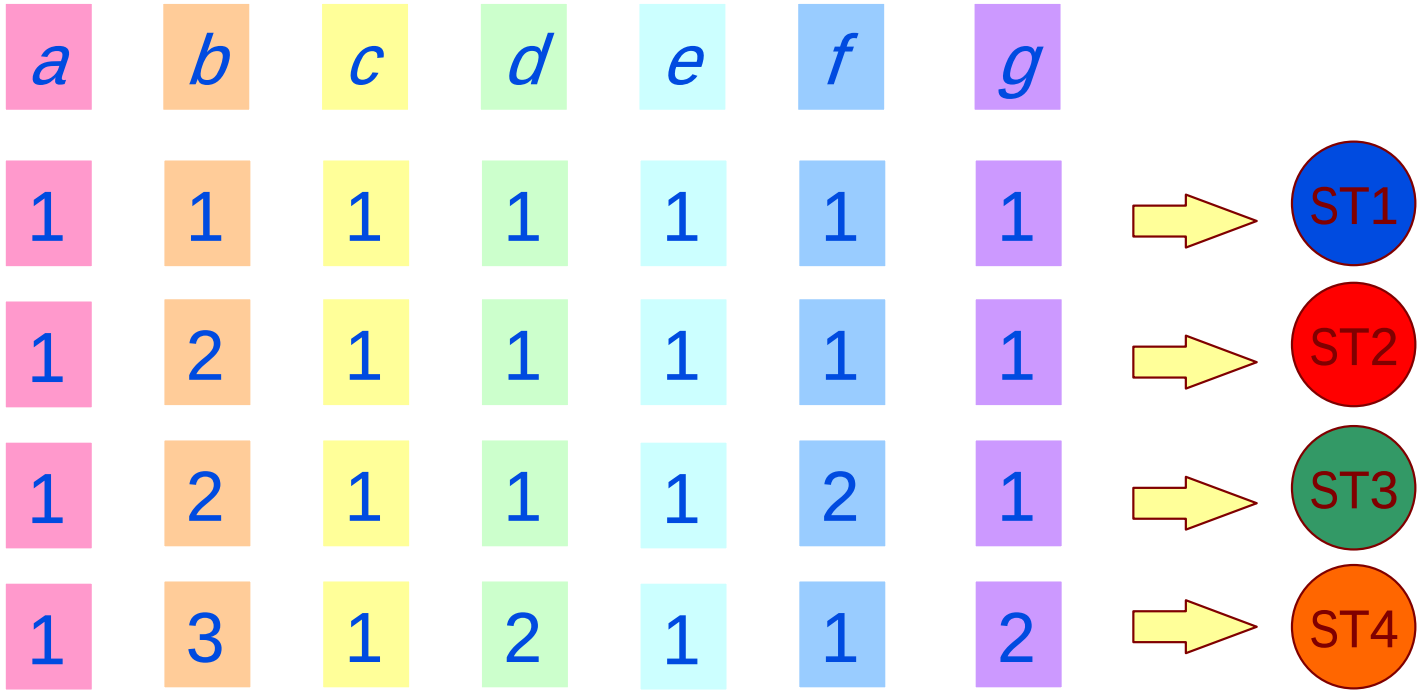
SEQUENCE THE SEVEN GENE FRAGMENTS ON BOTH DIRECTIONS

COMPARE THE SEQUENCES OF EACH GENE FRAGMENT WITH THE KNOWN ALLELES AT THE LOCUS

ASSIGN ALLELES AT THE SEVEN LOCI TO GIVE THE ALLELIC PROFILE

COMPARE THE ALLELIC PROFILES, AND OBTAIN SEQUENCE TYPE

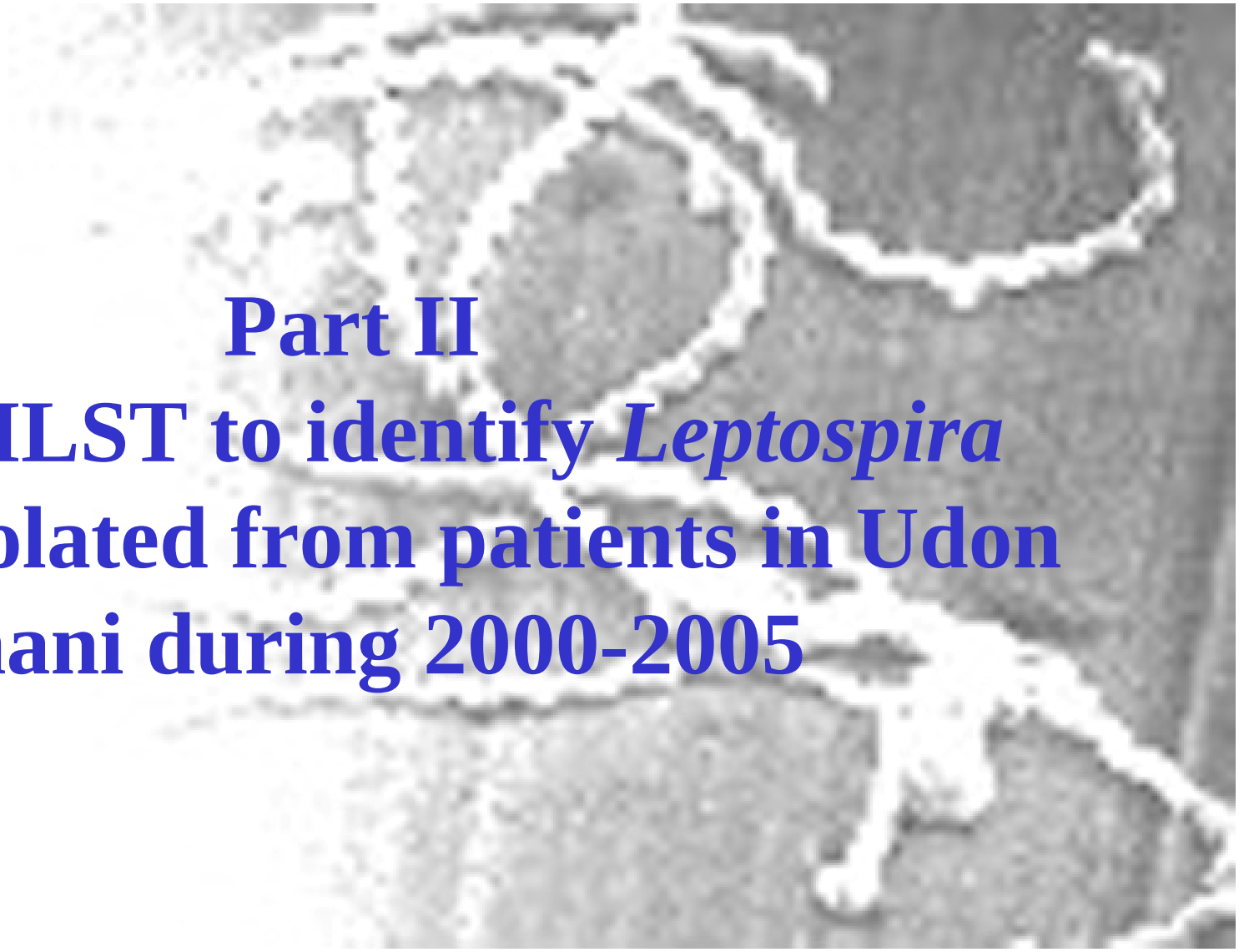
Designation of sequence type based on variability in loci



Seven selected house-keeping genes

Gene*	Function	TIGR Cellular role category	Location of sequence used to define MLST locus
pntA	NAD(P) transhydrogenase subunit alpha	Energy metabolism: Electron transport	56347-56871
sucA	2-oxoglutarate dehydrogenase decarboxylase component	Energy metabolism: TCA cycle	1227474-1227920
pfkB	Ribokinase	Energy metabolism: Sugars	1386553-1386984
tpiA	Triosephosphate isomerase	Energy metabolism: Glycolysis/gluconeogenesis	1694673-1694248
mreA	Rod shape-determining protein rodA	Cell envelope: Biosynthesis and degradation of murein sacculus and peptidoglycan	2734550-2734116
glmU	UDP-N-acetylglucosamine pyrophosphorylase	Cell envelope: Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides	3784955-3784512
fadD	Probable long-chain-fatty-acid--CoA ligase	Not known	83570-83115

- MLST using seven selected loci were able to define sequence type for ***L. interrogans*** and ***L. kirschneri.***
- ***L. borgpetersenii*** amplified at only one or two loci (*glmU* and *fadD*)



Part II
Using MLST to identify *Leptospira* strains isolated from patients in Udon Thani during 2000-2005

A prospective study in Udon Thani

- **A prospective study was undertaken between mid-October 2000 to October 2005 at Udon Thani General Hospital, Northeast Thailand (consecutive months from 2000-2002, four months each rainy season during 2003-2005)**

- **Patients (>15 years) presenting with fever (>37.8 C) of unknown cause were recruited. Patients with a blood smear positive for malaria parasites or other definable infections were excluded**

- **Blood samples were taken for *Leptospira* culture and serology tests**

A prospective study in Udon Thani

- **Of 104 isolates, 99 isolates were *L. interrogans*, 3 were *L. borgpetersenii*, and 2 were *L. kirschneri*.**
- **All *L. borgpetersenii* serovar javanica were failed to amplified at 5 or 6 loci but were identical *glmU* allele to each other.**
- **101 isolates were resolved into 12 ST.**
  - **77 isolates were ST34, serovar autumnalis: a dominant clone**
  - **8 isolates were ST46**
  - **4 isolates were ST49**
  - **Other nine ST comprised one or two isolates**

	2000	2001	2002	2003	2004	2005	total
Number of patients in Udon Thani cultured for *Leptospira*	122	551	503	124	184	174	1762
Number culture-positive for *Leptospira* in Udon Thani (% positive/year)	14 (11%)	47 (7%)	36 (7%)	8 (7%)	6 (3%)	4 (2%)	115 (6%)
Number of isolates available for multilocus sequence typing	14	39	34	8	6	3	104
Number of *Leptospira* isolated in Udon Thani belonging to ST34 (% of total per year)	12 (86%)	33 (85%)	23 (68%)	4 (50%)	4 (67%)	1 (33%)	77 (74%)

- **The numbers of cases of culture proven leptospirosis were greatest during 2001 (there were only two study months during 2000)**

- **A significant reduction over time in the proportion of patients presenting with fever who were leptospiremia (χ^2 for tend=15.3, p<0.0001)**

- **The proportion of ST34 causing leptospirosis fell over time significantly (χ2 for trend = 9.98, p=0.0016)**

(from 85% in 2000/2001 to 64% in 2002/2003 and 56% in 2004/2005)

These results indicate that ST34 clone was responsible for the 1999-2002 epidemic of leptospirosis in Udon Thani province, Thailand.

Part III
Distribution of ST34 across Thailand

Distribution of ST 34 across Thailand

Method

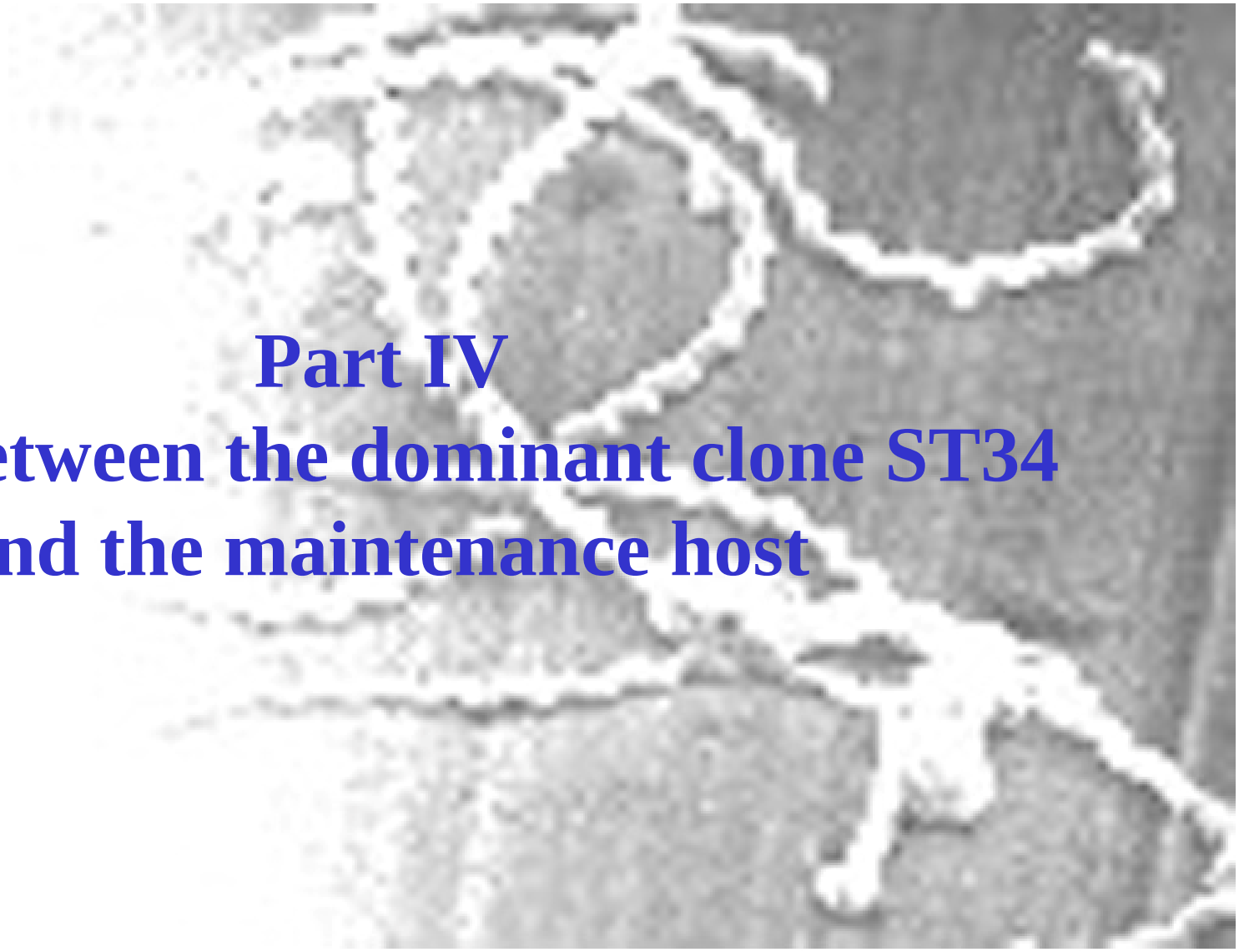
- MLST was performed on 24 unselected strains isolated from leptospirosis patients presenting to hospitals in 8 additional provinces in Thailand during the raining reason of 2003-2004.

- These strains were obtained from a culture collection.

Distribution of ST 34 across Thailand

- **2 isolates -> *L. borgpetersenii (*non-typable isolates)**
- **22 isolates were resolved into 6 STs**
- **The total proportion of isolates corresponding to ST34 was 17/22 (71%)**
- **This is not significantly different from the proportion of ST34 in isolates from Udon Thani in the same year (Fisher's exact p=0.3).**

This confirms that the epidemic clone ST34 is widely distributed throughout Thailand and formed the predominant virulent strain at the time of the epidemic



Part IV
A link between the dominant clone ST34 and the maintenance host

A link between ST 34 and maintenance host

- **Of 1,126 rodents trapped in Nakhon Ratchasima during 2004, 8 rodents were kidney culture positive for *Leptospira***

- **7/8 (88%) strains were *L. interrogans* ST34.**
  - **6 were isolated from *Bandicota indica***
  - **1 was isolated from *Bandicota savilei***

- **The remaining one isolate from *Rattus rattus* was ST49**

Bandicota indica

Bandicota savilei

Rattus rattus

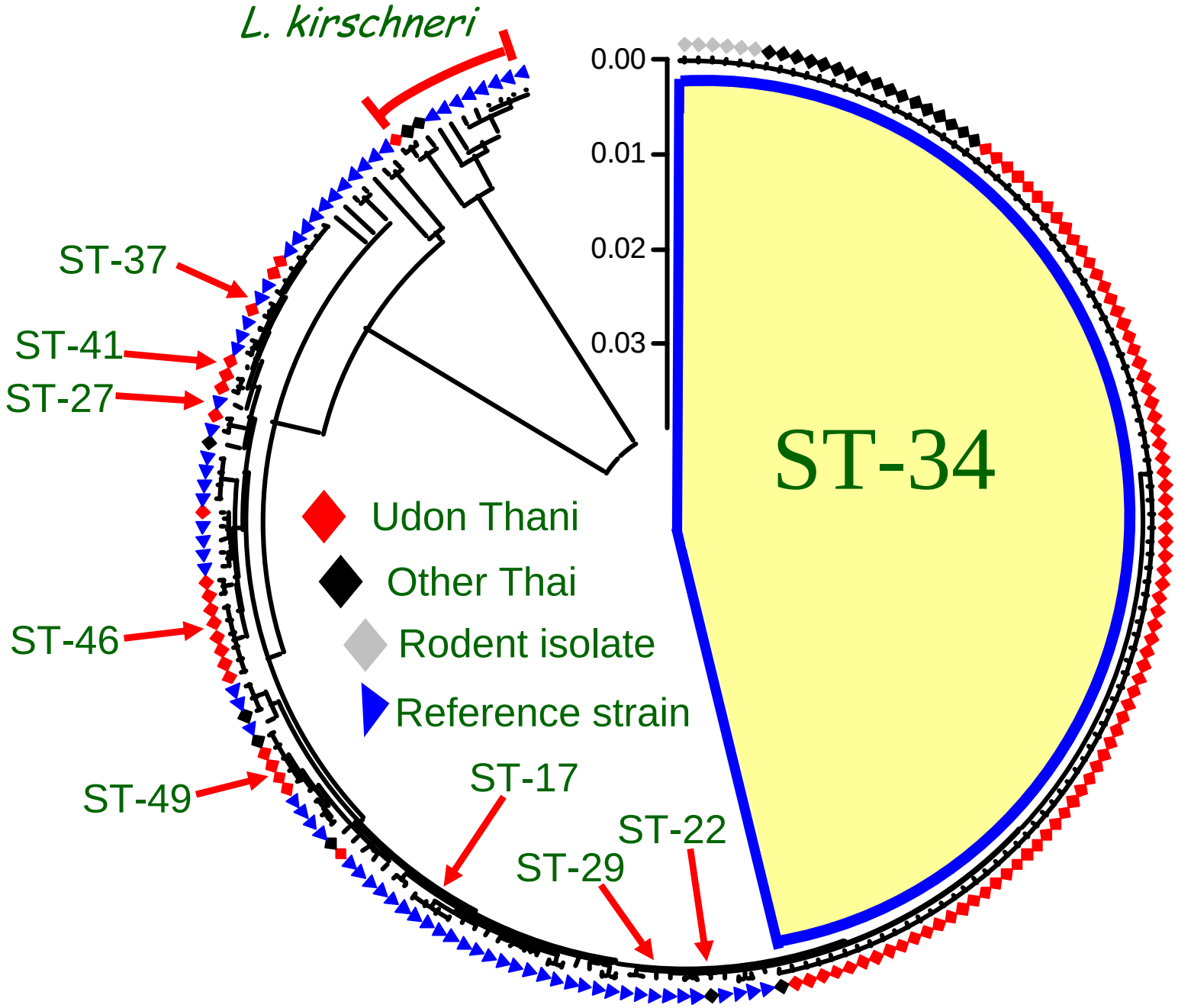
This confirms the predominance of the outbreak strain (ST34) in the maintenance host, bandicoot rats.

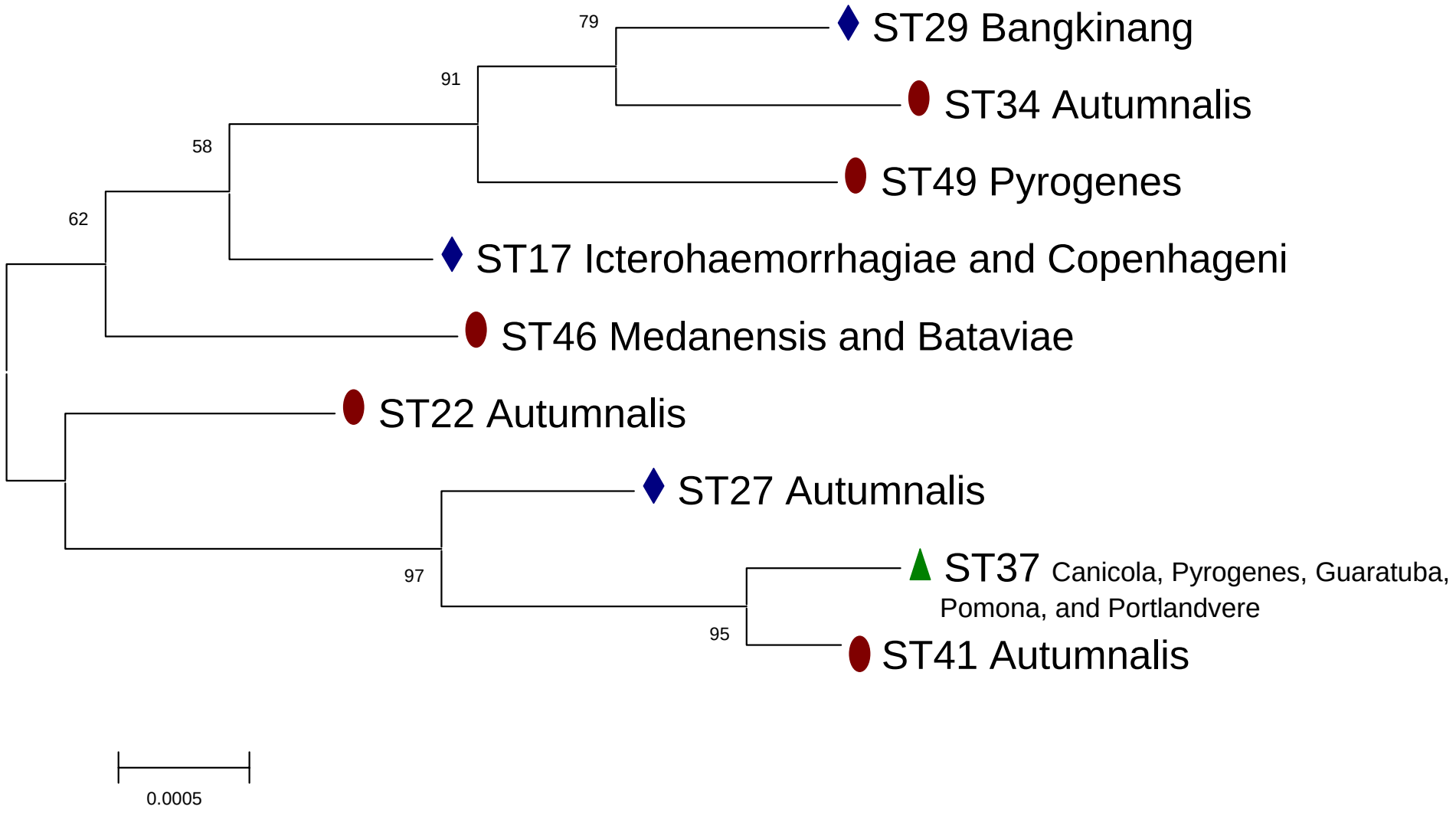
Part V
Thai strains within a global context

Thai strains within a global complex

- **MLST was performed on 73 reference strains isolated from diverse hosts in diverse geographical regions**
  - ***L. interrogans* = 65**
  - ***L. kirschneri* = 8**
- **73 reference strains -> 59 STs (0.81 ST per isolate)**
  123 Thai isolates -> 16 STs (0.13 ST per isolate)

This demonstrates Thai isolates are clonally restricted compared with reference collections

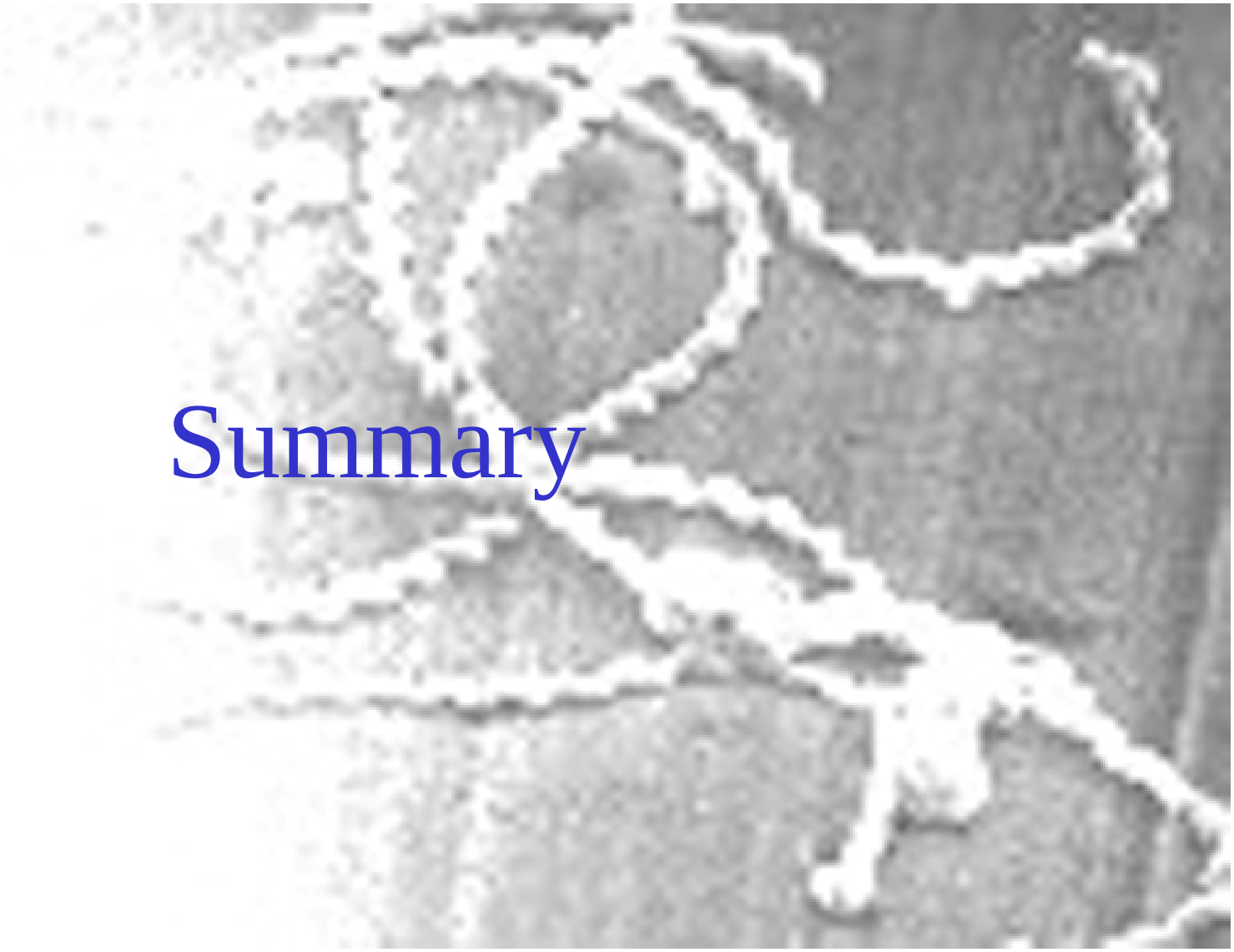

L. kirschneri
0.00
0.01
0.02
0.03
ST-37
ST-41
ST-27
ST-46
ST-49
ST-17
ST-29
ST-22
ST-34
Udon Thani
Other Thai
Rodent isolate
Reference strain


79
91
58
62
ST29 Bangkinang
ST34 Autumnalis
ST49 Pyrogenes
ST17 Icterohaemorrhagiae and Copenhageni
ST46 Medanensis and Bataviae
ST22 Autumnalis
ST27 Autumnalis
97
ST37 Canicola, Pyrogenes, Guaratuba, Pomona, and Portlandvere
95
ST41 Autumnalis
0.0005
Reference isolates
Thai isolates
Reference and Thai isolates

- The different clones sampled from Thailand did not from a single cluster but were dispersed throughout the tree. This indicates that they have not all diverged from a common Thai ancestor.

- The lack of evidence for strong geographical structure is consistent with high rates of migration via rodent (or possibly human) host.

Summary



Summary

Development of MLST for *L. interrogans* revealed that a single ecologically successful pathogenic clone of *L. interrogans* (ST34 serovar autumnalis) predominated in the rodent population, and was associated with a sustained outbreak of human leptospirosis in Thailand

Reference

Thaipadungpanit J., Wuthiekanun1 V., Chierakul W., Smythe L.D., Petkanchanapong W., Limpaiboon R., Apiwatanaporn A., Slack A.T., Suputtamongkol Y., N.J.White, E.J.Feil, N.P.J.Day, and S.J.Peacock. 2007. A dominant clone of *Leptospira interrogans* associated with an outbreak of human leptospirosis in Thailand. PLoS Negl Trop Dis 1(1):e56. doi:10.1371/journal.pntd.0000056

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Question?