WGS OF *M. TUBERCULOSIS* REVEAL STRONG ASSOCIATIONS BETWEEN GENOTYPES AND ETHNICITY: ITS IMPLICATION IN TB CONTROL

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AREEYA DISRATTHAKIT, DMSC, MOPH

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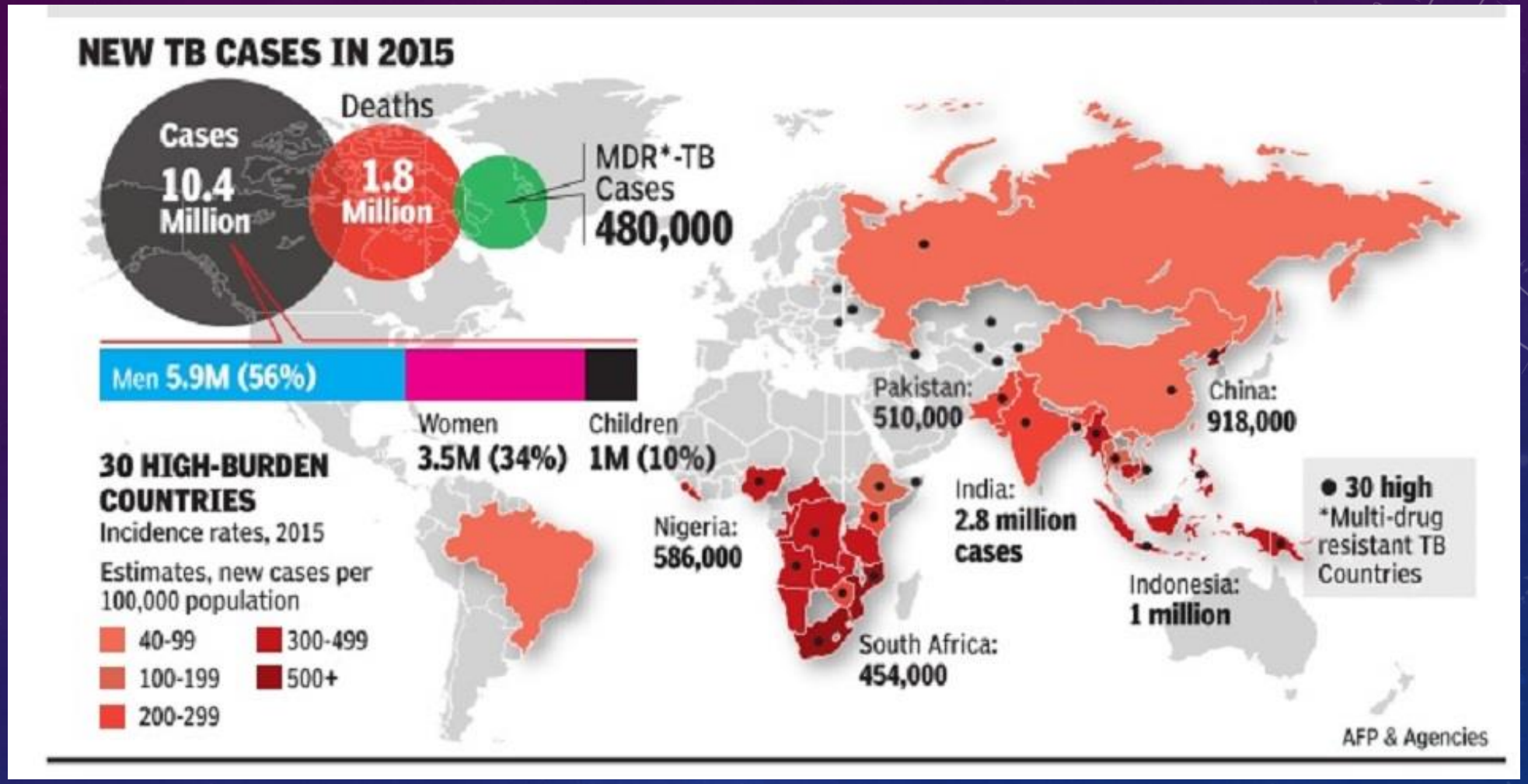
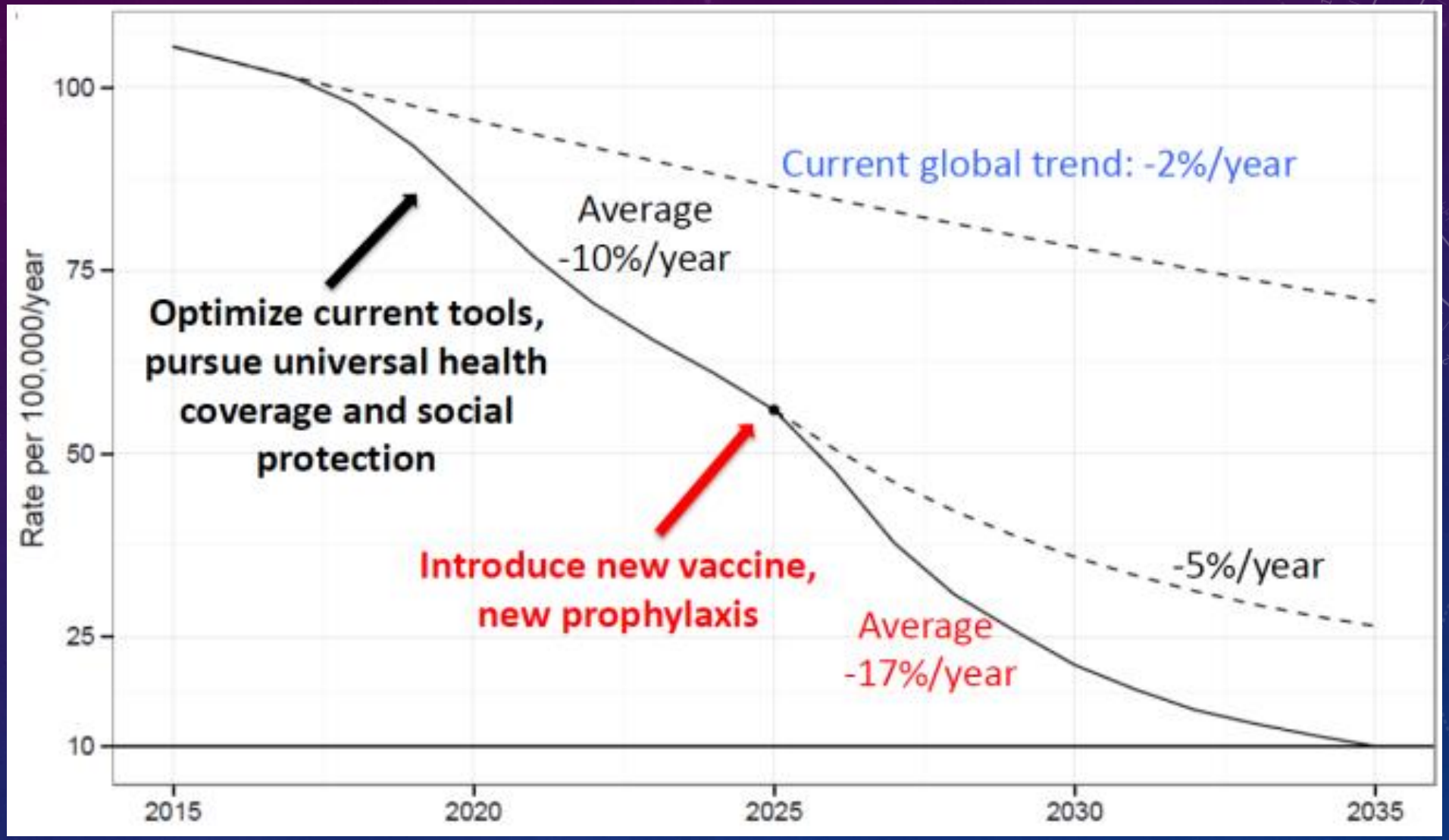
ET AL.


NEW TB CASES IN 2015
Cases
10.4
Million
Deaths
1.8
Million
MDR*-TB
Cases
480,000
Men 5.9M (56%)
Women
3.5M (34%)
Children
1M (10%)
30 HIGH-BURDEN COUNTRIES
Incidence rates, 2015
Estimates, new cases per 100,000 population
40-99
100-199
200-299
300-499
500+
Pakistan:
510,000
China:
918,000
India:
2.8 million
cases
Nigeria:
586,000
Indonesia:
1 million
South Africa:
454,000
● 30 high
*Multi-drug resistant TB Countries
AFP & Agencies



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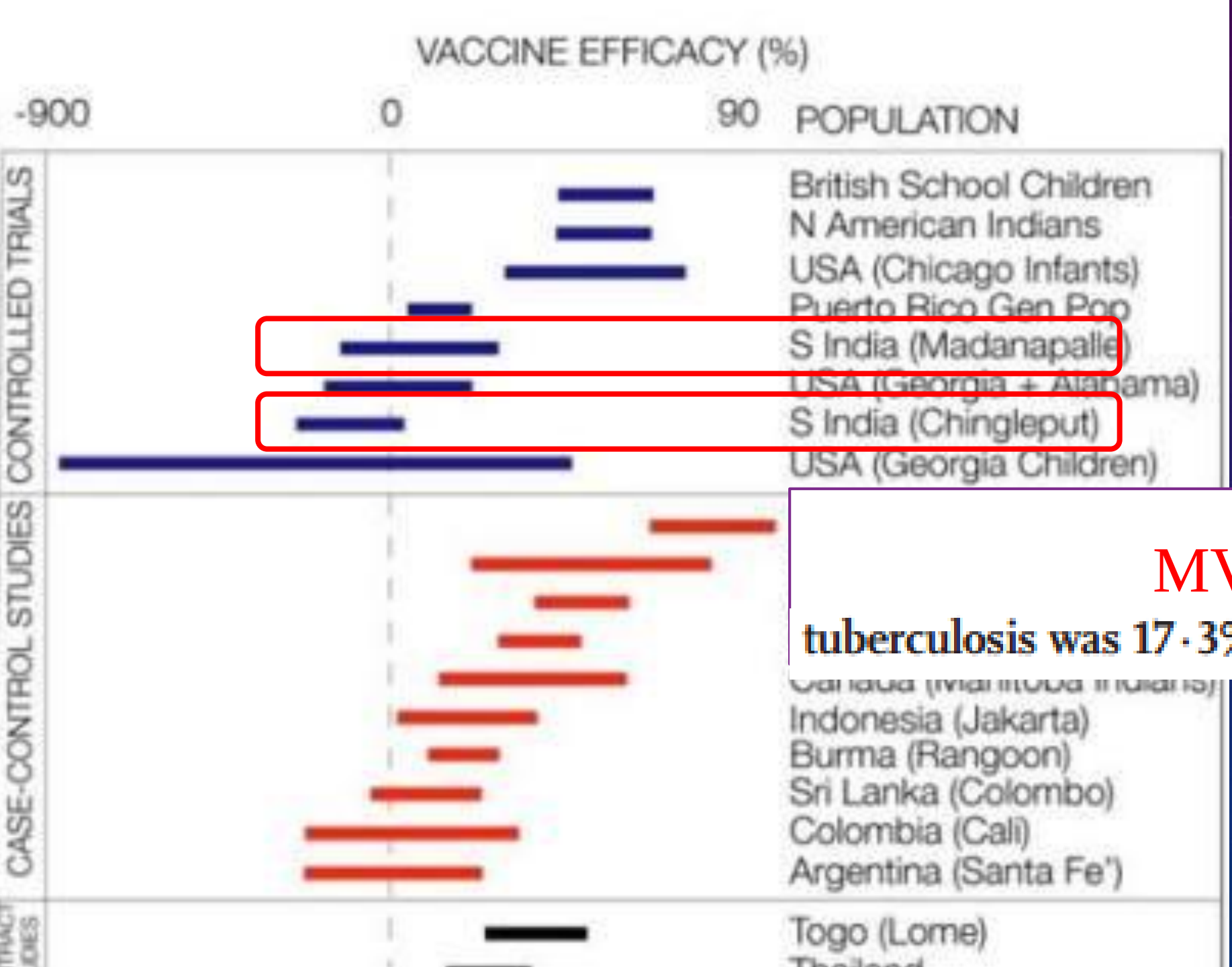
WHY IS IT SO HARD TO DEVELOP TB VACCINES?



MVA85A **Efficacy against tuberculosis was 17·3% (95% CI –31·9 to 48·2)**

SUPPORTED BY

SATREPS

Science and Technology Research Partnership for Sustainable Development Program

COLLABORATION OF

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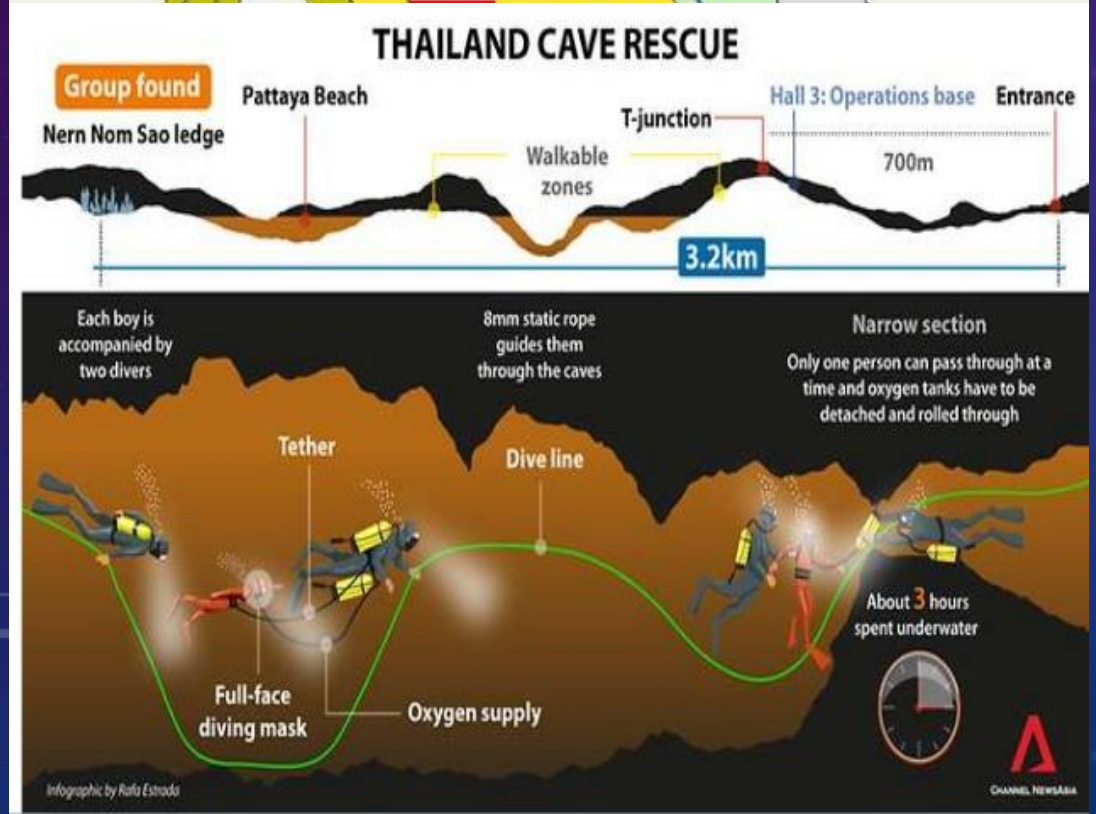
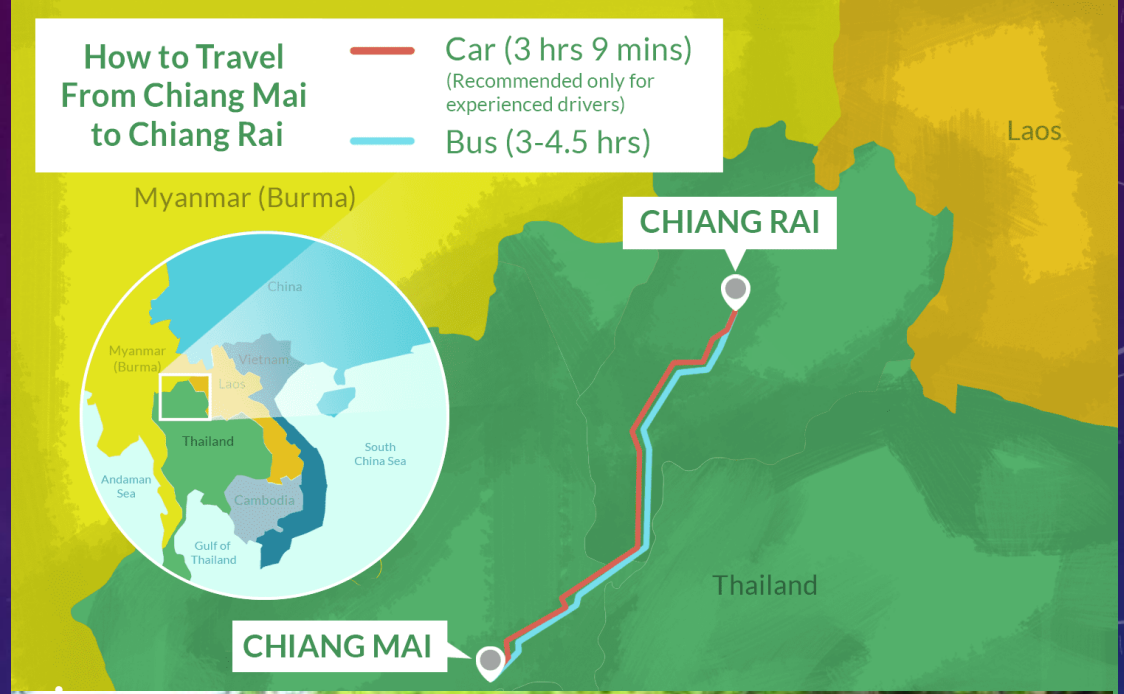

THAILAND CAVE RESCUE
Group found
Nern Nom Sao ledge
Pattaya Beach
T-junction
Hall 3: Operations base
Entrance
Walkable zones
700m
3.2km
Each boy is accompanied by two divers
8mm static rope guides them through the caves
Narrow section
Only one person can pass through at a time and oxygen tanks have to be detached and rolled through
Tether
Dive line
About 3 hours spent underwater
Full-face diving mask
Oxygen supply
Infographic by Rafa Estrada
CHANNEL NEWSASIA


How to Travel From Chiang Mai to Chiang Rai
Car (3 hrs 9 mins)
(Recommended only for experienced drivers)
Bus (3-4.5 hrs)
Laos
Myanmar (Burma)
CHIANG RAI
China
Myanmar (Burma)
Vietnam
Laos
Thailand
South China Sea
Andaman Sea
Cambodia
Gulf of Thailand
Thailand
CHIANG MAI

SETTINGS

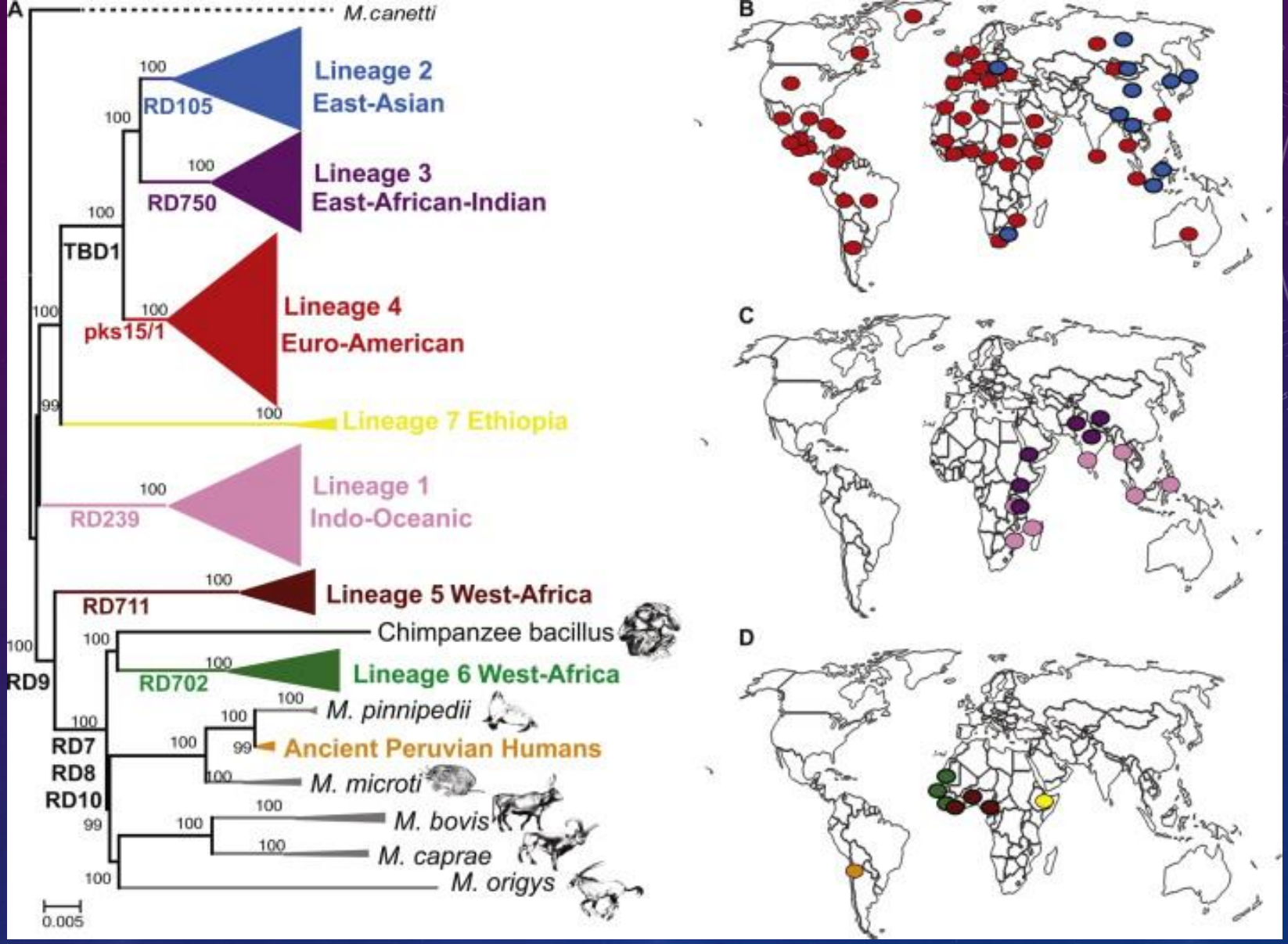
Chiangrai background

- Population: mainly Thais
  - Ethnic minorities: Tibeto-Burman (Ahka and Lahu), Hmong-Mien, etc.
- History: Settlement since 7th century, becoming Lanna Kingdom and then occupied by various tribes: Myanmar, Lao, Thai.
- Current: Tourist destination and transportation hub
- Populations: 1.2 M
- TB Incidence 2011: 152.6/100,000

Study Methods

- 1170 pulmonary tuberculosis patients, 2003-2010
- Bacteria:
  - Phenotypic drug susceptibility
  - Experimental LSP and spoligotyping
  - WGS by Illumina Hi-Seq at Sanger Institute
- Patients:
  - Clinical profiles
  - High density genotyping (Illumina HumanOmniExpressExome-8 v1.2 BeadChip, 938764 SNPs)

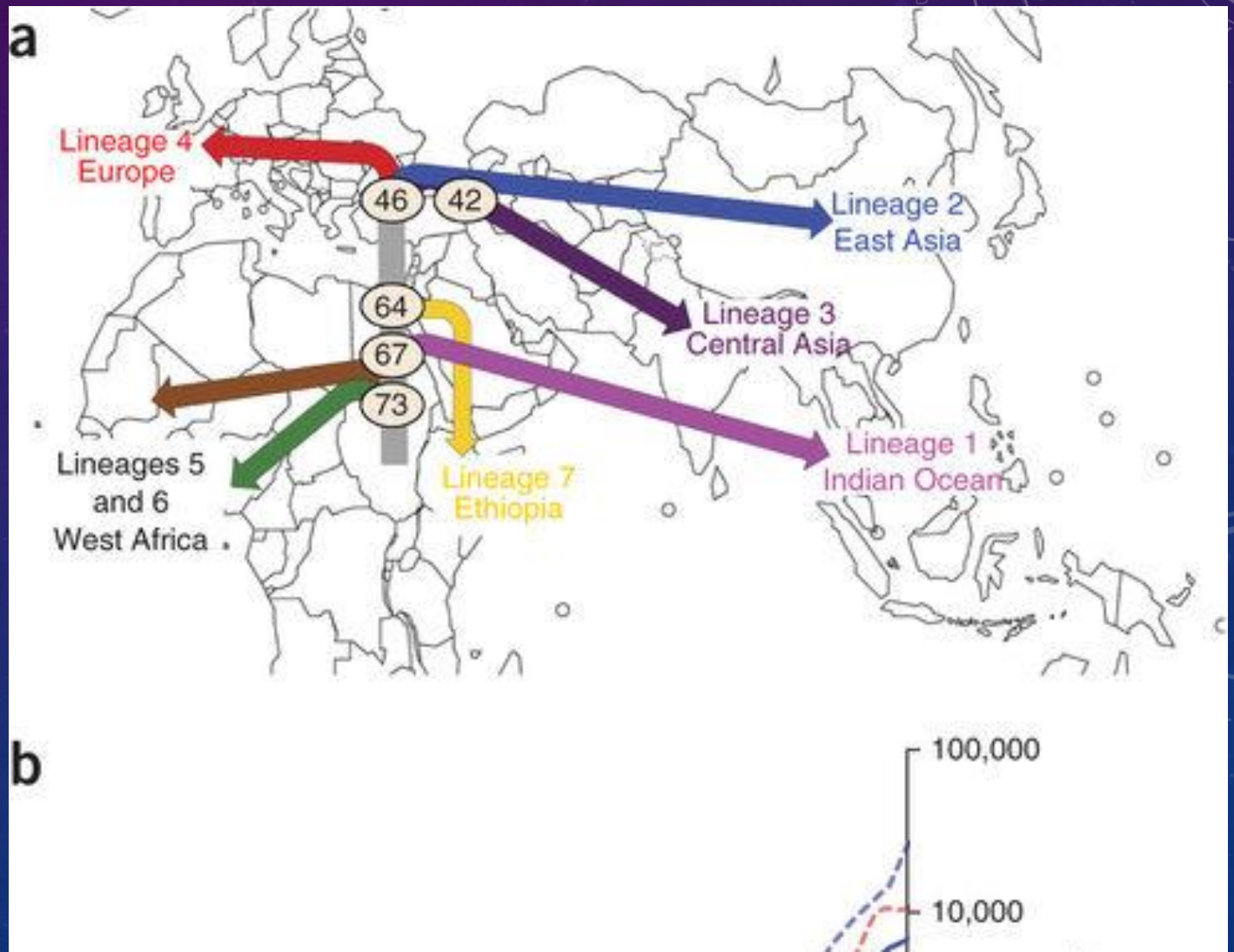
RESULTS



A: LSP and global phylogeny for *MTB* define 6 main lineages. Countries where the predominant lineages are B. 2 and 4, C. 1 and 3, D. 5 and 6 Coscolla and Gagneux. Sem Immunol 2016.

THE POSSIBLE CAUSES OF PHYLOGEOGRAPHIC ASSOCIATIONS

- Founder Effects
- Co-evolution



CORRELATION BETWEEN SNP GENOTYPES AND SPOLIGOTYPIC CLADES REVEALED THAT MANY CLADES AND TYPES ARE HOMOPLASTIC, THAT IS, GENOTYPIC MEANINGLESS

SCIENTIFIC REPORTS

OPEN

Evidence for Host-Bacterial Co-evolution via Genome Sequence Analysis of 480 Thai *Mycobacterium tuberculosis* Lineage 1 Isolates

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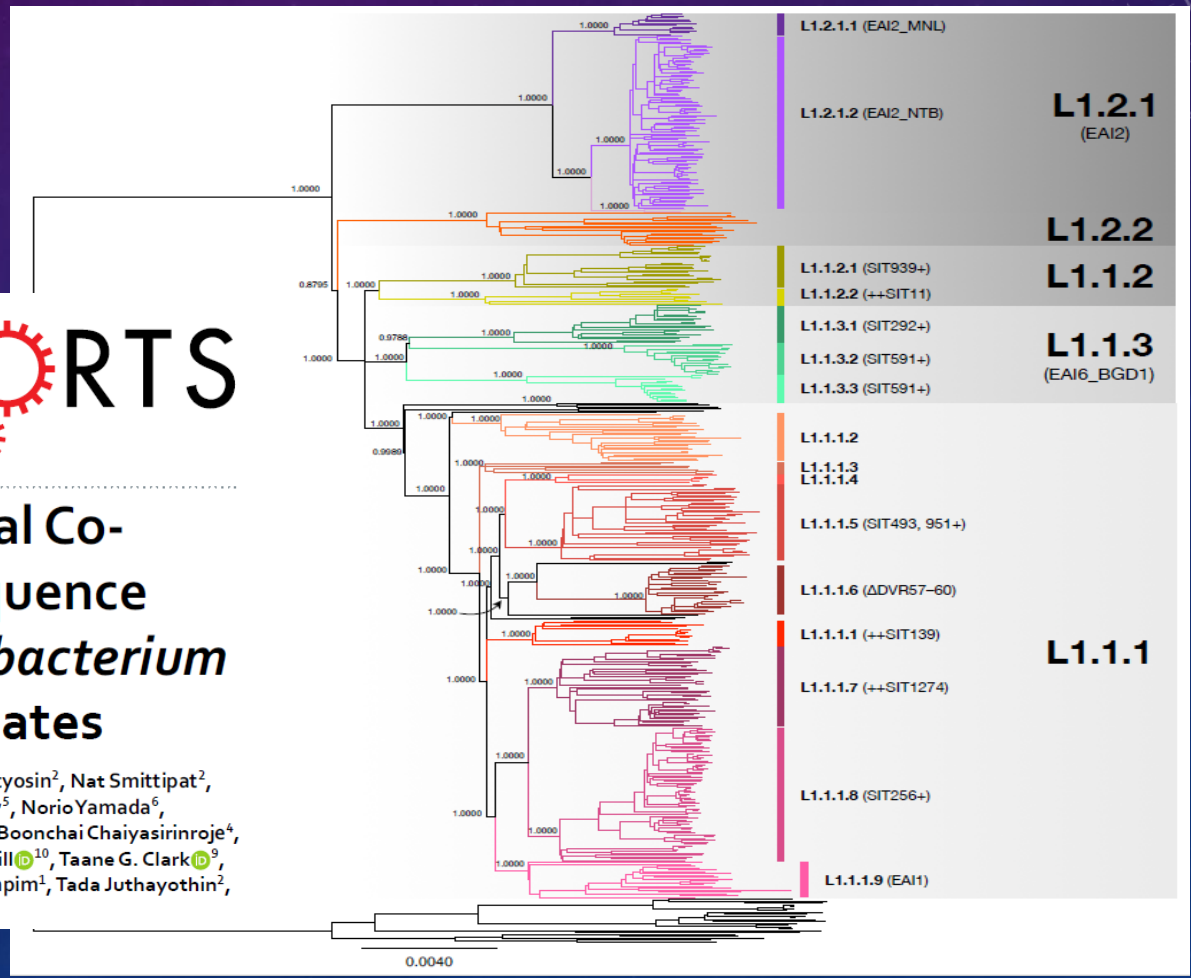
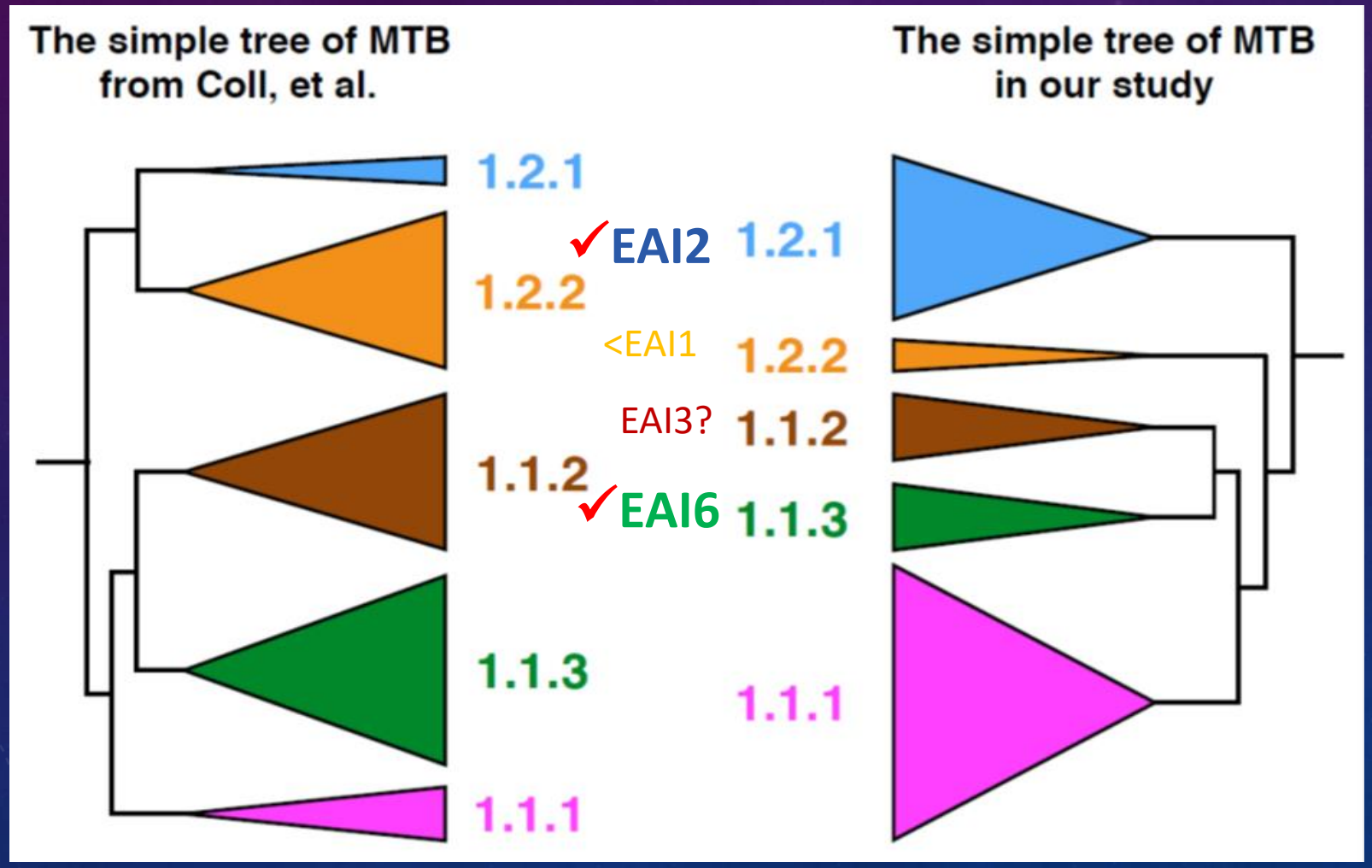


Table 2. The number of isolates with various experimental spoligotypes identified in each sublineage, listed by the ascending order of the octal codes. The spoligotypes that are found in more than one sublineage are indicated in bold typeface. There was no unclassified spoligotypes that appeared in two sublineages. The reporting country names were from SITVITWEB online searching tool (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/query) and was given using ISO 3166-1 alpha-3 three letter country code. When published articles are available, the references are given. (SIT is Spoligotype International Type)

Major Sublineages	Sublineages	Number	Number of isolates x Known spoligotypes	SIT	Reporting Countries (clade: numbers reported in SITVITWEB, reported countries(published references)	Number of isolates x Unclassified spoligotypes
1.1.1	1.1.1.1	14	1x677777777413771 **2x777737777413771** 2x777777774413771 **8x777777777413771**	342 **618** 139 **236**	EAI5: 13, IND(Shanmugam et al. 2011), GMB, GHA, GIN, NGA, MAR, BEL, DEN, NLD, DEU, USA EAI5: 11, IDN(Sasmono et al. 2012), VNM, THA, FRA, NLD, USA, EAI4_VNM: 323, VNM(Buu et al. 2009a; Duong et al. 2009; Nguyen et al. 2012), KHM(Zhang et al. 2011), THA(Yorsangsukkamol et al. 2009), IRN(Merza et al. 2010) EAI5:130, SAU(Al-Hajoj et al. 2007), PAK(Tanveer et al. 2008), IND(Narayanan et al. 2008; Shanmugam et al. 2011; Thomas et al. 2011; Joseph et al. 2013; Devi et al. 2015; Sharma et al. 2017), BGD(Rahim et al. 2007; Banu et al. 2012), MMR(Phyu et al. 2009), THA(Yorsangsukkamol et al. 2009), KHM(Zhang et al. 2011), VNM(Duong et al. 2009; Nguyen et al. 2012), MYS(Ismail et al. 2014), IDN(Sasmono et al. 2012; Chaidir et al. 2016), MOZ(Viegas et al. 2010), AUS, NZL, GNB, SEN, ZWE, BEL, DEN FRA, GBR, ITA, NDL, NOR, SWE, USA,	1x717777777003371
	1.1.1.2	26	**1x737777777413771** 3x777737777413731 **1x777737777413771** **2x777777777413731** **1x777777777413671** **11x7777777774137711** x777777777773771	**204** 349 **618** **48** **256** **236** 100	EAI5: 10, KHM(Zhang et al. 2011), VNM, THA(Yorsangsukkamol et al. 2009), IND(Sharma et al. 2017), FRA, USA EAI1: 18, BGD(Rahim et al. 2007; Banu et al. 2012), IND(Joseph et al. 2013), MYS(Ismail et al. 2014), MMR(Phyu et al. 2009), IDN(Chaidir et al. 2016), MOZ(Viegas et al. 2010), GUF, TUN, GBR, USA EAI5: 11, VNM(Nguyen et al. 2012), KHM(Zhang et al. 2011), THA, FRA, NLD, USA, EAI1:313, SAU(Al-Hajoj et al. 2007), IND(Narayanan et al. 2008; Arora et al. 2009; Shanmugam et al. 2011; Joseph et al. 2013; Devi et al. 2015;	1x737777770003771 1x777717777413771 1x777777777411771*

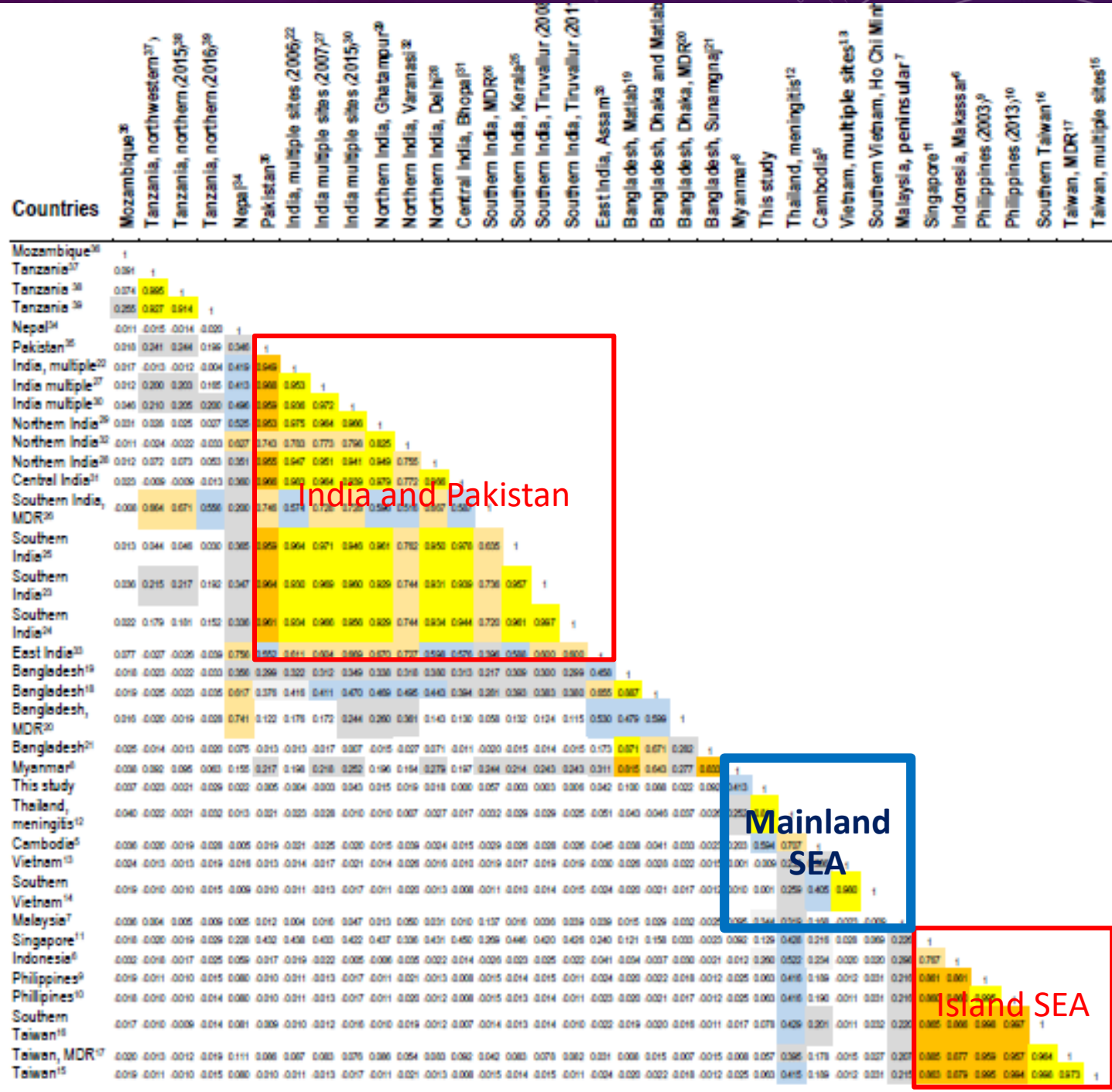
FREQUENCY DISTRIBUTION OF L1 WAS DIFFERENT FROM GLOBAL SAMPLES



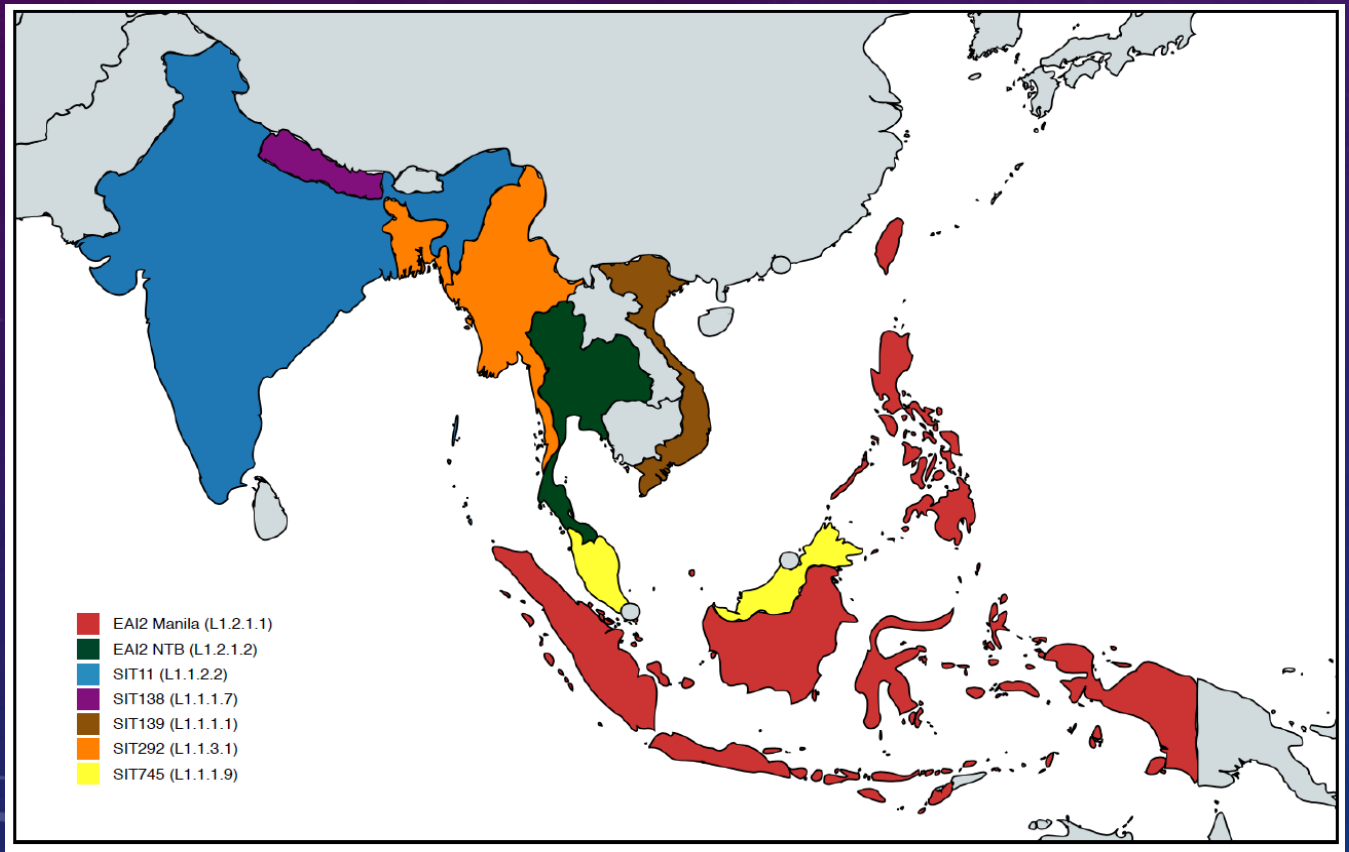
EAI1, EAI4 and EAI5 are homoplastic

CORRELATION OF NON-HOMOPLASTIC SPOLIGOTYPE PROFILE OF L1 BETWEEN COUNTRIES

- Excluding homoplastic spoligotypes and studies on special bact. pop. such as MDR or Epul TB.
- Correlations in the same countries were high
- No correlation between East Africa and SEA and within Mainland SEA
- Correlations between neighboring countries were intermediate but exceptionally high in
  - India-Pakistan
  - Bangladesh and Myanmar
  - ISEA (Indonesia, the Philippines), Singapore and Taiwan



DISTRIBUTION OF L1 SUBLINEAGES CORRELATES WITH LANGUAGE FAMILIES OF OFFICIAL LANGUAGES OF COUNTRIES IN SEA: AN EVIDENCE OF CO-EVOLUTION



Bangladesh: Indo-Arayan

Myanmar: Tibeto-Burman

Thailand: Tai-Kadai

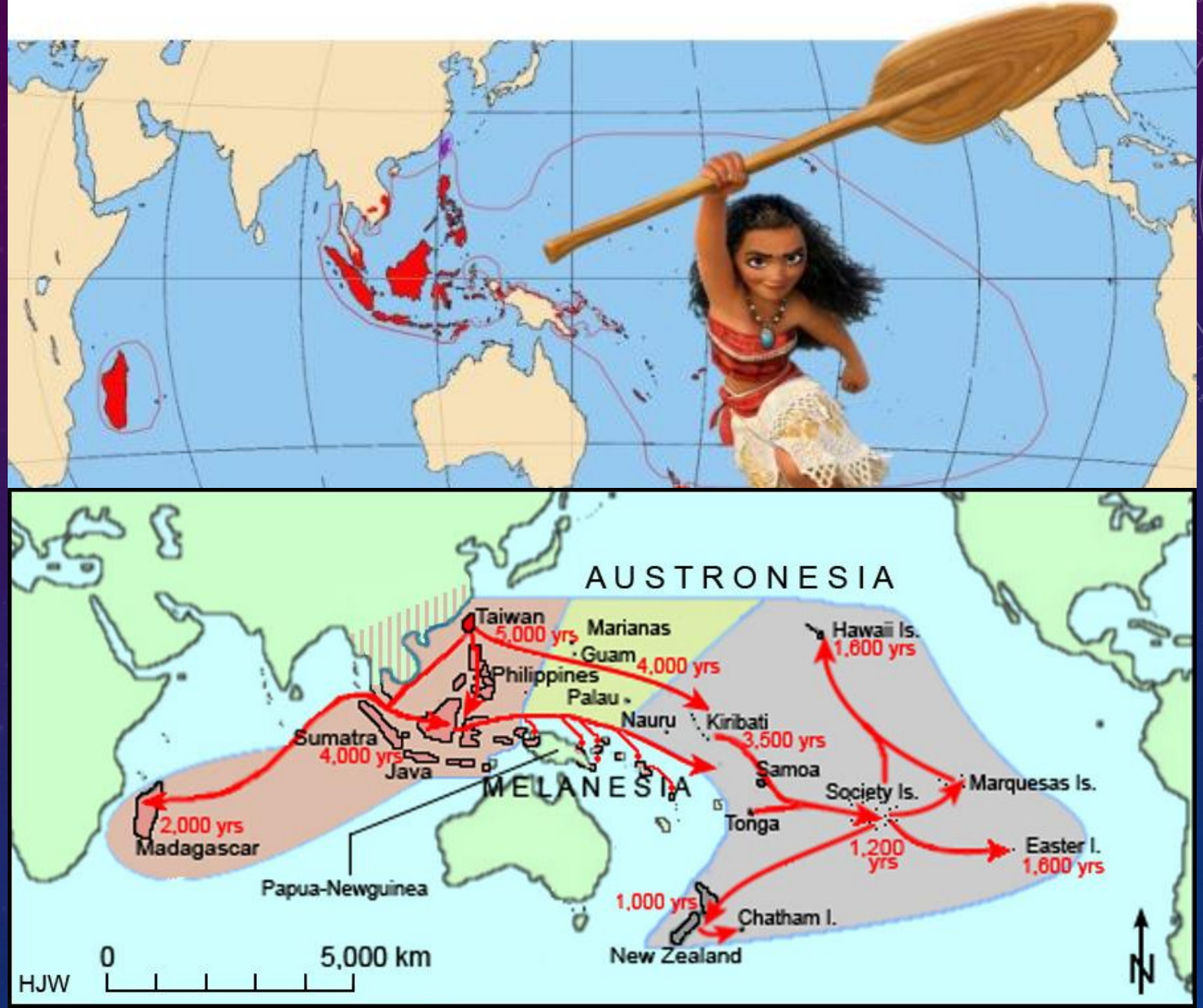
Vietnam: Austroasiatic (Vietic: Kinh)

Malaysia: Austronesian

Indonesia: Austronesian

Philippines: Austronesian

Southern Taiwan: Austronesian



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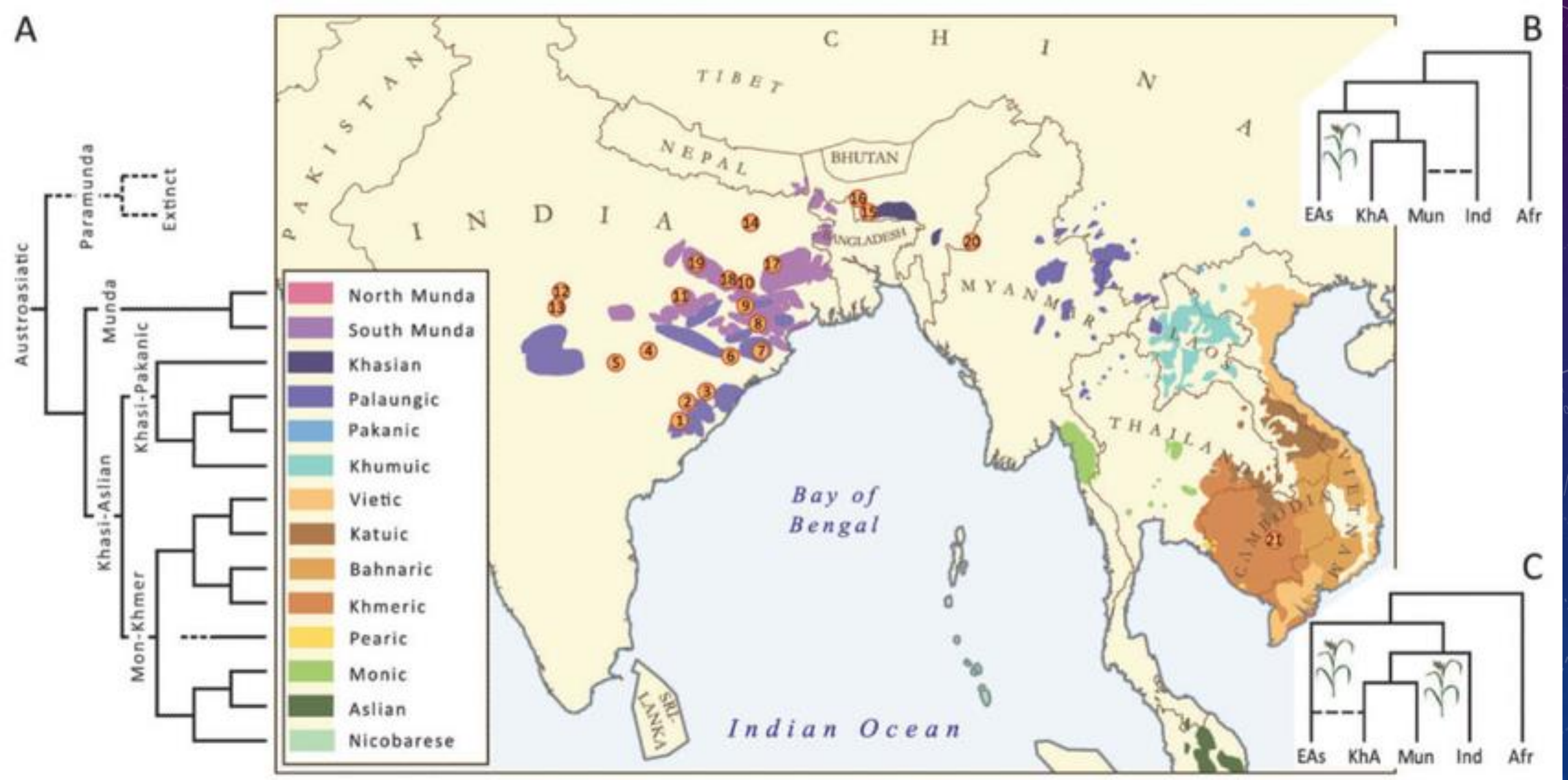
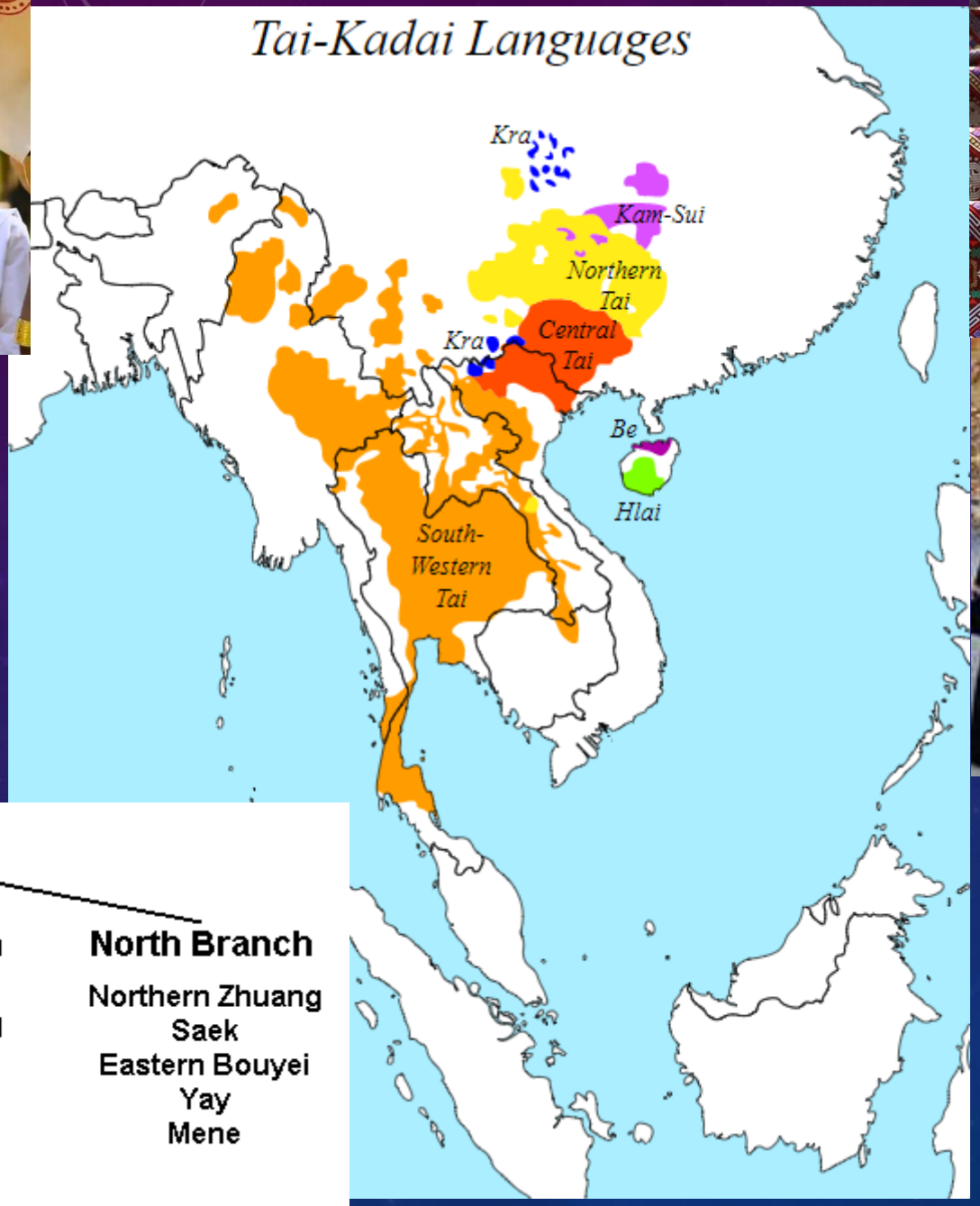


https://www.researchgate.net/profile/Jueri_Parik/publication/47555877/figure/fig1/AS:339573449216000@1457972027148/A-Language-tree-of-the-major-subgroups-of-the-Austroasiatic-AA-language-family.jpg

Shan



Zhuang

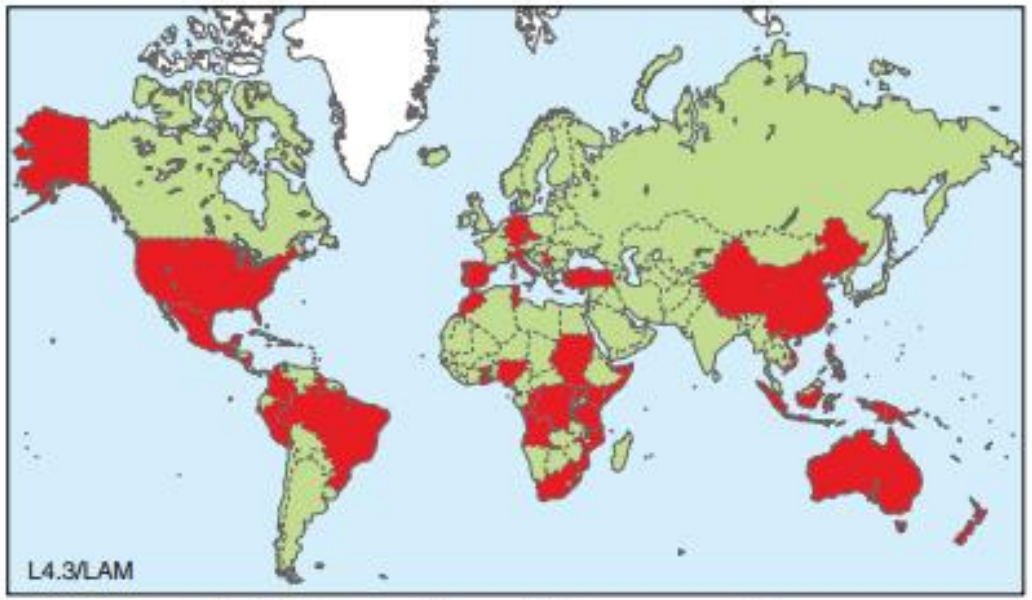
Tai

Southwest Branch	Central Branch	North Branch
Thai	Nung	Northern Zhuang
Lao	Southern Zhuang	Saek
Shan	Tay (Tho)	Eastern Bouyei
Black Tai		Yay
White Tai		Mene
Red Tai		
Ahom		

Human host range of *Mycobacterium tuberculosis*

Ruth Hershberg

A new study demonstrates that the most widespread lineage of the causative agent of tuberculosis consists of both globally distributed and geographically restricted sublineages. The geographically restricted sublineages are likely able to infect only specific human populations, whereas the globally distributed ones likely have a broader human host range.



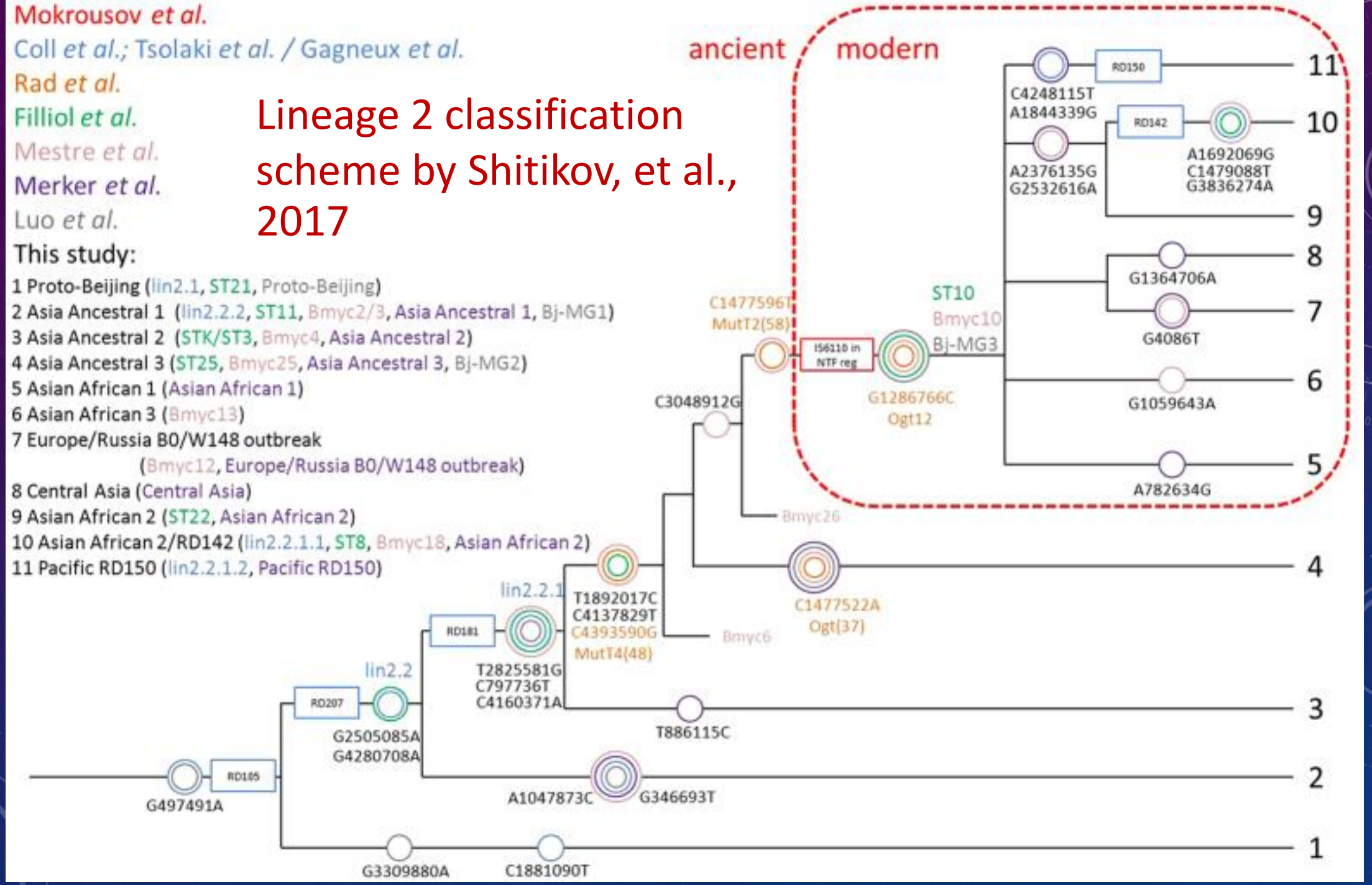
eages are restricted to a specific geographical location, with each of these present only within a specific region of Africa or Asia. The remaining three sublineages show an intermediate degree of geographical spread.

The African geographically restricted sublineages have been shown to have existed for several centuries and perhaps even for several millennia[7]. This suggests that lack of time in which to spread cannot explain the geographical restriction of these sublineages. Rather, Stucki *et al.* suggest that the sublineages

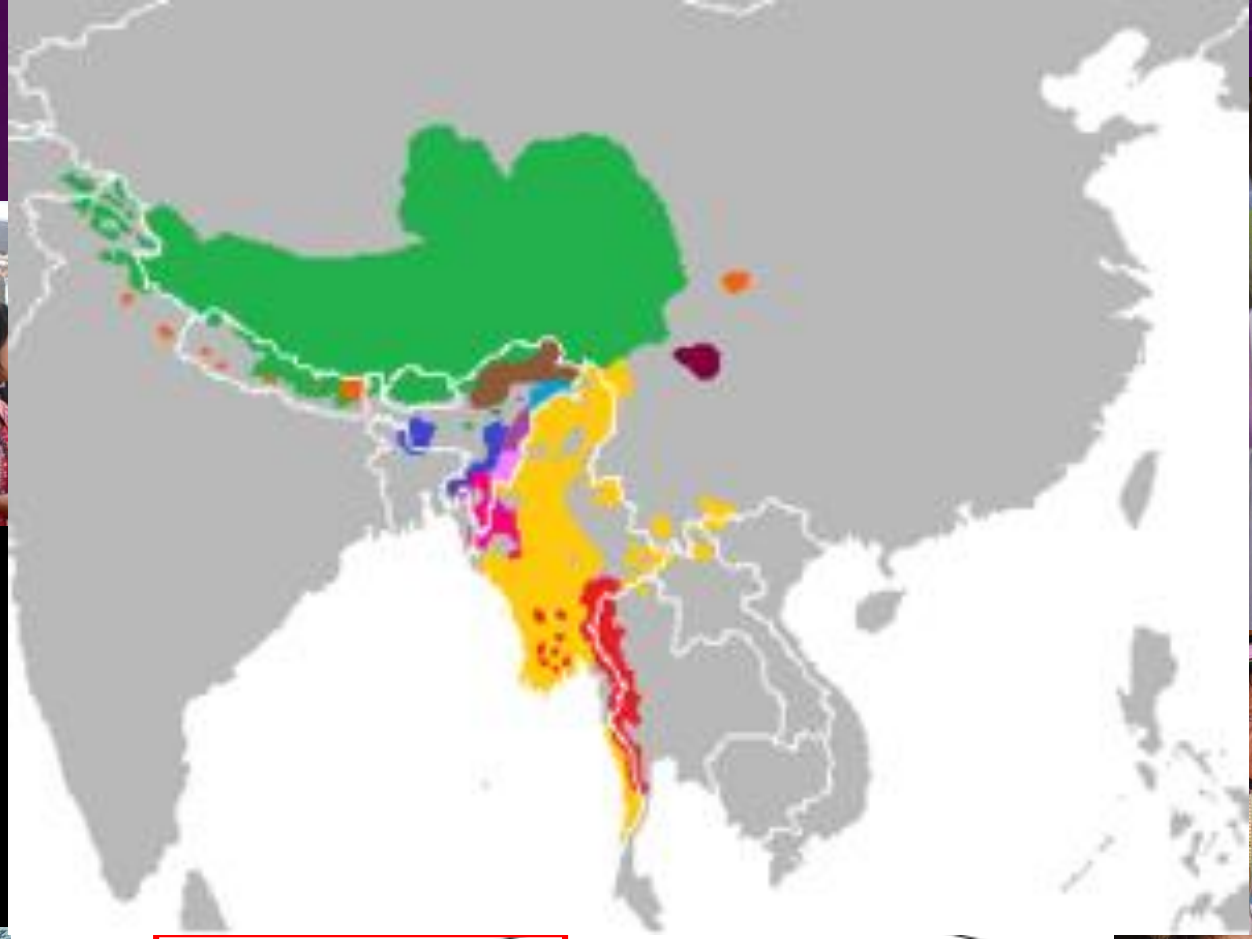
Ruth Hershberg is in the Rachel and Menachem Mendelovitch Evolutionary Processes of Mutation and Natural Selection Research Laboratory, Department of Genetics and Developmental Biology, Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.
e-mail: ruthersh@tx.technion.ac.il

Figure 1 Examples of the distributions of a globally distributed MTBC lineage 4 sublineage (L4.3/LAM) and a geographically restricted sublineage (L4.6.1/Uganda). Presence of each sublineage is indicated by red.

Lineage 2 classification scheme by Shitikov, et al., 2017



Lahu

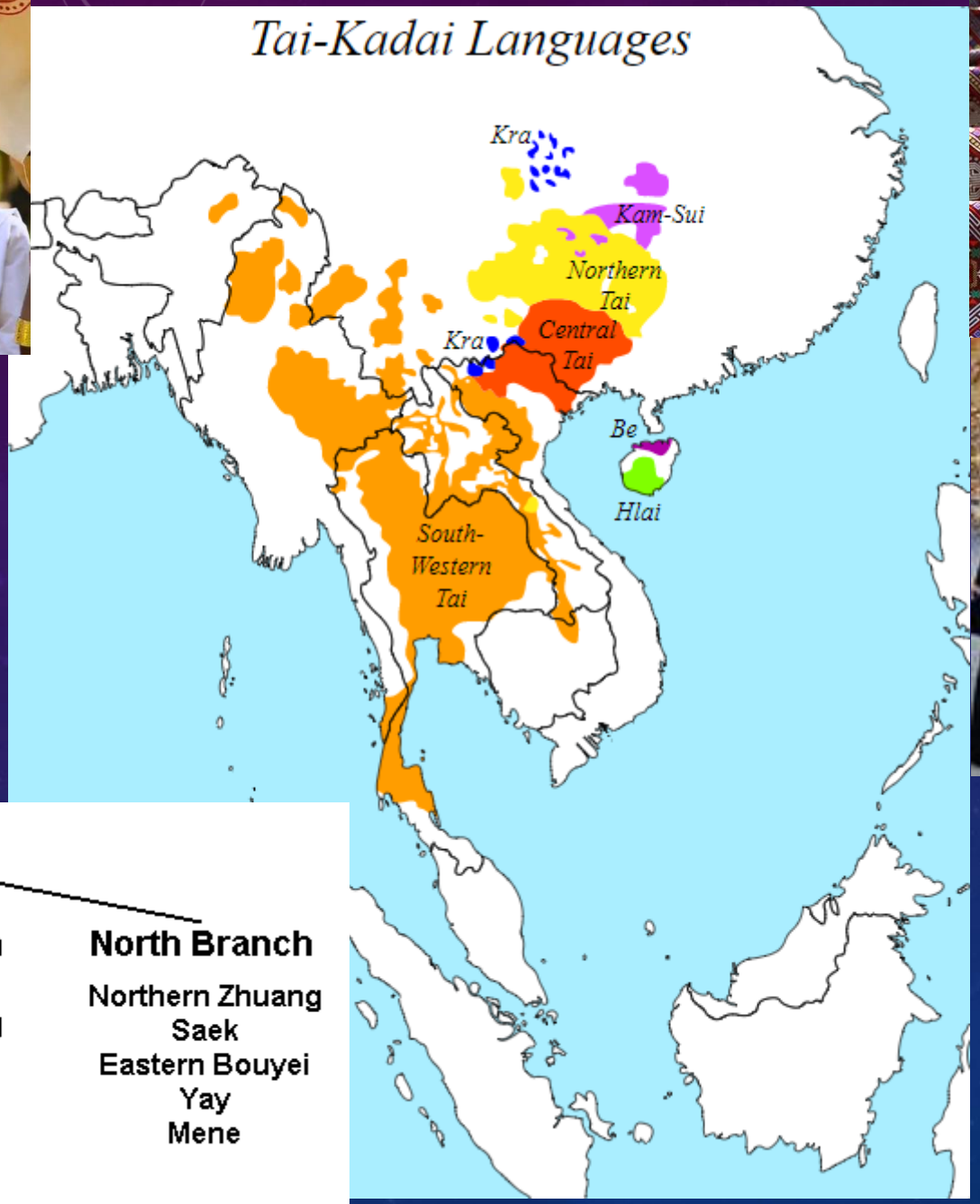


Tibeto-Burman

- Kamarupan — NE India, W Burma
- Himalayish — Tibet, Nepal, Bhutan, Sikkim
- Qiangic — Sichuan, Yunnan
- Jingpho-Nungish-Luish — N Burma, Yunnan
- Baic — Yunnan
- Karenic — Burma, Thailand
- Lolo-Burmese-Naxi — SW China, Burma, Thailand, Laos, Vietnam

Chinese

Kayaw



Tai

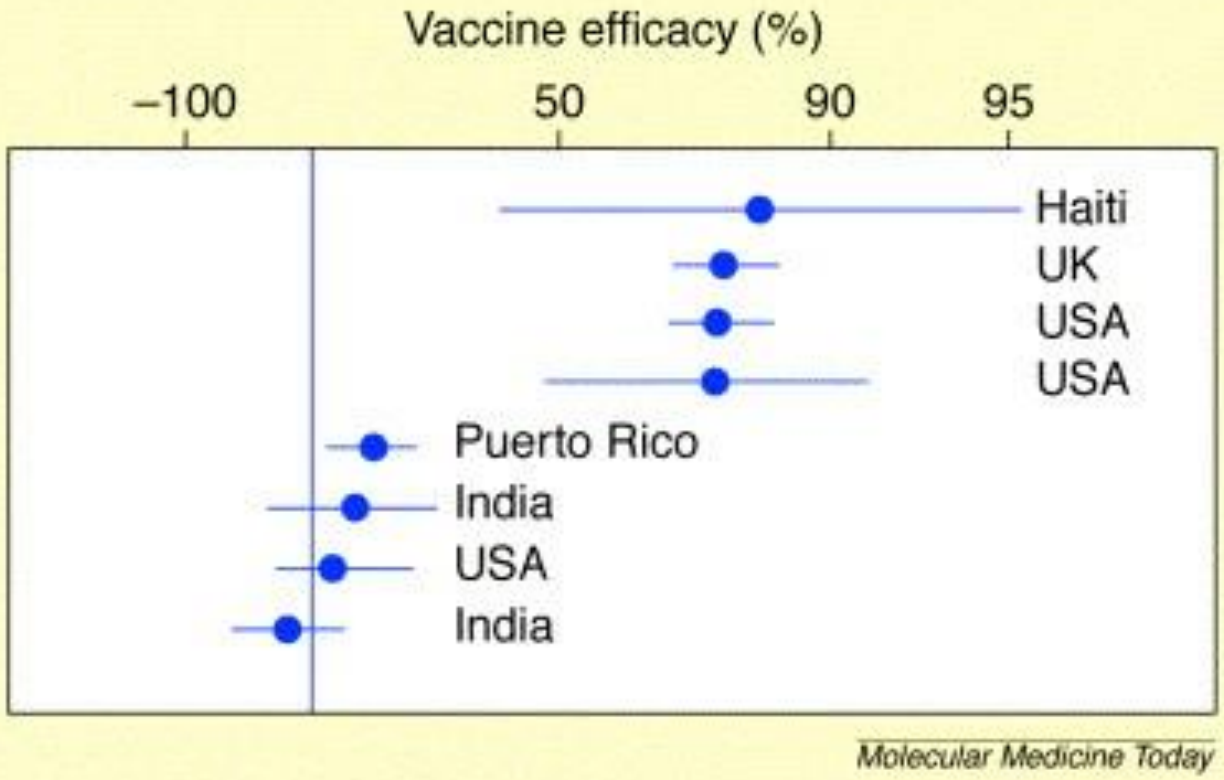
Southwest Branch	Central Branch	North Branch
Thai	Nung	Northern Zhuang
Lao	Southern Zhuang	Saek
Shan	Tay (Tho)	Eastern Bouyei
Black Tai		Yay
White Tai		Mene
Red Tai		
Ahom		

IMPLICATIONS IN TB CONTROL PROGRAM

- MDR-associated sublineages: indicated an evolutionary adaptive clones that cause primary MDR-TB, necessitate university drug susceptibility testing or at least genotypic screening.
- High mortality lineages may explain failure to achieve WHO-targeted cure rates, even with apparently reasonable TB control programs.
- Interactions between human and MTB are poly-mechanistic. Co-evolutionary hypothesis implied differential importance for different strains and different genetic populations.

TB VACCINES IN NEW LIGHT

- The universal functioning TB vaccines needs to be polygenic and poly-antigenic
- Particular attention should be given to the testing and trials in targeted populations with very high-burden. At this moment it means
  - India and Pakistan: L1.1.1, L1.1.2 and L3
  - China: Modern Beijing (L2.2.1/modern)
  - Indonesia and Philippines: Modern Beijing and Manila (L1.2.1.1) strains
- Challenging strain for vaccines development should not be H37Rv strain anymore (L4.9).
- Not even a single mice challenge test with L1 has ever reported!



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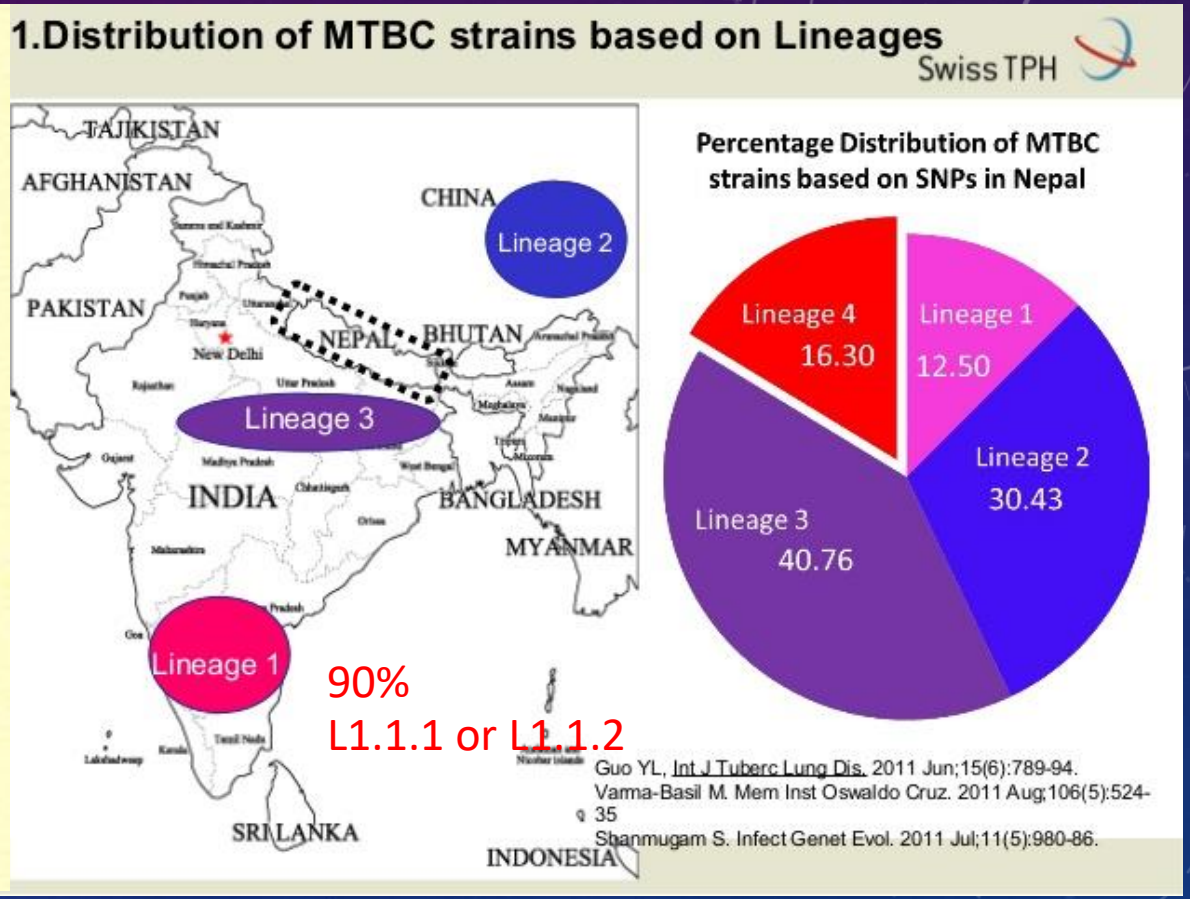

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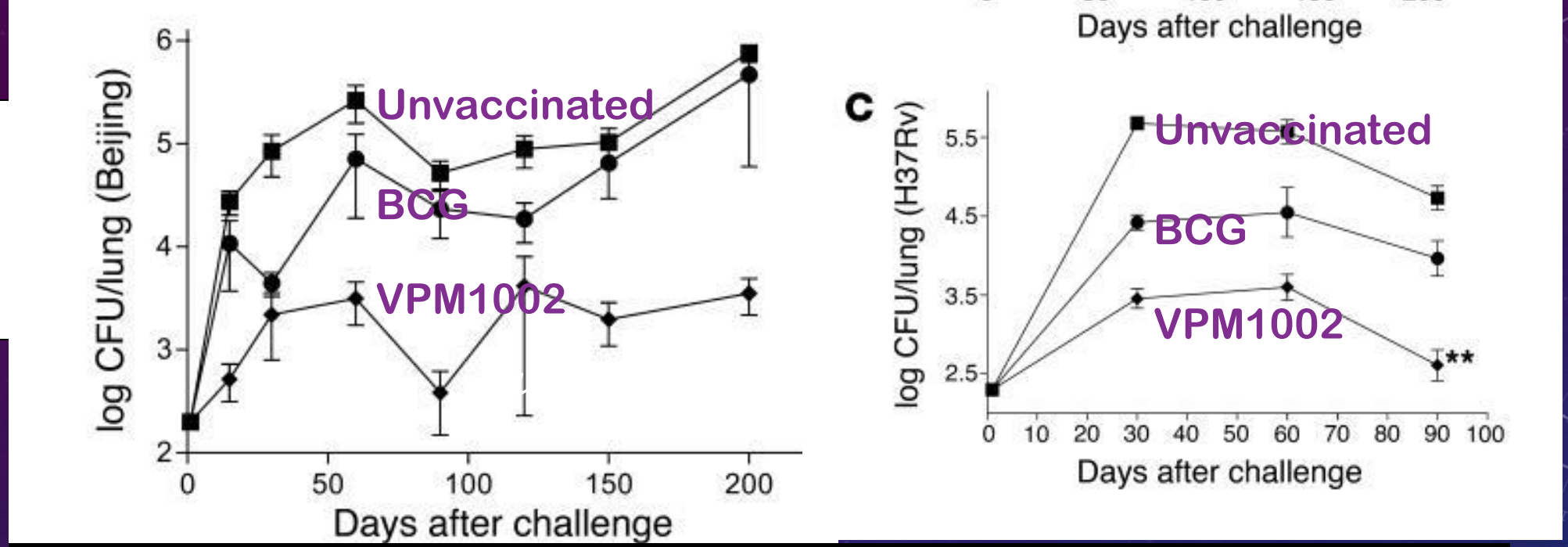

Beijing (200 cfu aerosol)

H37Rv (200 cfu aerosol)

Protection of BCG and VPM1002 (rBCG *ΔUreC::hly*) against H37Rv and a Beijing isolate in mice

(Grode L, et. al. Increased vaccine efficacy against tuberculosis of recombinant BCG mutants that secrete listeriolysin. J Clin Invest 2005)

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- Dr. Supalert Nedsuwan

All the collaborators and patients in this project

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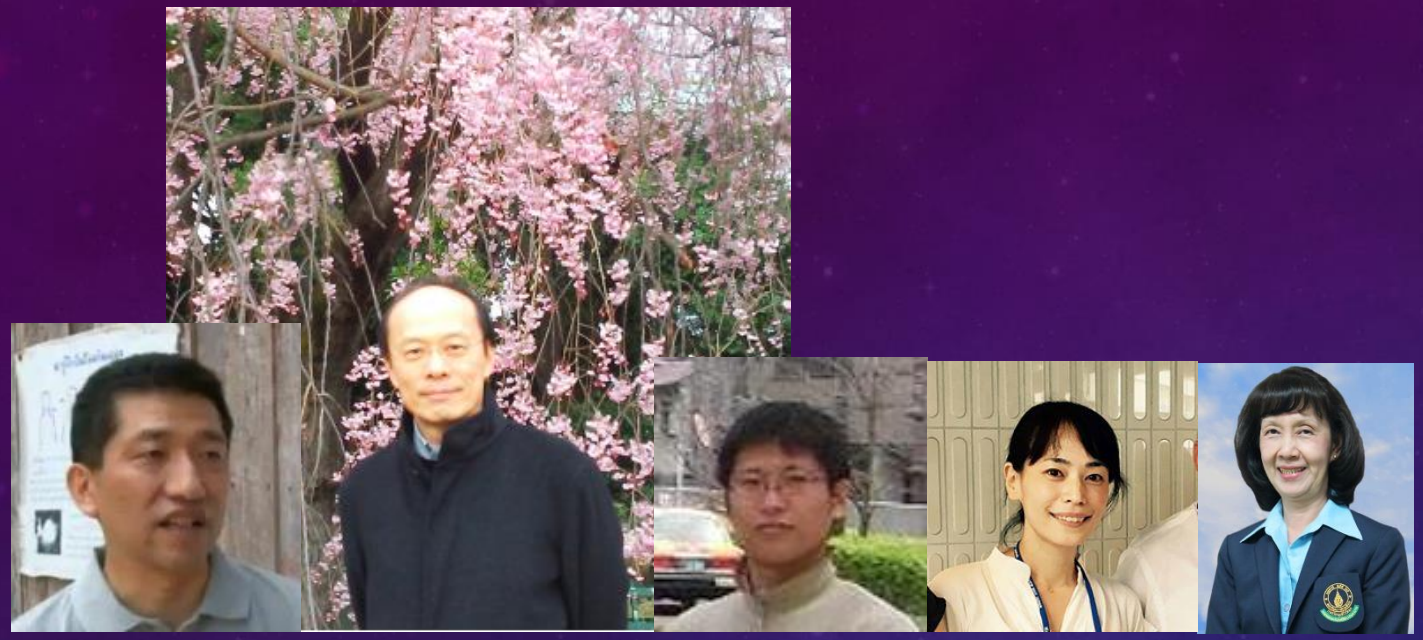
OUR TEAM



Katsushi Tokunaga