Studi di caratterizzazione genetica

Samples
• Biopsia cutanea di cavallo
• Liquido articolare di cavallo
• Omogenato di scroto di coniglio
• *T. equiperdum* OVI strain
• *T. evansi* strain (Kazakistan)

KDNA PCR Targets
• Subunit 6 ATP synthase
• Subunit 7 NAD dehydrogenase
• Subunits 4 NADH dehydrogenase
• Subunits 5 NADH dehydrogenase
• Murf 2- COX1
• Subunit 2 Cytochrome oxidase
• 12S ribosomal RNA

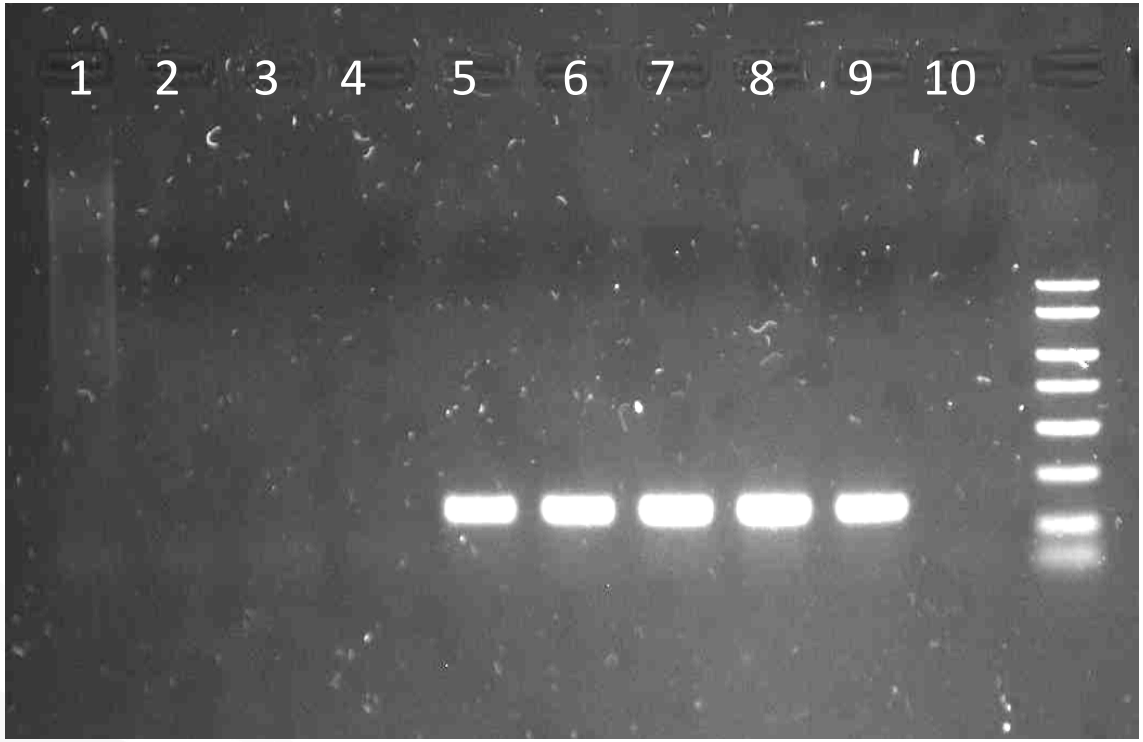
Nuclear DNA PCR Targets
• RoTat 1.2 VSG gene
• Dihydrolipoamide dehydrogenase (LipDH)
• ATP synthase γ subunit (γATPase)

Maxi circoli del kDNA

PCR	*T. equiperdum* ICT (IZSAM)	*T. equiperdum* OVI
6 ATP synthase	Negative	Positive
7 NAD	Negative	Positive
4 NAD	Negative	Positive
5 NAD	Negative	Positive
MURF2-CO1	Negative	Positive
CO2	Negative	Positive

Target nucleare

RoTat 1.2 VSG gene (Claes F. et al 2004)



1) DNA from rabbit scrotum

2) DNA from rabbit scrotum 1:10

3) DNA from rabbit scrotum 1:100

4) *T. equiperdum* OVI

5) *T. evansi (*Kazakistan)

6) *T. evansi (*Kazakistan) 1:10

7) *T. evansi (*Kazakistan) 1:100

8) *T. evansi* (Venezuela)

9) *T. evansi* (Venezuela) 1:100

10) NTC

Ann T 59 ° C

Caratterizzazione genetica

- *T. equiperdum* Italy è risultato negativo a tutte le PCR per target presenti sui maxicircoli del kDNA
- *T. equiperdum* Italy è risultato negativo alla PCR per RoTat1.2 VSG
- I prodotti PCR dei geni nucleari LipDH e γATPase sono stati clonati e sequenziati

IZSAM G.CAPORALE TERAMO

γATPase

Mutazioni nella regione C-terminal descritte in ceppi di *T. evansi* and *T. equiperdum*

S. Dean et al. 2013

Table 1. ATPase subunit γ sequence variations tested in this study

Mutation	Source (origin, host, year of isolation)	Source for mutation; isolate
L262P	*T. b. brucei* 164DK (USA, mouse, 1971)	17; 20
CTT→CCT		
A273P	*T. equiperdum* BoTat1.1 (Morocco, horse, 1924)	4; 3
GCG→CCG	*T. equiperdum* STIB784 (unknown)	4; 4
	T. equiperdum STIB842 (unknown)	4; 4
A281del	*T. equiperdum* ATCC30019 (France, horse, 1903)	This work; 3
TCTGCTATG→TCT—ATG	*T. equiperdum* ATCC30023 (France, horse, 1903)	This work; 3
	T. equiperdum STIB818 (China, horse, 1979)	4; 3
	T. evansi Antat 3/3 (South America, capybara, 1969)	This work; 51
	T. evansi C13 (Kenya, camel, 1982)	This work; 51
	T. evansi CPOgz1 (China, water buffalo, 2005)	This work; 4
	T. evansi E110 (Brazil, capybara, 1985)	This work; 44
	T. evansi E9/CO (Columbia, horse, 1973)	This work; 51
	T. evansi SS143M (Philippines, water buffalo, 2006)	This work; 4
	T. evansi SS73M (Philippines, water buffalo, 2006)	This work; 4
	T. evansi STIB805 (China, water buffalo, 1985)	4; 4
	T. evansi STIB807 (China, water buffalo, 1979)	This work; 4
	T. evansi STIB810 (China, water buffalo, 1985)	4; 4
	T. evansi Stock Kazakh (Kazakhstan, camel, 1995)	This work; 4
M282L	*T. evansi* KETRI2479 (Kenya, camel, 1981)	4; 51
ATG→TTG		

In all strains investigated, the L262P and A273P mutations are homozygous, whereas the A281del and M282L mutations are heterozygous.

Analisi della sequenza di γATPase

Le **mutazioni** descritte in *T. evansi* e *T. equiperdum* che compenserebbero la perdita totale o parziale del kDNA **non sono presenti nel ceppo italiano**

AA	T. eq OVI	T. eq BoTat 1.1	T. eq Italy (IZSAM)	T. ev STIB 810	T. ev KETRI 2479
262	L/L	L/L	L/L	L/L	L/L
273	A/A	**A/P**	A/A	A/A	A/A
281	A/A	A/A	A/A	**A/-**	A/A
282	M/M	M/M	M/M		**M/L**

Single point mutations in ATP synthase compensate for mitochondrial genome loss in trypanosomes

Samuel Dean[1,2], Matthew K. Gould[1], Caroline E. Dewar, and Achim C. Schnaufer[3]

Institute for Immunology and Infection Research and Centre for Immunity, Infection and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom

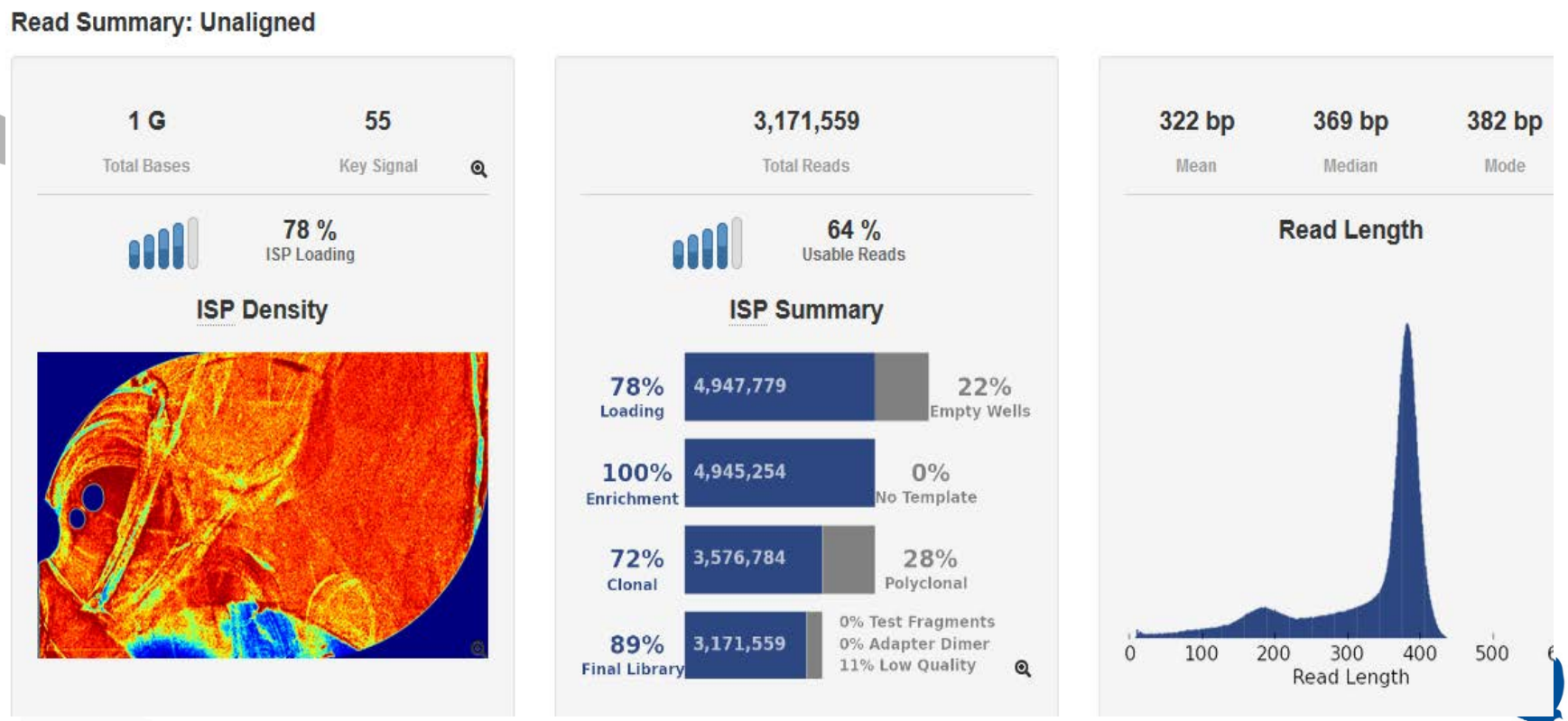
IZSAM G.CAPORALE TERAMO

Ion Personal Genome Machine® (PGM™) System (Ion Torrent™)

Ion PGM™ 400 Sequencing Kit

Ion 316 ™ v2 Chip

PGM Run summary



- **Contaminazione da DNA dell'ospite (ratto per T. *eq.* OVI, coniglio per T. *eq.* Italy)**
- **Basso coverage**

Risultati

Assemblaggio effettuato usando come genoma di referenza *T. brucei brucei* 927

- ***T. equiperdum* OVI**
  - Assembled nucleotides: 19 Mbp
  - N. contigs: 7603
  - Average contig length: 2508 bp
  - Maximum contig length: 51702 bp
  - N50: 6441

- ***T. equiperdum* Italy (IZSAM)**
  - Assembled nucleotides: 0.9 Mbp
  - N. contigs: 2676
  - Average contig length: 336 bp
  - Maximum contig length: 2018 bp
  - N50: 358

kDNA: maxi-mini circoli

T. equiperdum OVI

- Sequenza quasi completa (> 95%) dei maxi circoli
- Presenza dei mini circoli

T. equiperdum Italy (IZSAM)

- Nessuna evidenza di maxi circoli
- Nessuna evidenza di mini circoli

Analisi filogenetica

IZSAM G.CAPORALE TERAMO

OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

Genome and Phylogenetic Analyses of *Trypanosoma evansi* Reveal Extensive Similarity to *T. brucei* and Multiple Independent Origins for Dyskinetoplasty

J. Carnes et al. 2015

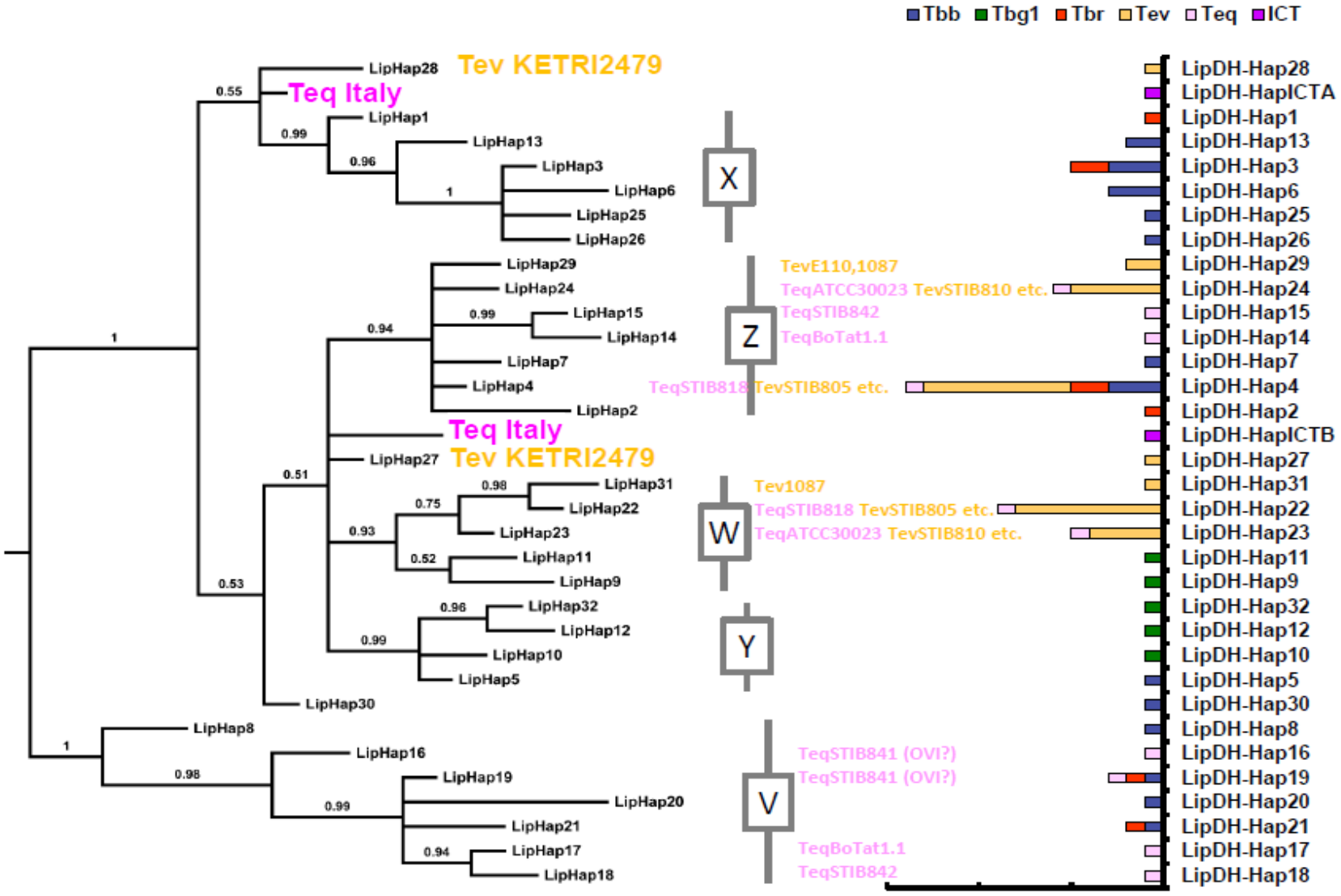
Jason Carnes[1], Atashi Anupama[1], Oliver Balmer[2], Andrew Jackson[3], Michael Lewis[4], Rob Brown[1], Igor Cestari[1], Marc Desquesnes[5,6], Claire Gendrin[1], Christiane Hertz-Fowler[7], Hideo Imamura[8], Alasdair Ivens[9], Luděk Kořený[10,11¤], De-Hua Lai[10,11,12], Annette MacLeod[13], Suzanne M. McDermott[1], Chris Merritt[1], Severine Monnerat[1], Wonjong Moon[1], Peter Myler[1], Isabelle Phan[1], Gowthaman Ramasamy[1], Dhileep Sivam[1], Zhao-Rong Lun[12]*, Julius Lukeš[10,11,14]*, Ken Stuart[1,15]*, Achim Schnaufer[9,16]*

Table 4. Proposed grouping of *T. evansi*/*T. equiperdum* isolates.

Group	1	2	3	4
Examples (code)	*T. ev.* STIB805 (27) *T. ev.* Kazakh (33) *T. ev.* AnTat3/3 (36) *T. eq.* STIB818 (24) *T. eq.* ATCC30023 (25)	*T. eq.* STIB842 (22) *T. eq.* BoTat1.1 (21)	*T. eq.* STIB841[1] (23) *= T. Eq OVI*	*T. ev.* KETRI2479 (42)
ATPase γ C-term.	A281del	A273P	WT	M282L
dominant minicircle class	A[2]	C[3]	n/a	B[4]
LipDH genotype	W/Z	V/Z	V/V	atypical
COX1 haplotype	23	22	14	n/a[5]
microsatellite PCA	Tev	Tbb	Tbb Kiboko	Tbb
RoTat1.2	+[6]	-[7]	-[7]	-[8]

Analisi filogenetica

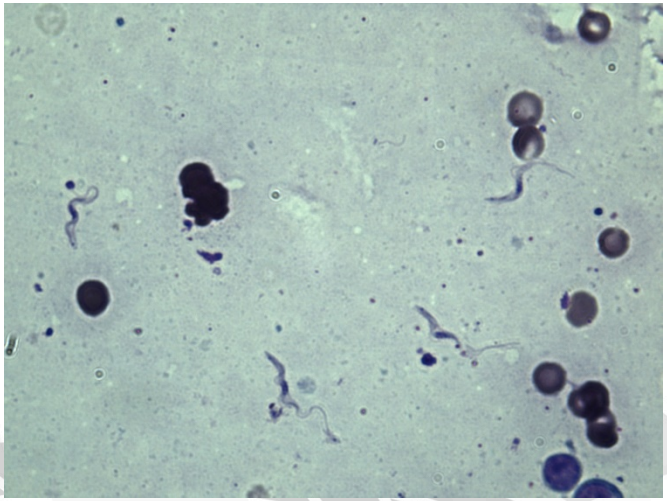
Bayesian phylogeny of Trypanozoon isolates based on the LipDH gene (Tb927.11.16730)



analysis by Michael Lewis (LSHTM)

Caratterizzazione genetica

T. eq. 2011	
• ATPase γ C-term	• WT
• Dominant minicircle class	• n/a
• LipDH genotype	• atypical
• COX1 haplotype	• n/a : ICT 2011 lacks COX1
• Microsatellite PCA	• Tbb
• RoTat 1,2	• -

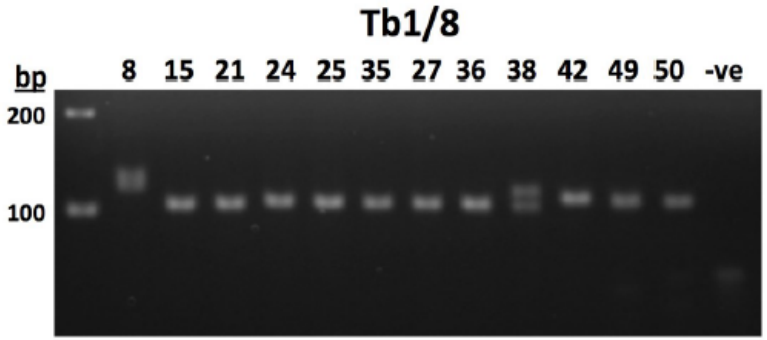


Analisi di microsatelliti

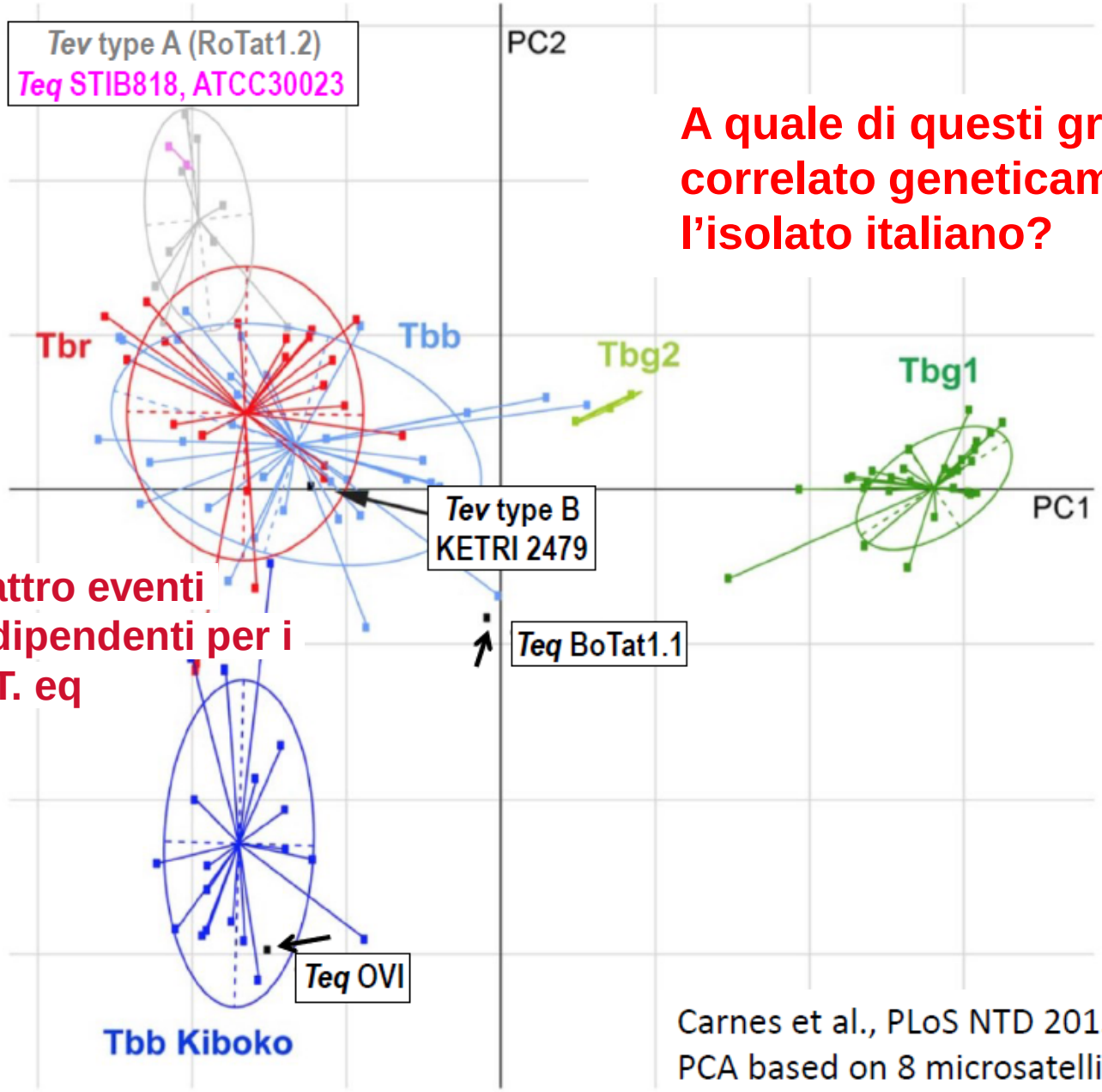
Pannello di 17 loci

R. Echodu et al 2015

Locus	Forward Primer	Reverse Primer	Motif	Size range (bp)	Chrom. Loc.
Tryp51	[FAM]-TGACCCGTGAGAAGTGAAC	GCGCATCTACAGGCATAGAC	(ATT)	187-238	9
Tryp52	[ALEXA 532]-GCATCATTGACGTCGACCC	TAACAACCACTGGGACCGC	(GT)	201-231	11
Tryp53	[ALEXA 546]-GTACAGCCACGTGCAAACC	TGTACACAATCGGGTGGATG	(AC)	200-254	7
Tryp54	[ROX]-AGTCGGCGTGATGGTACTC	TTCAGCCCACAAACAACCG	(AAAT)	144-176	10
Tryp55	[FAM]-AATTCAACCCCAACAGCCC	CTCGTTCAATGACTTGCCCC	(GT)	208-246	5
Tryp59	[ALEXA 532]-GAGGCAATCGCAGTGTGTG	CGCACGTTTCACCATCCTC	(GT)	209-225	9
Tryp61	[ALEXA546]-ACTCGCGACAGACCATGAG	ACAGGAGAGTGTTGTGAGTG	(ATT)	179-215	11
Tryp62	[ROX]-AAGGCGACCAACTTCAACC	GTTGTCATCGGCTTGCTCC	(AC)	153-177	11
Tryp65	[ALEXA 546]-GGAGGTAAACTTGATTCGGGTG	ACGACAACAGCGACAAAGC	(ATT)	207-234	9
Tryp66	[ROX]-TCCTCGTACCTTTTCTCTCAC	ACGAAATTTAGGTGTGAAAGCTG	(ATT)	384-396	5
Tryp67	[FAM]-GTTGCTGAGGTGCAACTGG	GTCGTCAGGCACCAAAACG	(GTT)	151-178	7
TB1/8	[FAM]-AGGTTTAGTGCATGTCGGA	CCTGTTGTACGGAGGTCA	(CA)	97-117	1
TB5/2	[HEX]-CAACCGAAAGTAAGGGGAAC	TCTCGCCTTCTTTGCCC	(AT)	83-107	5
TB6/7	[HEX]-AAGCTGACAGGTGGTTGA	GAACATGCGTGCGTGTG	(AT)	104-136	6
TB9/6	[HEX]-TGATTCATTGGTTAAGACAGG	AATGATAACTGCGGATTACAC	(AC)	124-158	9
TB10/5	[FAM]-AAAGGCGATATGTTATTATTGA	ATTGGGTATACTGTCCCTCA	(TA)	79-115	10
TB11/13	[FAM]-CAAGAACTCTGCATTGAGC	ATCTGTTGGCGATGGTGA	(AT)	125-161	11



A quale di questi gruppi è correlato geneticamente l'isolato italiano?



Almeno quattro eventi evolutivi indipendenti per i ceppi T. ev/T. eq

Carnes et al., PLoS NTD 2015
PCA based on 8 microsatellite markers

Ceppi analizzati

T. equiperdum Italy

***T.ev./T.eq.*group 1**
Tev_STIB805_CD
Tev_STIB810_CD
Tev_Colombian
Tev_Kazakstan
Tev_Philippine
Tev_RoTat1.2
Tev_Vietnam
Tev_Antat3/3
Tev_Can86/Brazil
Tev_C13
Teq_ATCC30019
Teq_ATCC30023
Teq_American
Teq_AnTat4.1
Teq_Canadian
Teq_Hamburg
Teq_STIB818
Teq_SVP

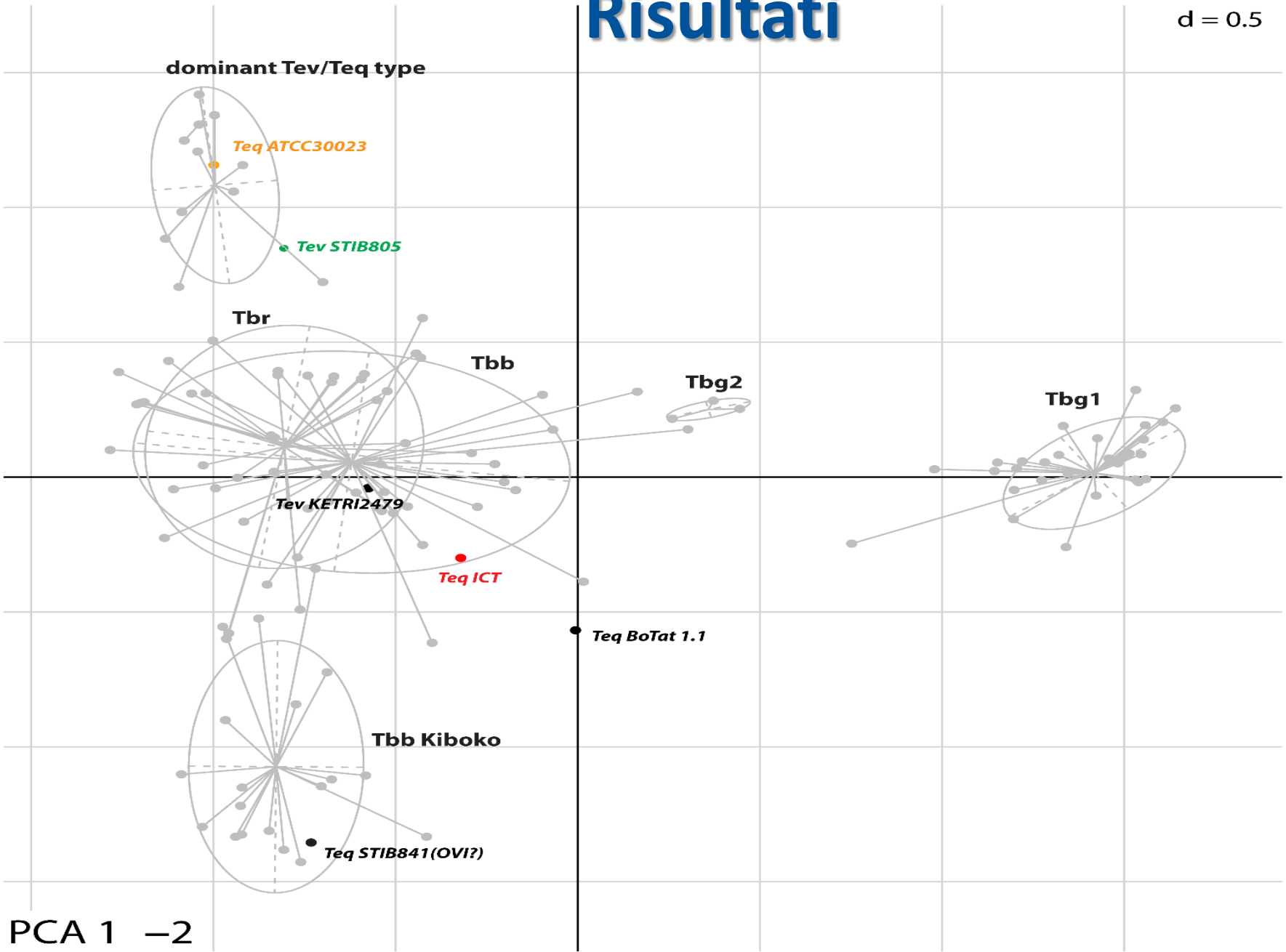
***T.ev./T.eq.*group 2**
Teq_BoTat1.1_1
Teq_BoTat1.1_2

***T.ev./T.eq.*group 3**
Teq_OVI

***T.ev./T.eq.*group 4**
Tev_KETRI2479

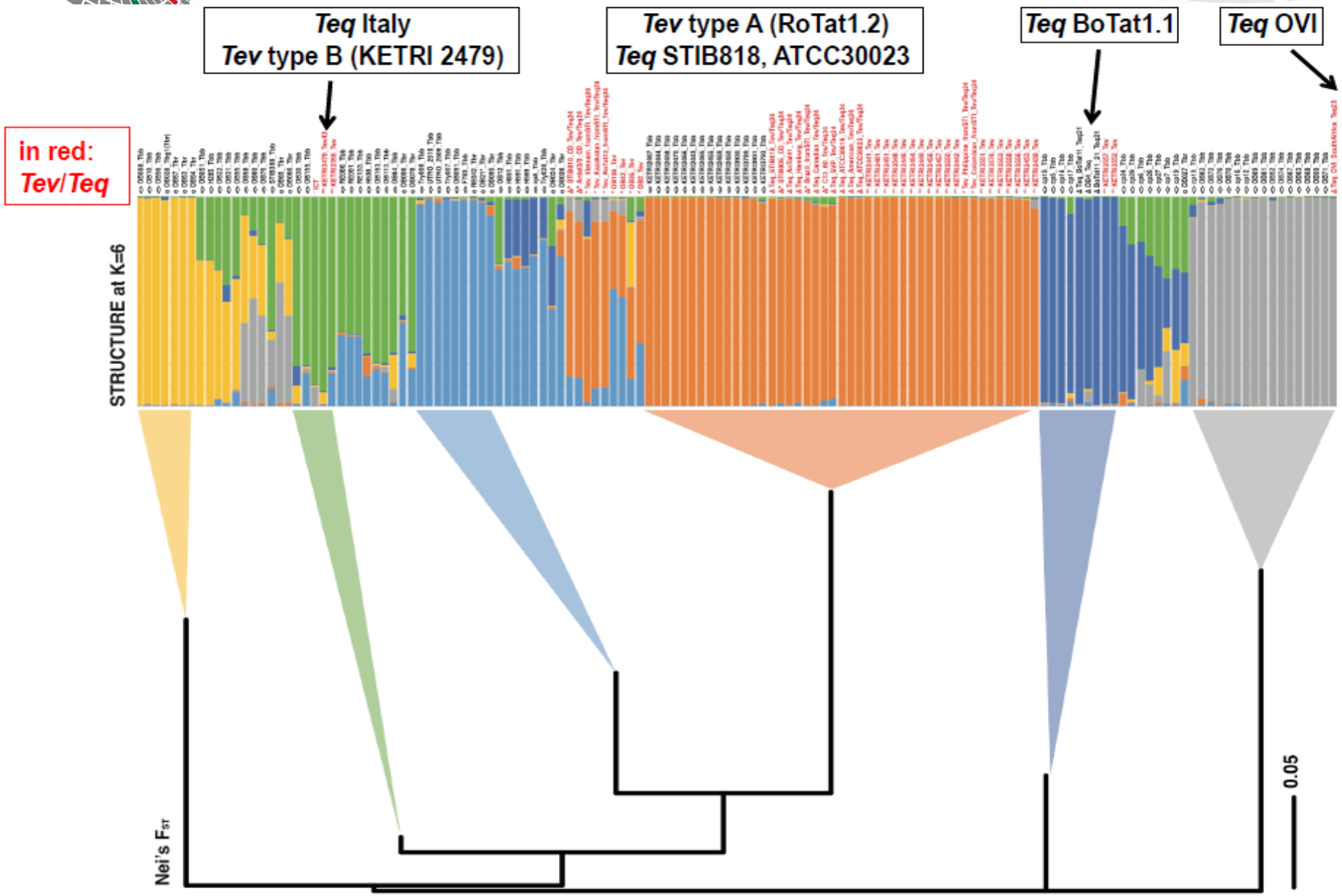
***T. b. brucei* controls (Yale)**
H285
H538
H569
H591
H851
Try006
Try007
Try020

Risultati


d = 0.5
dominant Tev/Teq type
Teq ATCC30023
Tev STIB805
Tbr
Tbb
Tbg2
Tbg1
Tev KETRI2479
Teq ICT
Teq BoTat 1.1
Tbb Kiboko
Teq STIB841(OVI?)
PCA 1 –2

IZSAM G.CAPORALE TERAMO

Risultati



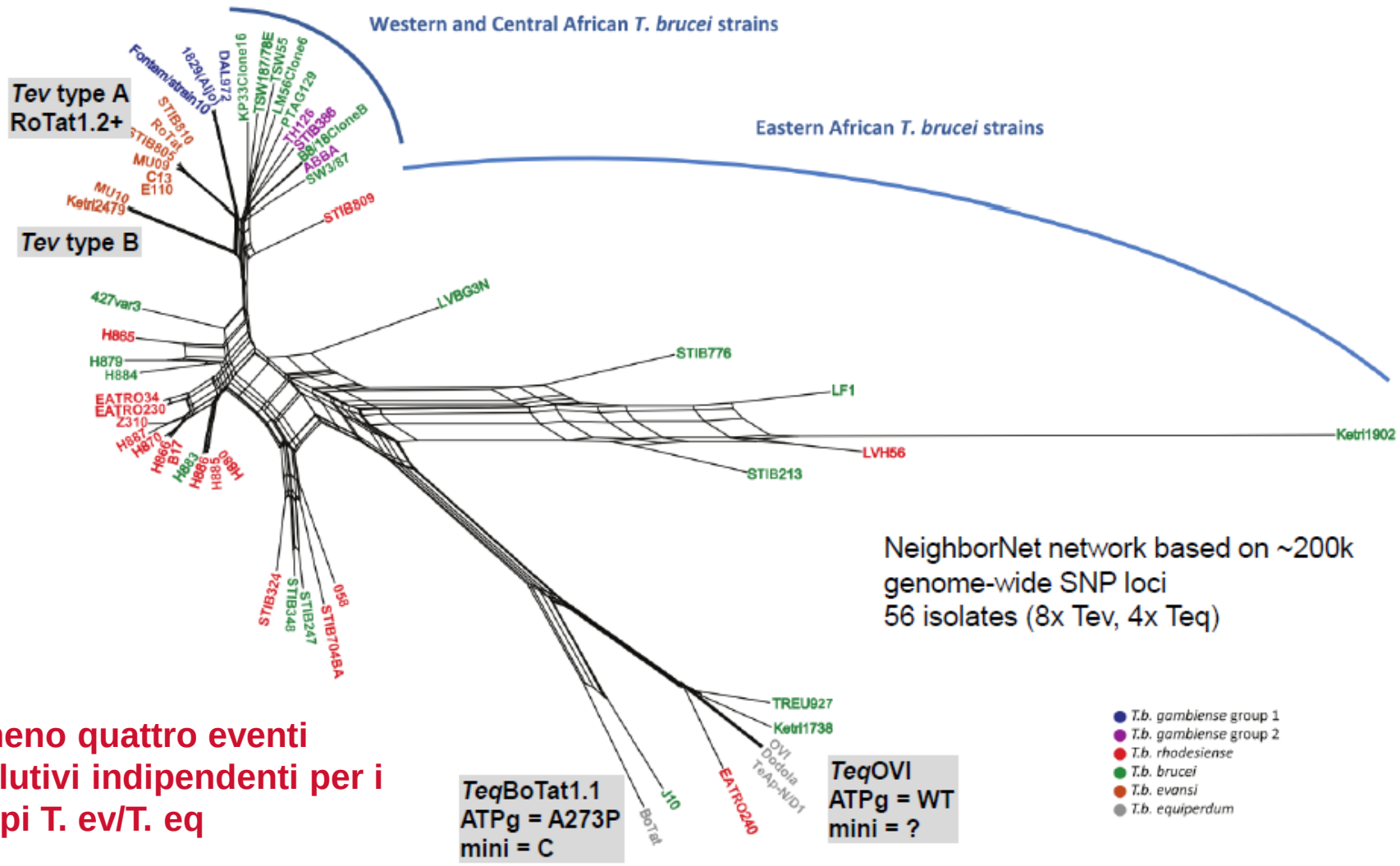
2017

GBE

Genome Biol. Evol. 9(8):1990–1997.

Genome-Wide SNP Analysis Reveals Distinct Origins of *Trypanosoma evansi* and *Trypanosoma equiperdum*

Bart Cuypers[1,2], Frederik Van den Broeck[1], Nick Van Reet[1], Conor J. Meehan[1], Julien Cauchard[3], Jonathan M. Wilkes[4], Filip Claes[5], Bruno Goddeeris[6], Hadush Birhanu[7], Jean-Claude Dujardin[1,8], Kris Laukens[2], Philippe Büscher[1], and Stijn Deborggraeve[1,*]



NeighborNet network based on ~200k genome-wide SNP loci
56 isolates (8x Tev, 4x Teq)

Almeno quattro eventi evolutivi indipendenti per i ceppi T. ev/T. eq

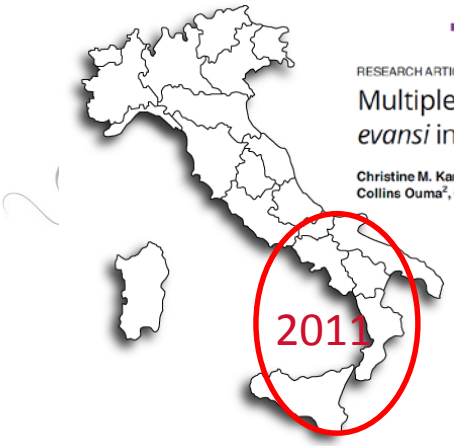
Conclusioni

- Il ceppo responsabile del focolaio di morbo coitale in Italia nel 2011 non è geneticamente correlato con nessun ceppo di *T. equiperdum* riportato in letteratura.

- Risulta strettamente correlato con un ceppo di *T. evansi* di tipo B (KETRI 2479) isolato nel 1980 in Kenya (Ngurunit area) e con uno di *T. b. bruceii* isolato nel 1977 in Uganda (Busoga area).

- Gli studi filogenetici evidenziano come l'evoluzione di ceppi appartenenti al sottogenere *Trypanozoon* con capacità di trasmissione attraverso insetti ematofagi o il coito sia avvenuta almeno in quattro diverse occasioni

Considerazioni



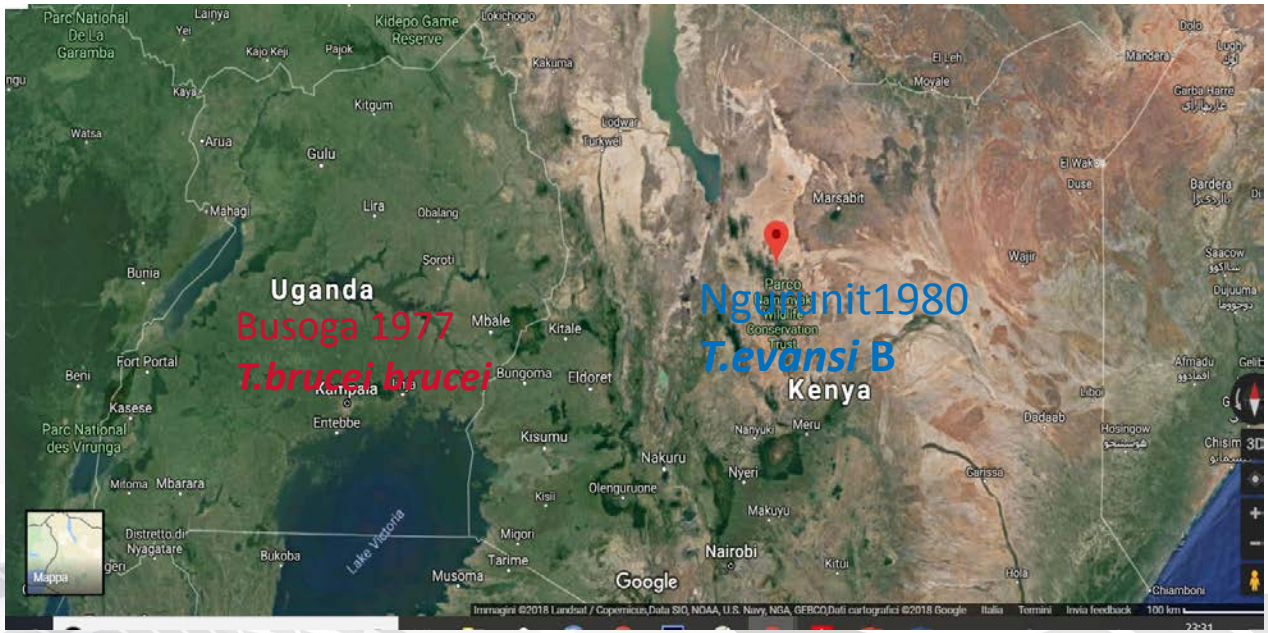
PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Multiple evolutionary origins of *Trypanosoma evansi* in Kenya

Christine M. Kamidi[1,2,3¶*], Norah P. Saarman[4¶*], Kirstin Dion[4], Paul O. Mireji[1,3,5], Collins Ouma[2], Grace Murilla[1], Serap Aksoy[3], Achim Schnaufer[6], Adalgisa Caccone[3,4]

- Multiple evolutionary origins of *T. b. rhodesiense* from different strains of *T. brucei brucei.*
  T. b. brucei strains from different genetic backgrounds apparently can become either parasites of a lethal human disease or become able to be transmitted by a variety of hematophagous insects.
- Few reported cases of *T. evansi* infecting humans.
- The acquisition of the SRA gene requires sexual recombination in the tsetse fly.



Ringraziamenti

- Maurilia Marcacci
- Valentina Curini
- Marco Di Domenico
- Andrea Di Provvido
- Vincenzo D'Innocenzo
- …e tutto il gruppo di lavoro MCM dell'IZSAM

- **Achim Shnaufer**
- Julie Wallis

- **Adalgisa Caccone**
- Norah Saarman
- Christine Muhonja
- Kirstin Dion

Yale University
Department of Ecology & Evolutionary Biology

- **Michel Lewis**