

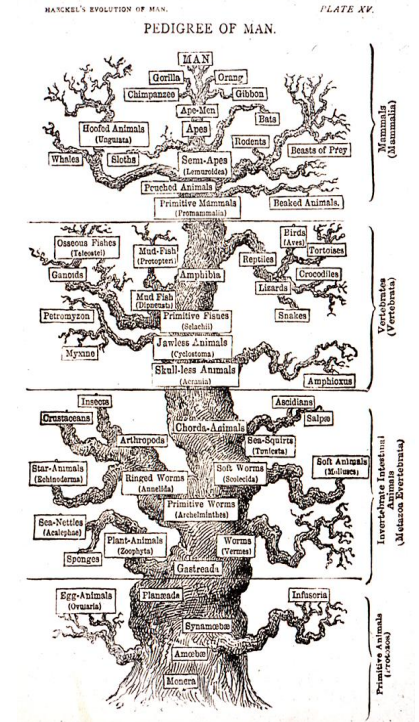
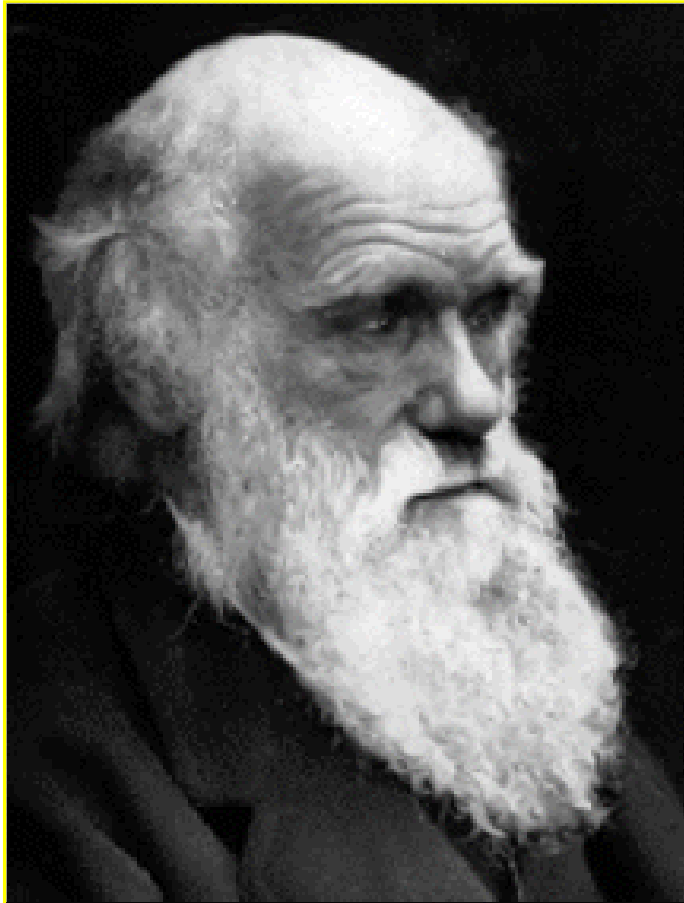
# **GENETIC DIVERSITY AND PHYLOGENETIC RELATIONSHIPS OF CCHF VIRUS**

Anna Papa, MD, PhD  
Aristotle University of Thessaloniki, Greece

Istanbul 2008

# Charles Darwin

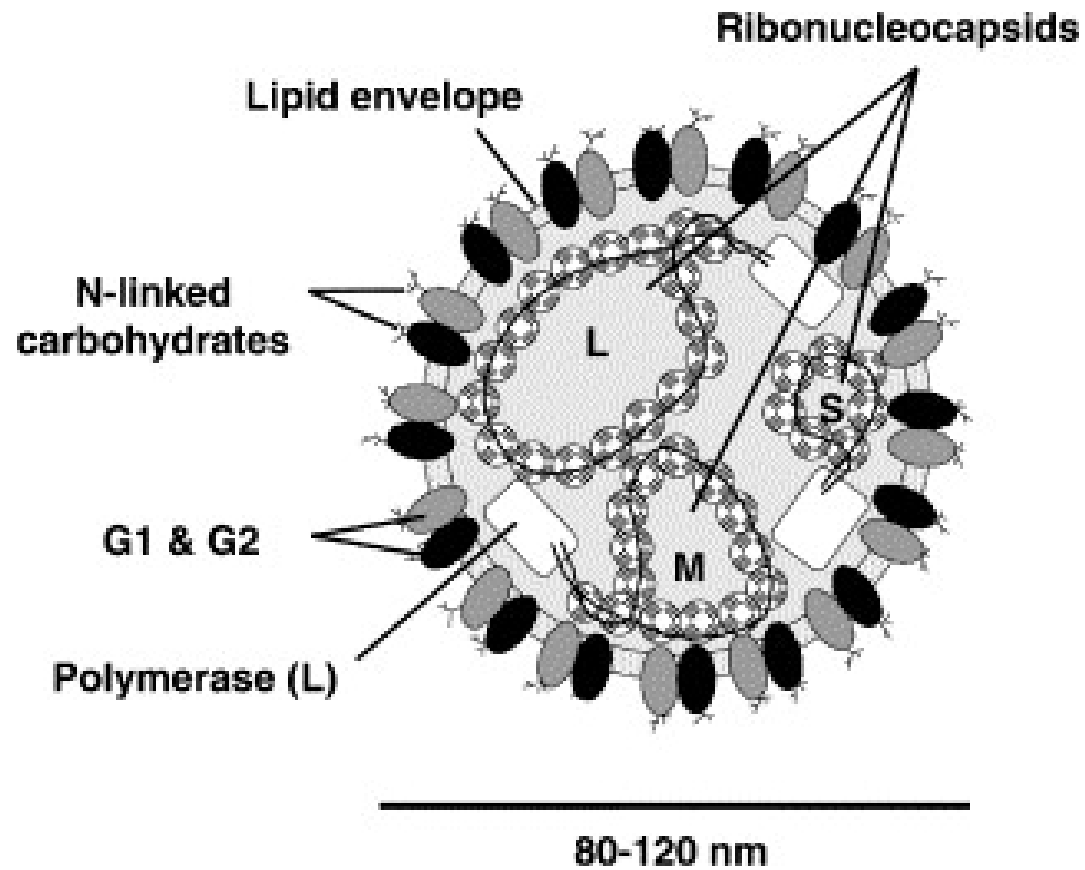
1809-1882



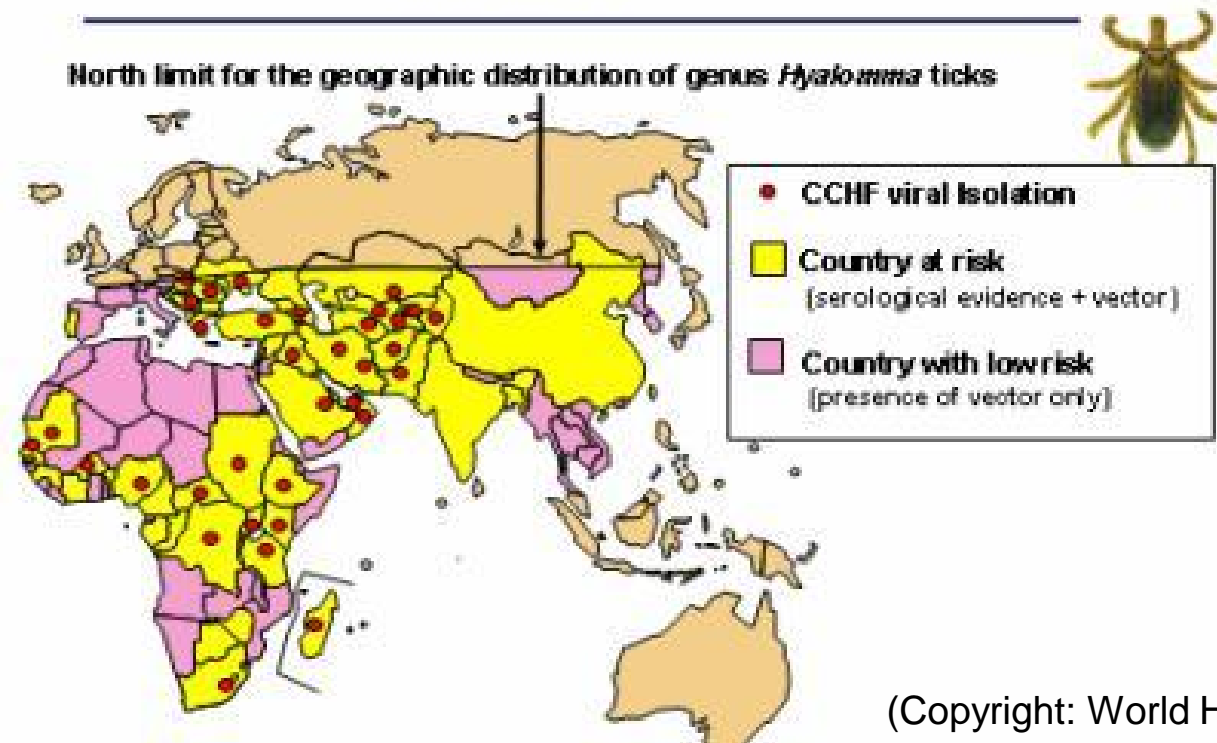
'I have called this principle, by which each slight variation, if useful, is preserved, by the term Natural Selection'

On the Origin of Species by Means of Natural Selection (1959)

# A *Bunyaviridae* virion



# CCHF: geographic distribution



Vector: *Hyalomma* genus ticks, mainly *H. marginatum marginatum* (*Mediterranean Hyalomma*) which has a northern geographic limit of 48° north latitude.

# BIBLIOGRAPHY OF TICKS AND TICKBORNE DISEASES

FROM HOMER (ABOUT 800 B.C.) TO 31 DECEMBER 1976

BY

HARRY HOOGSTRAAL, Ph.D., D.Sc.

With the editorial assistance of  
ALICE DJIGOUNIAN

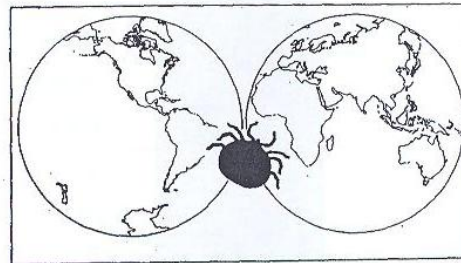
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SPECIAL PUBLICATION

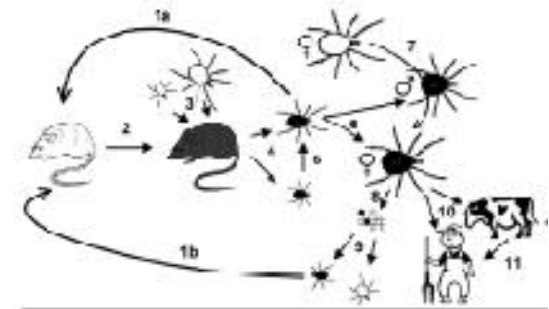
APRIL 1978

UNITED STATES NAVAL MEDICAL RESEARCH UNIT NUMBER THREE  
(NAMRU-3), CAIRO, EGYPT

*H. marginatum marginatum* is considered the main vector of CCHFV in most parts of the Palaearctic region

RNA viruses show high rates of accumulation of mutations.

Arthropod RNA viruses evolution rate is remarkably lower than other RNA viruses (double filter concept- fitness maintenance in both arthropods and vertebrates).



Life cycle of CCHFV  
Turell 2007

## CCHF virus presents high level of genome diversity

### Nucleotide variation

S RNA segment : 20%

M RNA segment: 31%

L RNA segment: 22%

### Amino acid variation

N protein: 8%

GPC: 27% (↑not silent mutations)

L protein: 10%

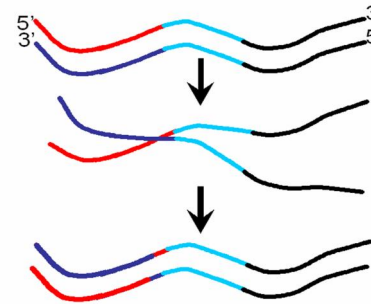
However, overall genome RNA segment and ORF lengths and important motifs are well conserved.

# MEANS OF GENETIC VARIATION

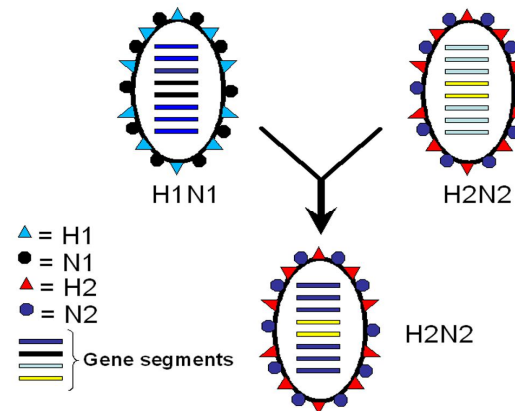
## 1. Mutations

5'	AUG	CGA	UUA	UAC	GGG	AAA	3'
	Met	Arg	Leu	Tyr	Gly	Lys	
↓							
Silent 5'	AUG	CGA	UUA	UAU	GGG	AAA	3'
	Met	Arg	Leu	Tyr	Gly	Lys	
↓							
Missense 5'	AUG	CGA	UUA	UAC	GGG	AAA	3'
	Met	Arg	Leu	Tyr	Gly	Lys	
↓							
	AUG	CGA	UUA	UUC	GGG	AAA	3'
	Met	Arg	Leu	Phe	Gly	Lys	
↓							
Nonsense 5'	AUG	CGA	UUA	UAC	GGG	AAA	3'
	Met	Arg	Leu	Tyr	Gly	Lys	
↓							
	AUG	CGA	UUA	UAA	GGG	AAA	3'
	Met	Arg	Leu	Stop	Gly	Lys	

## 2. Recombination



## 3. Reassortment



# First published CCHFV sequence data

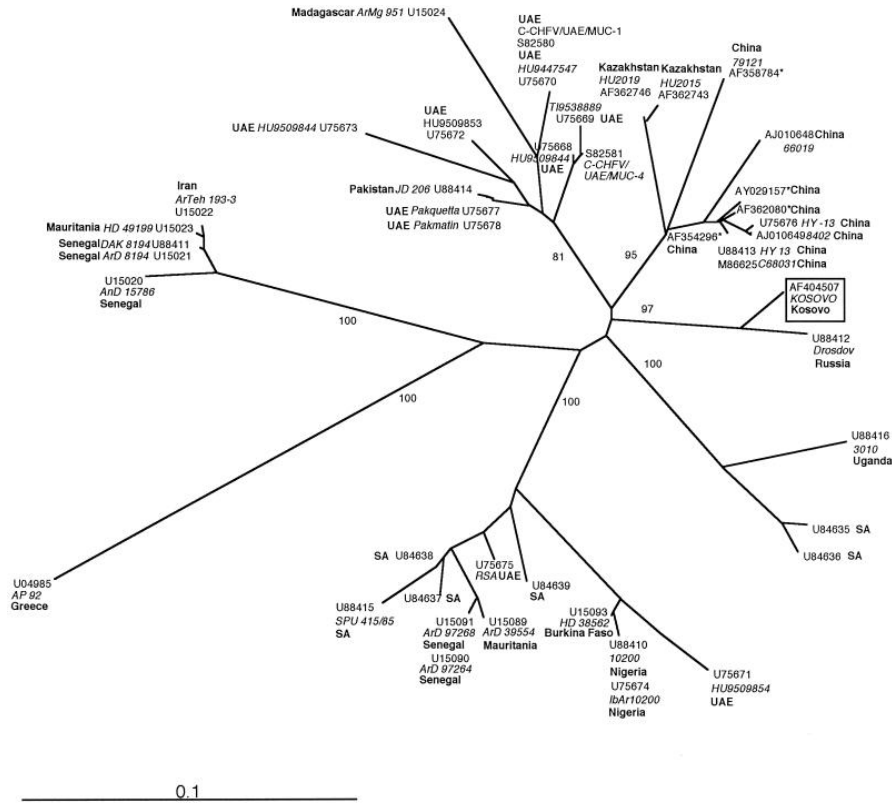
**S RNA segment:** Chinese strain C68031 isolated in 1968 from sheep (Marriott and Nuttall, 1992).

**M RNA segment:** Chinese strains BA66019 and BA8402 isolated in 1965 and 1984 from a CCHF patient and *Hyalomma asiaticum asiaticum* ticks, respectively (Papa et al. 2002).

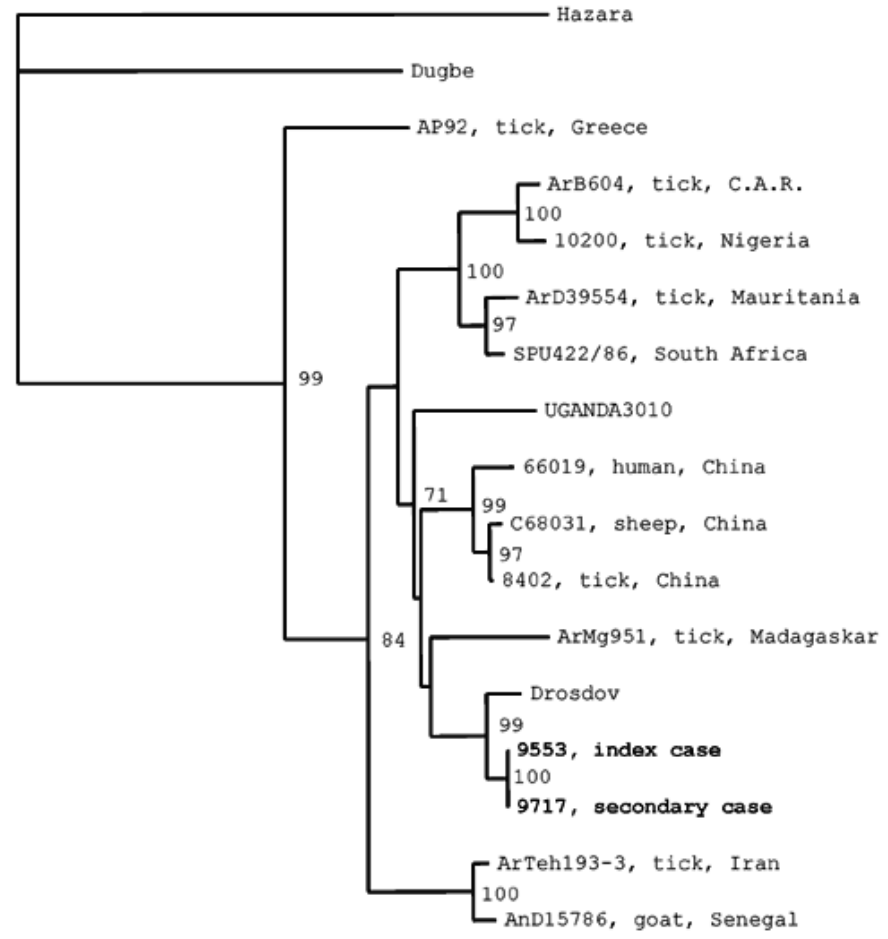
**L RNA segment:** Nigerian strain IbAr10200 isolated in 1966 from *Hyalomma excavatum* ticks (Honig et al. 2004, Kinsella et al. 2004).



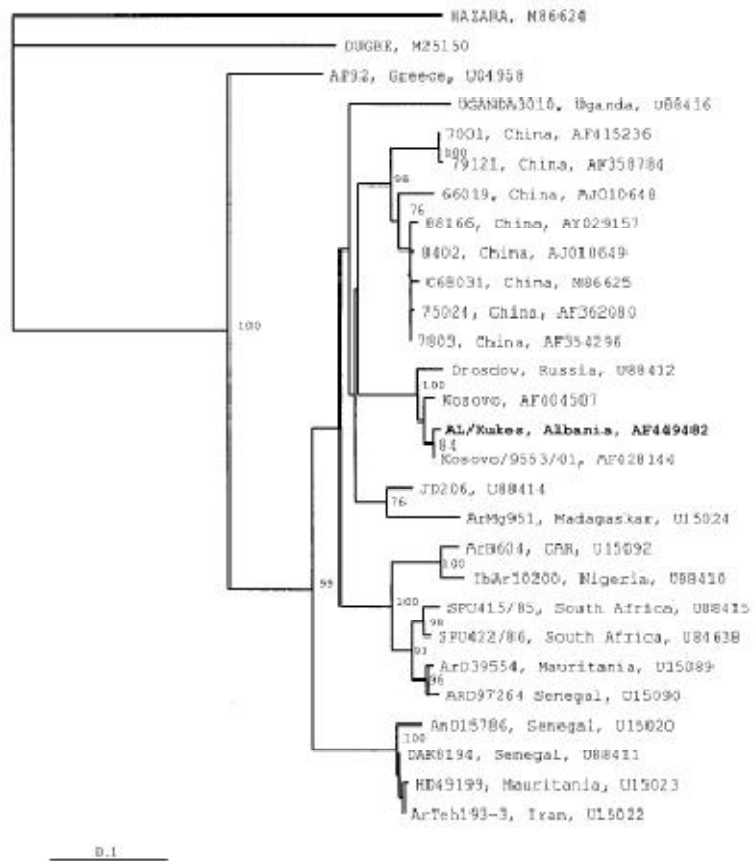
# Building up the Europe/Turkey clade



Drosten et al. JCM 2002

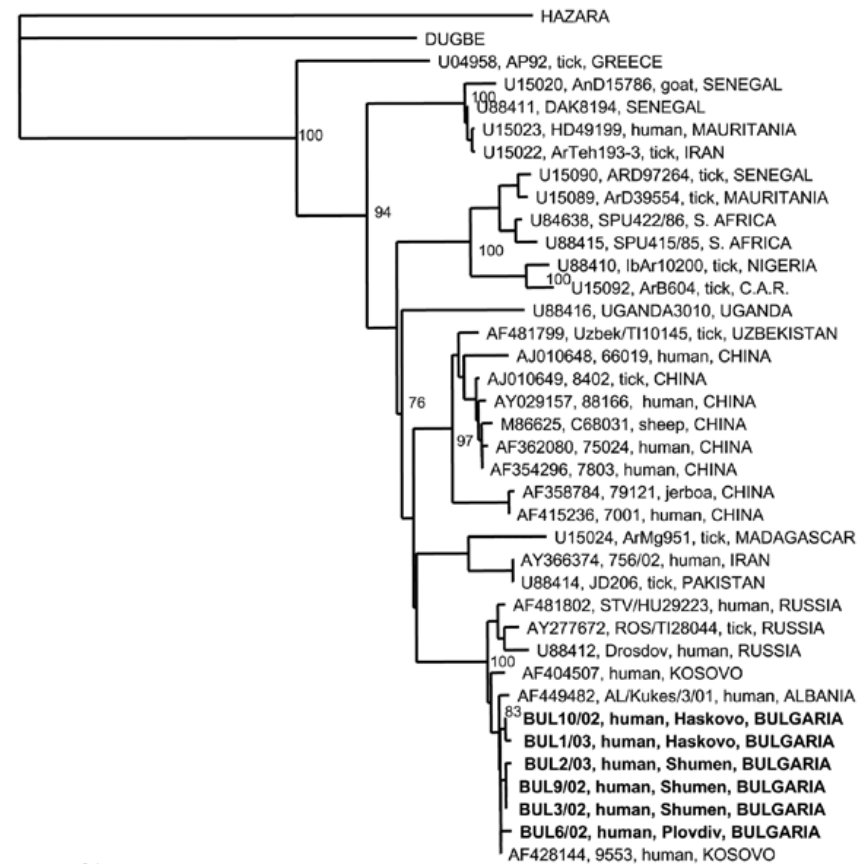


Papa et al. EID 2002  
Kosovo 9553/01 – Drosdov: 4%

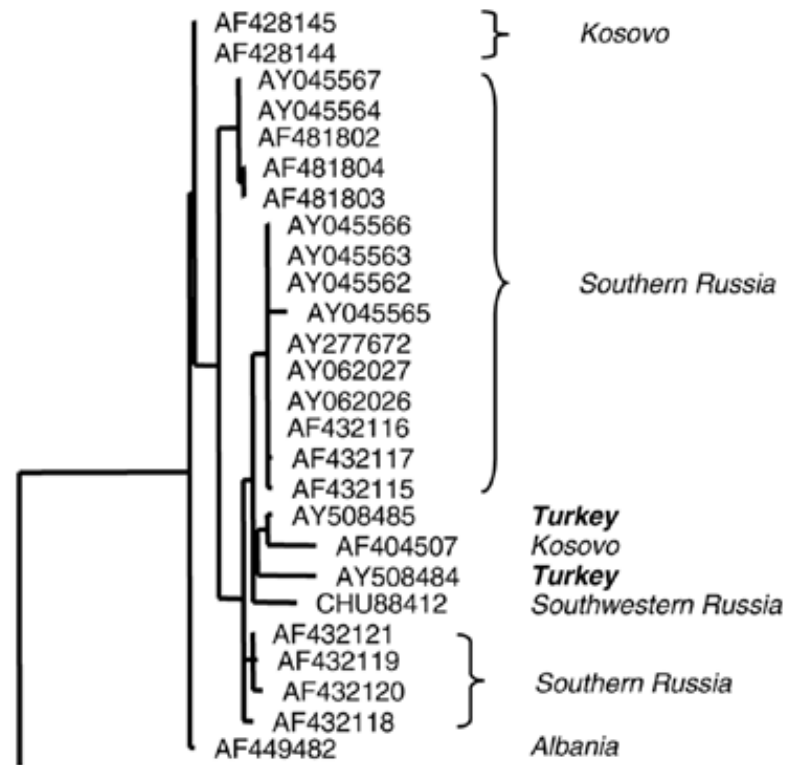


Papa et al. EVID 2002

- AL/Kukes – Kosovo 2001: 0.7%
- AL/Kukes – Kosovo 2000: 2.3%
- AL/Kukes – Drosdov: 4.8%

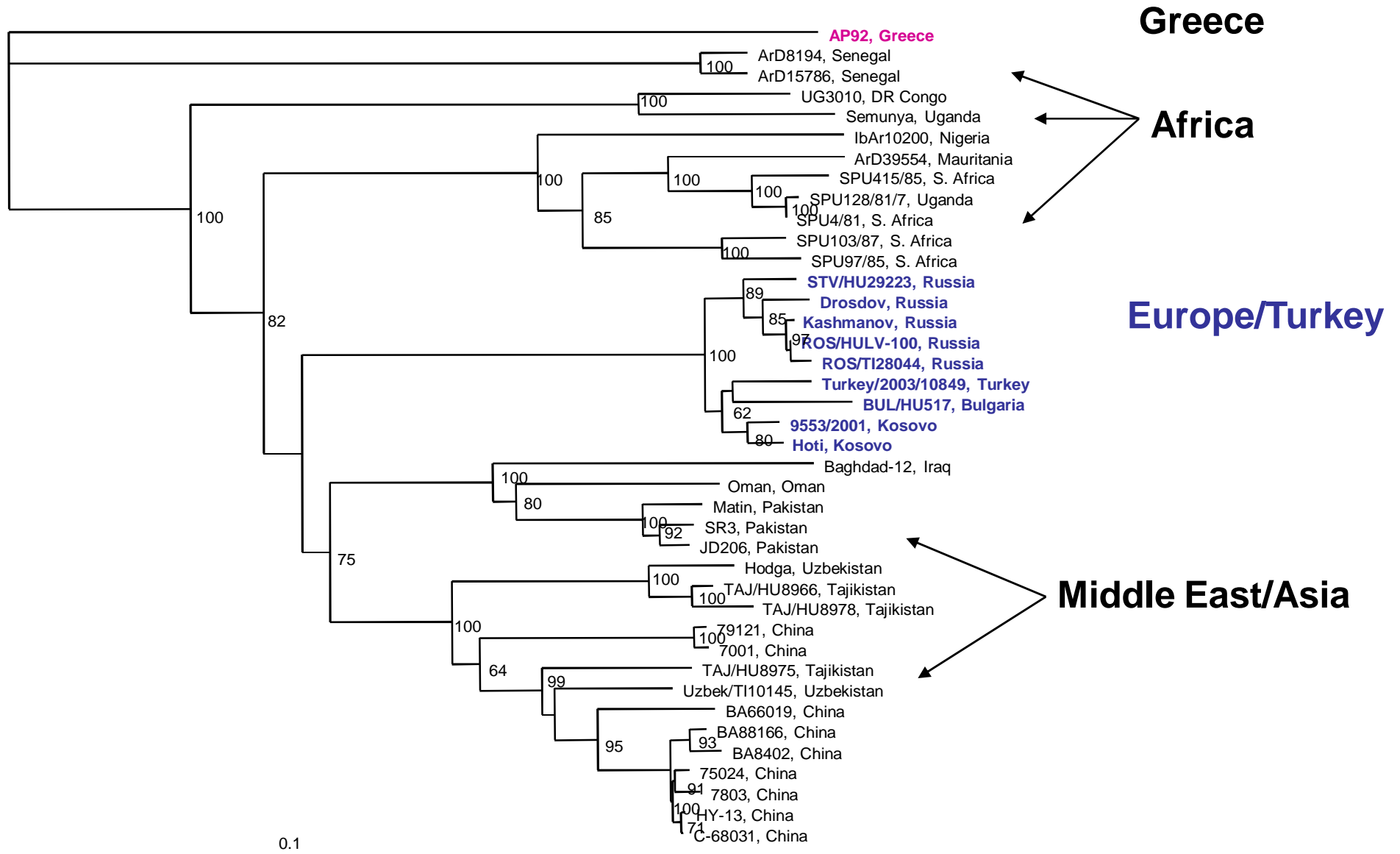


Papa et al. EVID 2004

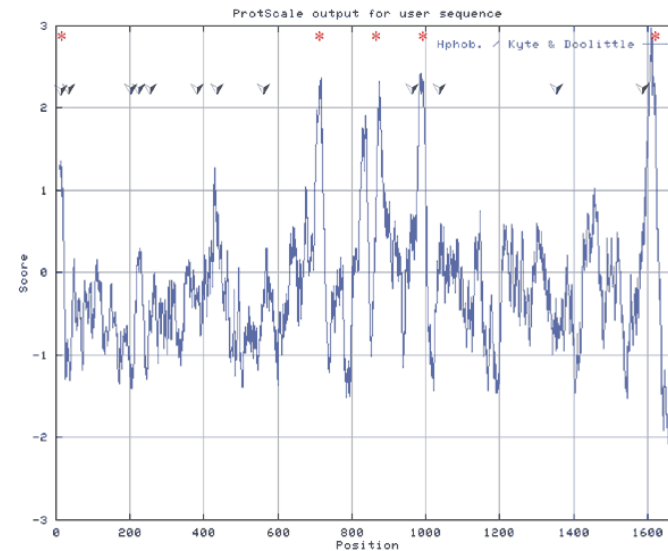
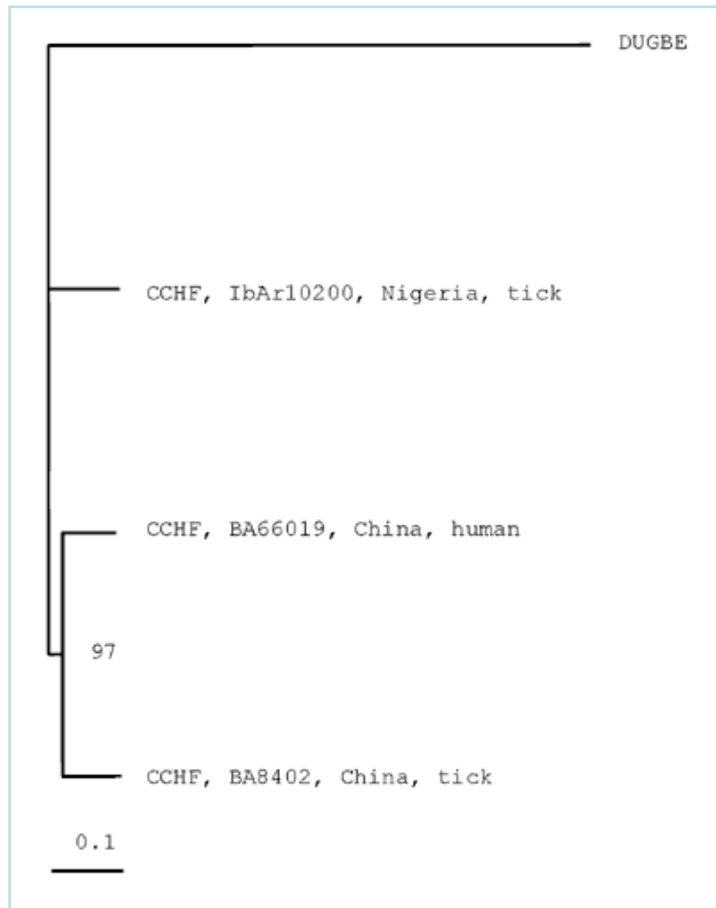


Karti et al. EID 2004

# Phylogenetic tree of all available (40) sequences of whole S RNA segment



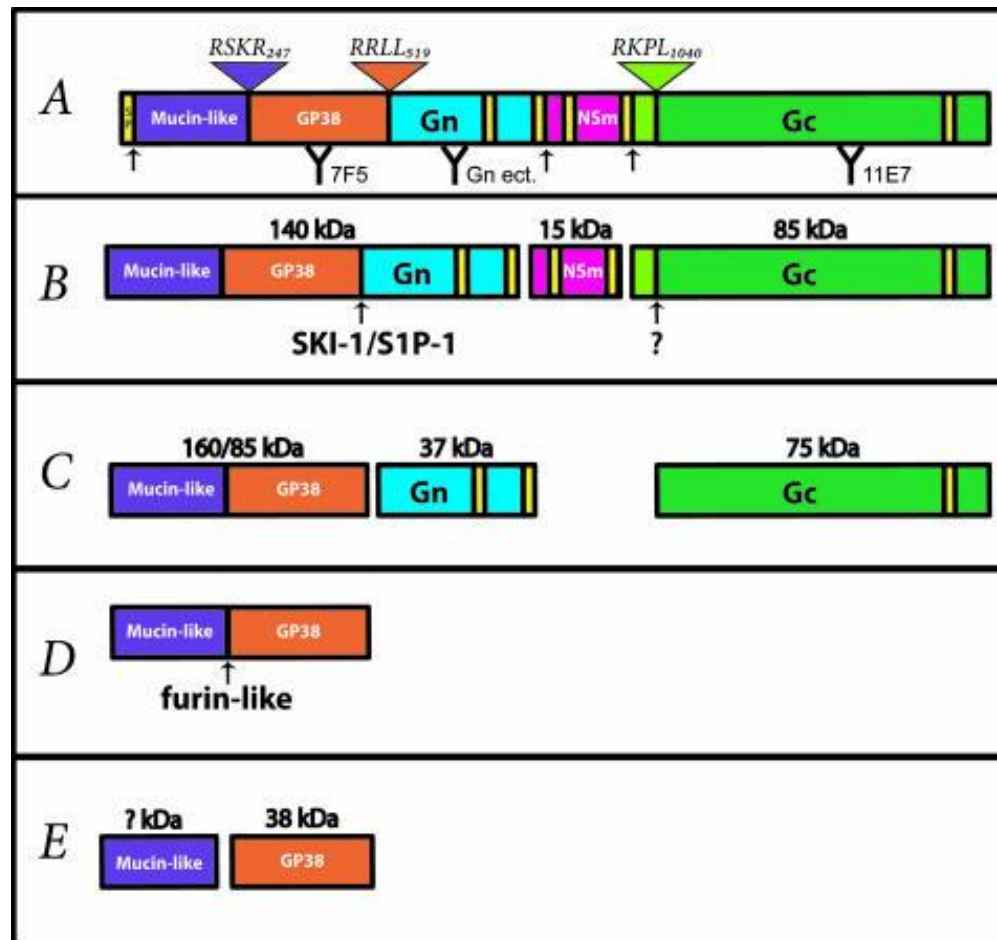
# The first phylogenetic tree of the CCHFV M RNA segment



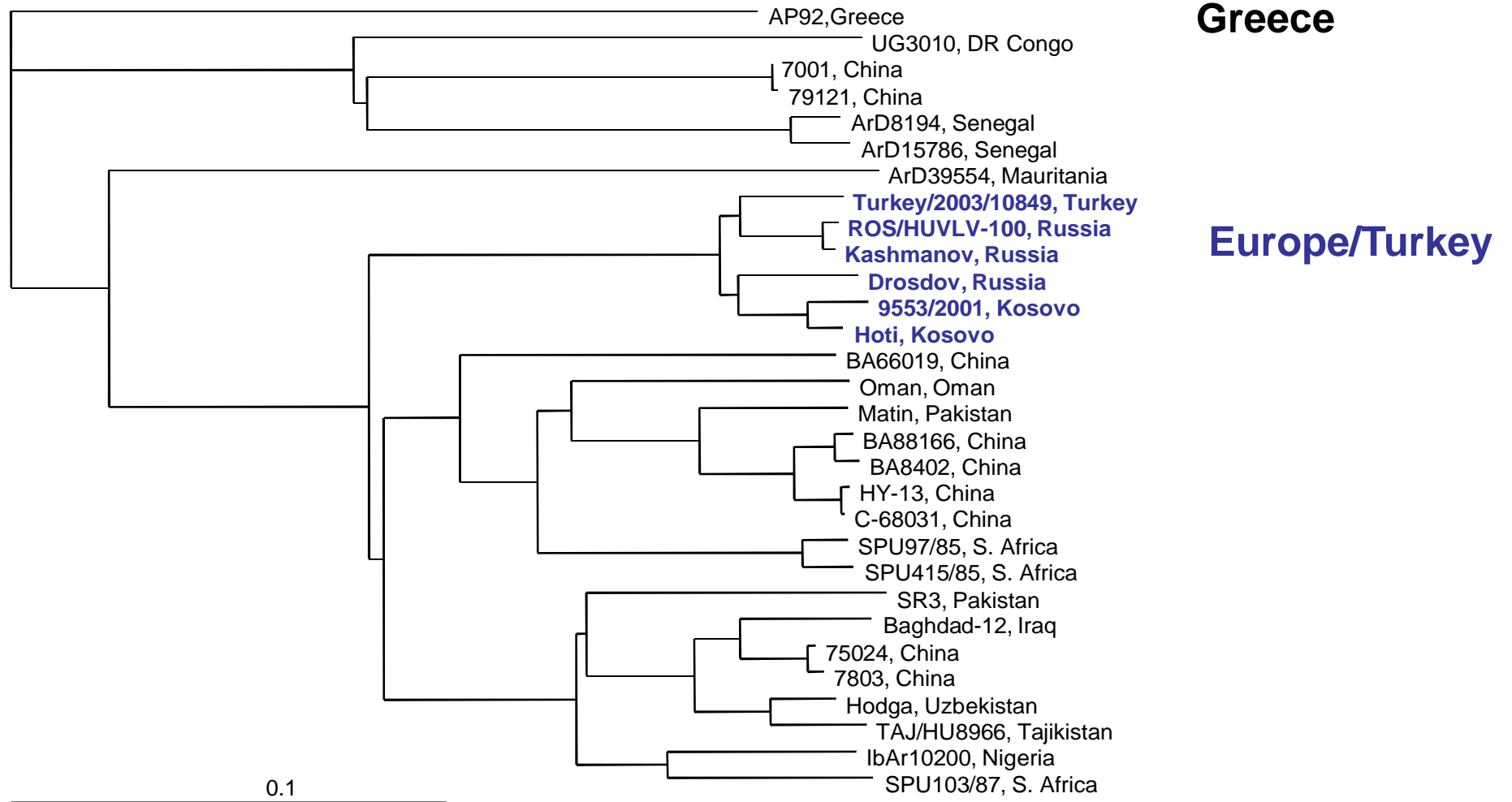
Hydropathy plot of strain BA66019 M segment ORF (1689 aa). 12 potential N-linked glycosylation sites are indicated. Five strongly hydrophobic regions are marked with an asterisk.

- Hypervariable region in the aminoterminal region of ORF M (what is now named *mucin-like region*)
- Conserved region.

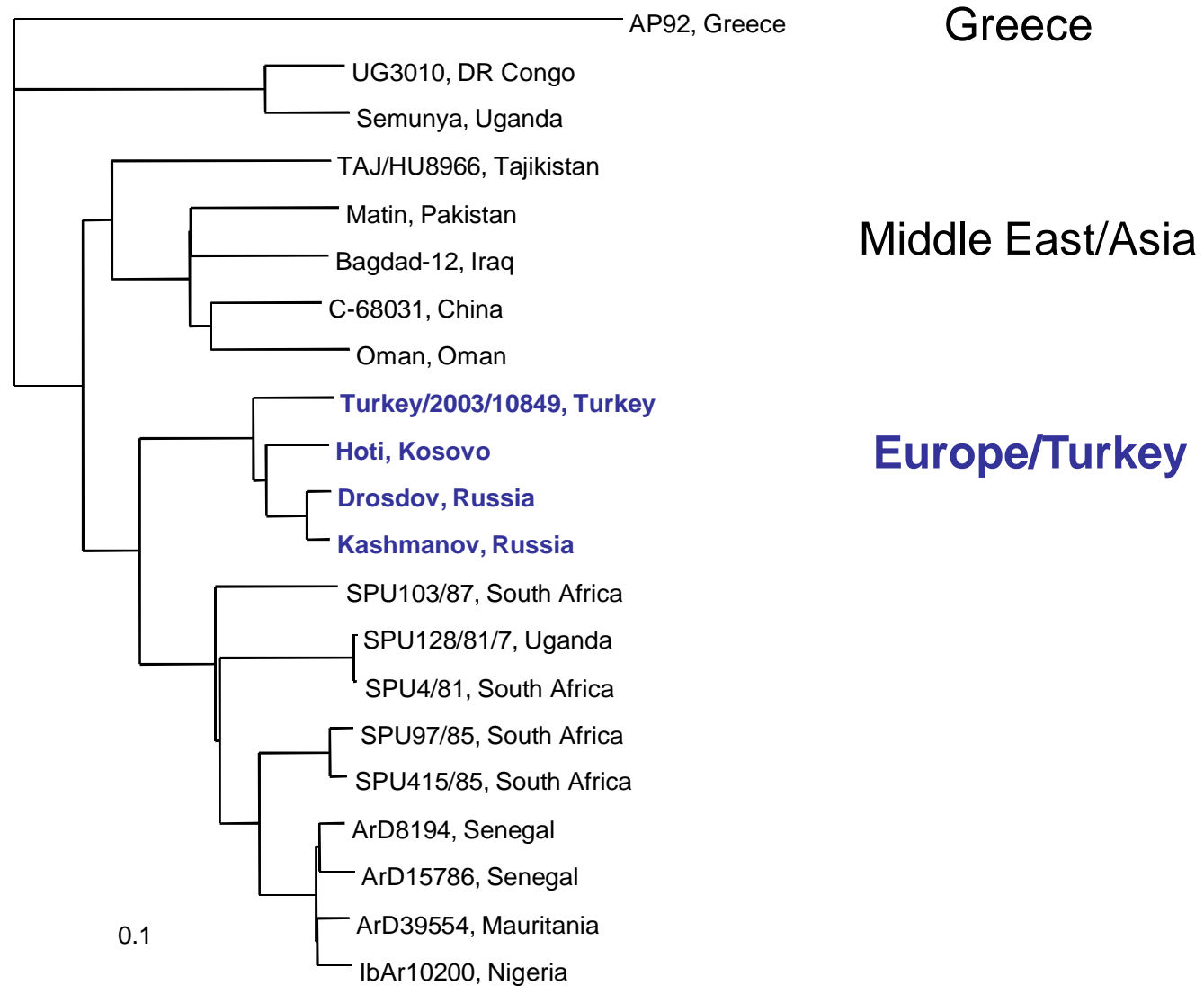
# Schematic representation of CCHFV M-encoded polyprotein domains and proteolytic processing



# Phylogenetic tree of all available (30) sequences of whole M RNA segment



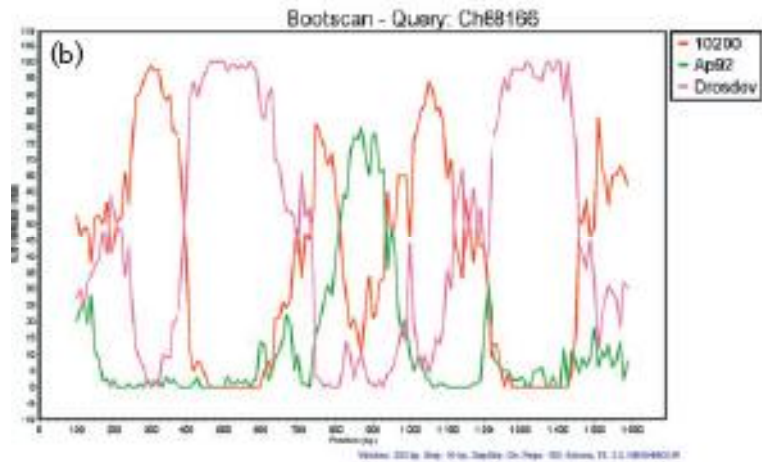
# Phylogenetic tree of all available (21) sequences of whole L RNA segment





# RECOMBINATION

## Similarity plots



S segment



L segment

# REASSORTMENT

RNA viruses with segmented genomes have the capacity to reassort their genomic segments into **new** genetically distinct viruses if the target cells are subject to dual infection.  
Example for CCHFV: Matin strain.

## Reassortment events

- M segment : frequently
- L segment : less often
- S segment : seldom

## **Factors affecting virus dispersion**

- Migratory birds infested with ticks carrying CCHFV
- Livestock trade

Most likely occurs **within ticks** coinfecting with different types of CCHFV since in ticks the virus persists for long periods.

Greatest accumulation of mutations: in the surface glycoprotein encoding the M RNA segment (31% nt - 27% aa divergence).

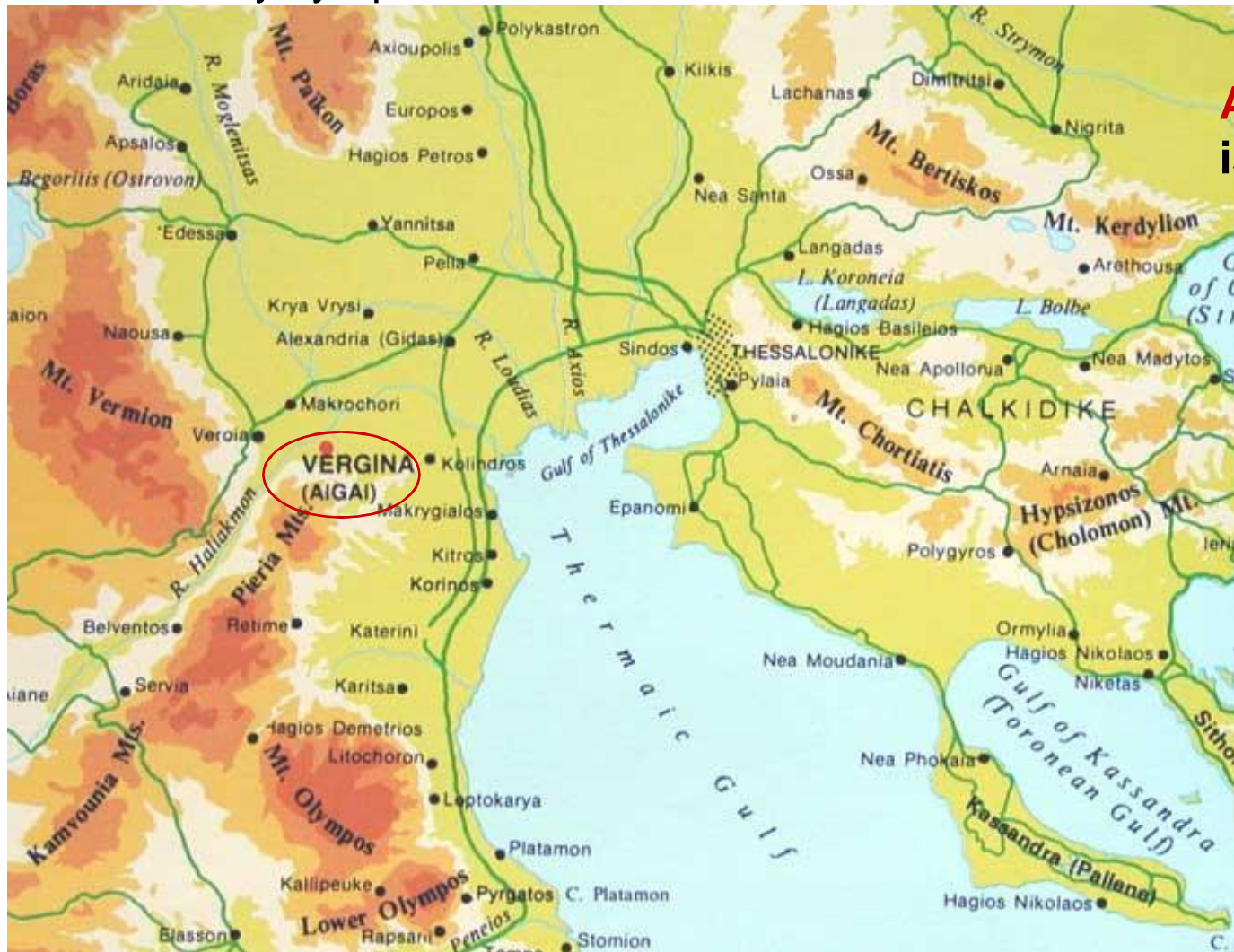
## Reasons for higher genetic diversity

Varying positive selection:

- immune selection
- selection for efficient attachment to **different combinations** of arthropod and vertebrate host cells in **different natural cycles** throughout the virus **geographic range**.

# The Greek AP92 strain

Isolated from *R. bursa* ticks, collected in May 1975 from goats of a flock in Vergina village, 80 km W. of Thessaloniki. The veterinarian who has been working with this strain presented high titers of antibodies against CCHF virus, but not any symptoms of the disease.



**AIGAI** in ancient Greek is the plural of 'goat' !

## QUESTIONS WAITING FOR AN ANSWER

**Pathogenicity.** Are certain CCHFV genotypes or certain mutations associated with increased virulence and severe disease?

**Transmission and persistence potential.** Are certain genotypes related with increased capability for transmission and persistence?

**Important sites.** Which are (if any) the important sites for the CCHFV biology and pathogenicity.

## IMPACT OF GENETIC DIVERSITY TO RESEARCH STRATEGIES

- Development of CCHF **diagnostic tools**
- Design of molecular tools for epidemiologic and forensic **analysis of outbreaks**
- Design of effective broad-spectrum **vaccine** (although target group is limited)

## CONCLUSIONS

- Existence of high genetic diversity among CCHFV strains.
- RNA segment reassortment appears to play a significant role in CCHFV evolution.
- Selective and constraining forces acting on CCHFV during its natural life cycle are the key factors for the genetic diversity.
- Knowledge of the levels of genetic diversity is valuable for further research strategies.

*Phylogenetic analysis has to be treated carefully*

***Phylogenetic analysis is frequently treated as a black box into which data are fed (often gathered at considerable cost) and out of which “The Tree” springs.***

Hillis, Moritz & Mable 1996, Molecular Systematics